CONTENTS

SYLLABUS

STANDARDIZATION AND STABILIZATION METHODS (QA) THEORY

M-255

1. General Methods

i. WHO guide lines of the standardization of Herbal raw materials and finished products.

ii. Morphological, microscopical, cytomorphological and chemical examinations of raw materials and finished products.

iii. Determination of Physical and chemical constants such as extractive values, moisture content, alcohol content, volatile oil content, ash values, bitterness values, foreign matters, and physical constants applicable to the lipid containing drugs.

iv. Microbial counts, bioburden and Pharmacopoeial microbial assays.

2. Standardization

i. Standardization of food products. Concepts of nutritional requirements at different age, sex, and in different conditions like normal, pregnancy and diseases like diabetes, hypertension and atherosclerosis, jaundice etc. Different types of additives used and analysis of these ingredients in ethical and non ethical foods.

ii. Standardization of cosmetics. Information on ingredients used in various cosmetics such as creams, powders, lotions, hair products nail polishes, lipstick, depilatories, toiletries etc. and their analysis.

iii. Standardization of Herbal products. Physicochemical characterization in whole form, separation and identification of active principles, excipients and their estimation by different techniques.

3. Stability

i. Factors affecting stability of a formulation, ICH guidelines. Methods of stabilizations and Methods of stability testing. Concept of development of stability indicating analytical methods.

PRACTICAL

The practical syllabus comprises of the exercises based on the topics mentioned in the Theory syllabus.

PART 1 : GENERAL METHODS

UNIT I: WHO GUIDELINES HERBAL FORMULATIONS

NOTES

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- give the guidelines for herbal formulations.
- define the limits for microbial contamination.
- discuss about the modern herbal ayurvedic monographs.
- describe the biological activity evaluation.

1.1 INTRODUCTION

Pharmaceutical research is aimed at meeting the medical needs of the population for whom appropriate therapeutic remedies are not available or at those that are available are unsafe for prophylactic use for various disorders. While meeting medical needs, research also has to ensure that market needs for such exist and that the product will command sales and profits proportionate to investments. In cases where there are mismatches between these two, the products suffer the status of orphan drugs. The selection of an appropriate R&D portfolio is a strategic management exercise for a company, which should take into account apart from medical needs, innovative potential for success and available resources.

1.2 WHO GUIDELINES FOR QUALITY STANDARDIZED HERBAL FORMULATIONS

1. Quality control of crude drugs material, plant preparations and finished products.

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2. Stability assessment and shelf life.
3. Safety assessment; documentation of safety based on experience or toxicological studies.
4. Assessment of efficacy by ethnomedical informations and biological activity evaluations.

The bioactive extract should be standardized on the basis of active principles or major compounds along with the chromatographic fingerprints (TLC, HPTLC, HPLC and GC). The standardization of crude drug materials include the following steps:

1. Authentication (Stage of collection, parts of the plant collected, regional status, botanical identity like phytomorphology, microscopical and histological analysis, taxonomical identity, etc.)
2. Foreign matter (herbs collected should be free from soil, insect parts or animal excreta, etc.)
3. Organoleptic evaluation (sensory characters - taste, appearance, odor, feel of the drug, etc.)
4. Tissues of diagnostic importance present in the drug powder.
5. Ash values and extractive values.
6. Volatile matter.
7. Moisture content determination.
8. Chromatographic and spectroscopic evaluation. TLC, HPTLC, HPLC methods will provide qualitative and semiquantitative information about the main active constituents present in the crude drug as chemical markers in the TLC Fingerprint Evaluation of Herbals (FEH). The quality of the drug can also be assessed on the basis of the chromatographic fingerprint.
9. Determination of heavy metals – *e.g.*, cadmium, lead, arsenic, etc.
10. Pesticide residue – WHO and FAO (Food and Agricultural Organization) set limits of pesticides, which are usually present in the herbs. These pesticides are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, toxaphene, aldrin cause serious side-effects in human beings if the crude drugs are mixed with these agents.
11. Microbial contamination – usually medicinal plants containing bacteria and molds are coming from soil and atmosphere. Analysis of the limits of *E. coli* and molds clearly throws light towards the harvesting and production practices. The substance known as afflatoxins will produce serious side-effects if consumed along with the crude drugs.

1.3 LIMITS FOR MICROBIAL CONTAMINATION

Microorganism	*Finished product*	*Raw materials*
E. coli	10^1	10^4
Salmonella	–	–
Total aerobic bacteria	10^5	–
Enterobacteria	10^3	–

Afflatoxins should be completely removed or should not be present.

12. Radioactive contamination – Microbial growth in herbals are usually avoided by irradiation. This process may sterilize the plant material but the

radioactivity hazard should be taken into account. The radioactivity of the plant samples should be checked accordingly to the guidelines of International Atomic Energy (IAE) in Vienna and that of WHO.

In order to obtain quality oriented herbal products care should be taken right from the proper identification of plants; season and area of collection, extraction, isolation and verification process. Chemical and instrumental analyses are routinely used for analyzing synthetic drugs to confirm its authenticity. In the case of herbal drugs, however the scene is different especially for polyherbal formulation, as there is no chemical or analytical methods available. Therefore biological-screening methods can be adopted for routine checkup of herbal drugs and formulations. In the case of herbal drugs, the quality of raw materials and products can be furnished by regular pharmacognostic identifications and phytochemical analysis. The herbal formulations in general can be standardized schematically as to formulate the medicament using raw materials collected from different localities and a comparative chemical efficacy of different batches of formulation are to be observed. The preparation with better clinical efficacy are to be selected. After all the routine physical, chemical and pharmacological parameters are to be checked for all the batches to select the final finished product and to validate the whole manufacturing process.

The stability parameters for the herbal formulations which includes physical parameters, chemical parameters, and microbiological parameters.

Physical parameters include color, appearance, odor, clarity, viscosity, moisture content, pH, disintegration time, friability, hardness, flowability, flocculation, sedimentation; settling rate and ash values.

Chemical parameters includes limit tests, extractive values, chemical assays, etc. Chromatographic analysis of herbals can be done using TLC, HPLC, HPTLC and GC, UV, Fluorimetry, GC-MS, etc.

Microbiological parameters include total viable content, total mold count, total enterobacterial and their count. Limiters can be utilized as a quantitative or semiquantitative tool to ascertain and control the amount of impurities like the reagents used during abstraction of various herbs, impurities coming directly from the manufacturing vessels, impurities from the solvents, etc.

Chemical decomposition of substances present in the formulation also produces several toxic or impure compounds during storage in undesirable conditions. Contaminants may come directly from the atmosphere also. This include mainly dust, sulfur dioxide, H_2S, CO_2, Arsenic, moisture, etc.

The Guidelines set by who can be Summarized as Follows

1. Reference to the identity of the drug. Botanical evaluation - sensory characters, foreign organic matter, microscopical, histological, histochemical evaluation, quantitative measurements, etc.
2. Reference to the physiochemical character of the drug. Chromatographic profiles, ash values, extractive values, refractive index, polarimetric readings, moisture content, volatile oil content, etc.
3. Reference to the pharmacological parameters. Biological activity profiles, bitterness values, haemolytic index, astringency, swelling factor, foaming index, etc.
4. Toxicity details - heavy metals like cadmium, lead, arsenic, mercury, etc. Pesticide residues.

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Maximum residue limits

$$= \frac{\text{Acceptable daily index} \times \text{body weight} \times \text{extraction factor}}{\text{Mean daily intake of drug} \times \text{safety factor} \times 100} \times \text{Therapeutic dose}$$

5. Microbial contamination – Total viable aerobic count, pathogenic bacteria like enterobacteria, *E. coli*, salmonella, *Pseudomonous aeruginosa*, *Staphylococcus aureus*, etc., and presence of afflatoxins etc.
6. Radioactive contamination.

1.4 MODERN HERBAL AYURVEDIC MONOGRAPHS

In the modern herbal ayurvedic monographs the standardization parameters are discussed in a comprehensive way. According to the modern ayurvedic monograph the quality control protocols include the following: Title, synonyms, publications related to that plant, constituents present, analytical methods. Descriptive evaluation: Description of the drug, phytomorphological, microscopical, organoleptic evaluations, foreign matter, foreign minerals, etc.

Physicochemical parameters identity: Physical and chemical identity, chromatographic finger prints, ash values, extractive values, moisture content. Strength: Ethanol and water extractive values, volatile oil and alkaloidal assays, quantitative estimation protocols, etc.

Biological activity evaluation: Bitterness values, astringency, swelling factor, form index, hemolytic index, etc.

Toxicological evaluation: Pesticide residues, heavy metals, microbial contamination like total viable aerobic count, pathogens like *E. coli*, Salmonella, *P. aeruginosa*, *S. aureus*, Enterobacteria, etc.

Aflatoxins: The presence of aflatoxins can be determined by chromatographic methods using standard aflatoxins B_1, B_2, G_1, G_2 mixtures. Aflatoxin is a product of the microbial strain *Aspergillus flavus*.

The subject of herbal drug standardization is massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function. For the purpose of research work on standardization of herbal formulations and neutraceuticals a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of utmost importance. India can emerge as the major country and play the lead role in production of standardized, therapeutically effective ayurvedic formulation. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization such as UV-visible, TLC, HPLC, HPTLC, GC-MS, spectrofluorimetric and other methods.

SUMMARY

- The radioactivity of the plant samples should be checked accordingly to the guidelines of International Atomic Energy (IAE) in Vienna and that of WHO.

- The stability parameters for the herbal formulations which includes physical parameters, chemical parameters, and microbiological parameters.
- Microbiological parameters include total viable content, total mold count, total enterobacterial and their count.

REVIEW QUESTIONS

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1. Discuss the WHO guidelines herbal formulations.
2. Know about the limits for microbial contamination.
3. Define the biological activity evaluation.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

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UNIT II: MORPHOLOGICAL, MICROSCOPICAL, CYTOMORPHOLOGICAL AND CHEMICAL EXAMINATION: POTENTIALLY HAZARDOUS CONTAMINANTS AND RESIDUES IN HERBAL MEDICINES

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- discuss about the general considerations.
- know about the chemical contaminants.
- give the example of mycotoxins and endotoxins.
- describe the agrochemical residues.

2.1 GENERAL CONSIDERATIONS

Herbal medicines are defined as herbal products in the medicines category in a national drug regulatory framework, and may include "herbs", "herbal materials", "herbal preparations" and "finished herbal products"/"herbal medicinal products".

In some countries, certain herbs and herbal materials may also be used as foods or as ingredients of foods. For this reason, the following terms have been adapted accordingly to address both regulatory categories of herbal medicines and food.

Table 2.1 shows examples of potentially hazardous contaminants and residues that may occur in herbal medicines. The summary table includes information on possible sources of contaminants and residues, as well as the manufacturing stages at which they may be detectable. Some of them are considered as unavoidable contaminants or residues of herbal medicines. Contaminants in herbal medicines are classified into physicochemical contaminants and biological contaminants. A variety of agrochemical agents and some organic solvents may be important residues in herbal medicines.

Contamination should be avoided and controlled through quality assurance measures such as Good Agricultural and Collection Practices (GACP) for medicinal plants, and Good Manufacturing Practices (GMP) for herbal medicines. Chemical and microbiological contaminants can result from the use of human excreta, animal manures and sewage as fertilizers. As noted in the WHO guidelines on GACP for medicinal plants (3), human excreta must not be used as a fertilizer, and animal manures should be thoroughly composted. Toxic elements and other chemical contaminants, including solvents originating from products intended for use in households and industrial chemicals, can be concentrated in composted sewage. Therefore, care should also be exercised with sewage management in agricultural areas. Foreign matter should be controlled. By far the majority of potentially hazardous contaminants and residues are found in the herbs and herbal materials. This results in their presence in the products, such as herbal preparations and finished herbal medicines. The level of some contaminants and residues present at the stage of the medicinal plant may change as a result of post-harvest processing (*e.g.*, drying), in herbal preparations such as extracts, and in finished herbal products during the manufacturing process. Each contaminant and residue is described in the following two subsections. Some concerns have been expressed in connection with the advancement of biotechnology, which, in the future, might be applied to medicinal plants produced using DNA technologies. This is an area that requires continuous monitoring for probable modification and new policy development. This subject, however, is beyond the scope of these guidelines.

Potentially hazardous contaminants and residues in herbal medicines WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues.

2.2 CHEMICAL CONTAMINANTS

Toxic Metals and Non-metals

Contamination of herbal materials with toxic substances such as arsenic can be attributed to many causes. These include environmental pollution (*i.e.*, contaminated

Table 2.1 Classification of major contaminants and residues in herbal medicines

Contaminants					
General classification	Group	Subgroup	Specific examples	Possible sources	Stage of production at which detectable[a]
Chemical contaminants	Toxic and hazardous materials	Toxic metals and non-metals	Lead, cadmium, mercury, chromium (arsenic, nitrite)	Polluted soil and water, during cultivation/ growth, manufacturing process	1, 2, 3, 4
		Persistent organic pollutants	Dioxin aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex	Polluted air, soil and water, during cultivation/growth	1, 2, 3, 4
		Radionuclide	Cs-134, Cs-137	Air, soil, water during cultivation/growth	1, 2, 3, 4
		Biological toxins	Mycotoxins	Post-harvest processing, transportation and storage	2, 3, 4
			Bacterial endotoxins	Post-harvest processing, transportation and storage	1, 2, 3, 4
Biological contaminants	Microorganisms	Bacteria	Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella species, Shigella species, Escherichia coli	Soil, post-harvest processing, transportation and storage	1, 2, 3, 4
		Fungi	Yeast, moulds	Post-harvest processing, transportation and storage	1, 2, 3, 4
	Animals	Parasites	Protozoa – amoebae, Helminths – nematoda	Soil, excreta; organic farming/cultivation, manufacturing process	1, 3, 4
		Insects	Cockroach and its parts	Post-harvest processing, transportation and storage	1, 2, 4
		Others	Mouse excreta, earthworms, acarus	Post-harvest processing, transportation and storage	1, 2, 4
Solvents		Organic solvents	Acetone, methanol, ethanol, butanol	Soil and water, during cultivation/growth, manufacturing process	1, 2, 3, 4

Residues					
General classification	Group	Subgroup	Specific examples	Possible sources	Stage of production at which detectable[a]
Agrochemical residues	Pesticides	Insecticides	Carbamate, chlorinated hydrocarbons, organophosphorus	Air, soil, water, during cultivation/growth, post-harvest processing	1, 2, 3, 4
		Herbicides	2, 4-D, 2, 4, 5-T	Air, soil, water, during cultivation/growth, post-harvest processing	1, 2, 3, 4
		Fungicides	Dithiocarbamate	Air, soil, water, during cultivation/growth	1, 2, 3, 4
	Fumigants	Chemical agents	Ethylene oxide, phosphine, methyl bromide, sulfur dioxide	Post-harvest processing	2, 3, 4
	Disease control agents	Antiviral agents	Thiamethoxam	During cultivation	1, 2, 3, 4
Residual solvents		Organic solvents	Acetone, methanol, ethanol, butanol	Manufacturing process	3, 4

[a] stage of production at which detectable: 1, medicinal plants; 2, herbal materials; 3, herbal preparations; 4, Finished herbal products.

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emissions from factories and leaded petrol and contaminated water including runoff water which finds its way into rivers, lakes and the sea, and some pesticides), soil composition and fertilizers. This contamination of the herbal material leads to contamination of the products during various stages of the manufacturing process.

Pesticides containing arsenic and mercury were widely used until a few years ago and they are still being used in some countries. As toxic substances are likely to be present in many foods, due to their abundance in nature, it is important to note that concomitant ingestion of herbal products would add to the total concentration of toxic metals consumed by people, even if best practice guidelines are followed.

Persistent Organic Pollutants

POPs include organic chemicals, such as the synthetic aromatic chlorinated hydrocarbons, which are only slightly soluble in water and are persistent or stable in the presence of sunlight, moisture, air and heat. In the past, they were extensively used in agriculture as pesticides. They are still generated inadvertently as by-products of combustion or industrial processes.

The use of persistent pesticides, such as DDT and benzene hexachloride (BHC), in agriculture has been banned for many years in many countries. However they are still found in the areas where they were previously used and often contaminate medicinal plants growing nearby. Also many of these substances are still being used for public health purposes, for example the control of disease vectors such as malaria-carrying mosquitoes, and are often applied near agricultural fields. The pesticide residues can then drift through the air on to the medicinal plant crops growing in nearby fields resulting in their contamination.

Thus care should be exercised with checking the quality of the medicinal plants grown in areas where these persistent pesticides are still being used. The Stockholm Convention on Persistent Organic Pollutants1 currently includes DDT and 11 other POPs including dioxin (a potent carcinogen), aldrin, chlordane, dieldrin, endrin, heptachlor, mirex, toxaphene and hexachlorobenzene.

Radioactive Contamination

A certain amount of exposure to ionizing radiation is unavoidable because many sources, including of radionuclides occur naturally in the ground and the atmosphere. Potentially hazardous contaminants and residues in herbal medicines. Dangerous contamination may be the consequence of a nuclear accident or may arise from other sources. WHO, in close collaboration with several other international organizations, has developed guidelines for use in the event of widespread contamination by radionuclides resulting from a major nuclear accident. Examples of such radionuclides include long-lived and short-lived fission products, actinides and activation products. In general the nature and the intensity of these radionuclides may differ markedly and depends on factors such as the source, which could be a reactor, reprocessing plant, fuel fabrication plant, isotope production unit or other.

These guidelines emphasize that the health risks posed by herbal medicines accidentally contaminated by radionuclides depend not only on the specific radionuclide and the level of contamination, but also on the dose and duration of use of the product consumed. An important consideration in the testing for radioactive substances in herbal materials and products is the availability of the appropriate

methodology and equipment. Member States would probably benefit from collaboration with countries where these facilities are available. Cross-contamination of radionuclide-free herbal materials should be totally avoided during all the stages of production, transportation and storage.

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2.3 MYCOTOXINS AND ENDOTOXINS

Mycotoxins

The presence of mycotoxins in plant material can pose both acute and chronic risks to health. Mycotoxins are usually secondary metabolic products which are nonvolatile, have a relatively low molecular weight, and may be secreted onto or into the medicinal plant material. They are thought to play a dual role, firstly, in eliminating other microorganisms competing in the same environment and secondly, helping parasitic fungi to invade host tissues. Mycotoxins produced by species of fungi including *Aspergillus, Fusarium* and *Penicillium* are the most commonly reported.

Mycotoxins comprise four main groups, namely, aflatoxins, ochratoxins, fumonisins and tricothecenes, all of which have toxic effects. Aflatoxins have been extensively studied and are classified as Group 1 human carcinogens by the International Agency for Research on Cancer.

Endotoxins

Endotoxins are found mainly in the outer membranes of certain Gram-negative bacteria and are released only when the cells are disrupted or destroyed. They are complex lipopolysaccharide molecules that elicit an antigenic response, cause altered resistance to bacterial infections and have other serious effects. Thus tests for their presence on herbal medicines should be performed in dosage forms for parenteral use, in compliance with the requirements of national, regional or international pharmacopoeias.

Solvents Occurring as Contaminants

Solvents used in industries other than the manufacturing of herbal medicines, are often detected as contaminants in water used in irrigation, for drinking and for industrial purposes and thus they find their way into medicinal plants and herbal materials at various stages of growth and processing.

2.4 BIOLOGICAL CONTAMINANTS

Microbiological Contaminants

Herbs and herbal materials normally carry a large number of bacteria and moulds, often originating in soil or derived from manure. While a large range of bacteria and fungi form the naturally occurring microflora of medicinal plants, aerobic spore-forming bacteria frequently predominate. Current practices of harvesting, production, transportation and storage may cause additional contamination and microbial growth. Proliferation of microorganisms may result from failure to control the moisture levels of herbal medicines during transportation and storage, as

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well as from failure to control the temperatures of liquid forms and finished herbal products. The presence of *Escherichia coli, Salmonella* spp. and moulds may indicate poor quality of production and harvesting practices.

Microbial contamination may also occur through handling by personnel who are infected with pathogenic bacteria during harvest/collection, post-harvest processing and the manufacturing process. This should be controlled by implementing best practice guidelines such as GACP and GMP.

Parasitic Contamination

Parasites such as protozoa and nematoda, and their ova, may be introduced during cultivation and may cause zoonosis, especially if uncomposted animal excreta are used. Contamination with parasites may also arise during processing and manufacturing if the personnel carrying out these processes have not taken appropriate personal hygiene measures.

2.5 AGROCHEMICAL RESIDUES

The main agrochemical residues in herbal medicines are derived from pesticides and fumigants. Pesticides may be classified on the basis of their intended use, for example as follows:

- insecticides;
- fungicides and nematocides;
- herbicides; and
- other pesticides (*e.g.*, ascaricides, molluscicides and rodenticides).

Examples of fumigants include ethylene oxide, ethylene chlorohydrin, methyl bromide and sulfur dioxide.

Pesticide Residues

Medicinal plant materials may contain pesticide residues, which accumulate as a result of agricultural practices, such as spraying, treatment of soils during cultivation and administration of fumigants during storage. It is therefore recommended that Potentially hazardous contaminants and residues in herbal medicines WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues every country producing medicinal plant materials should have at least one control laboratory capable of performing the determination of pesticides using a suitable method.

Classification of Pesticides

Different classifications of pesticides exist. A classification based on the chemical composition or structure of the pesticide is the most useful for analytical chemists, for example:

- chlorinated hydrocarbons and related pesticides: hexachlorocyclohexane (HCH) or benzene hexachloride (BHC), lindane, methoxychlor
- chlorinated phenoxyalkanoic acid herbicides: 2, 4-D, 2, 4, 5-T
- organophosphorus pesticides: carbophenothion (carbofenotion), chlorpyrifos and methylchlorpyrifos, coumaphos (coumafos), demeton, dichlorvos, dimethoate, ethion, fenchlorphos (fenclofos), malathion, methyl parathion, parathion

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- carbamate insecticides: carbaryl (carbaril)
- carbamoyl benzimidazoles: benomyl, carbendazim
- dithiocarbamate fungicides: ferbam, maneb, nabam, thiram, zineb, ziram
- amino acid herbicides: glyphosate
- inorganic pesticides: aluminium phosphide, calcium arsenate
- miscellaneous: bromopropylate, chloropicrin, ethylene dibromide, ethylene oxide, methyl bromide, sulfur dioxide
- pesticides of plant origin: tobacco leaf extract, pyrethrum flower, and pyrethrum extract; derris and *Lonchocarpus* root and rotenoids.

Only the chlorinated hydrocarbons and related pesticides (*e.g.*, HCH) and a few organophosphorus pesticides (*e.g.*, carbophenothion) have a long residual action. Although the use of many persistent pesticides has been widely discontinued, residues may still remain in the environment (*e.g.*, DDT). Thus the recording of all pesticide usage in countries should be strongly encouraged so as to enable cost-effective quality control of medicinal plants and of their products.

Pesticides based upon copper as the active agent *e.g.*, copper sulfate and mixtures of copper sulfate and hydrated lime were often used in the past and are still popular with farmers today. Such compounds are effective fungicides. Although copper is an essential nutrient for plants its levels must be controlled because if ingested at high levels, around 70 mg/day, it does have serious adverse effects on health. The likelihood of exposure to copper is also heightened by the fact that copper is strongly bioaccumulated in nature and therefore it is likely to persist in herbal materials, similarly to the heavy metals.

Most other pesticides have very short residual actions. Therefore it is suggested that, where the length of exposure to pesticides is unknown, the herbal materials should be tested for the presence of organically bound chlorine and phosphorus as a preliminary screening method which can be useful in predicting where a pesticide might be used.

2.6 RESIDUAL SOLVENTS

A range of organic solvents are used for manufacturing herbal medicines, and can be detected as residues of such processing in herbal preparations and finished herbal products. They should be controlled through GMP and quality control.

Solvents are classified by ICH (CPMP/ICH 283/95), according to their potential risk, into:

- Class 1 (solvents to be avoided such as benzene);
- Class 2 (limited toxic potential such as methanol or hexane); and
- Class 3 (low toxic potential such as ethanol).

SUMMARY

- A variety of agrochemical agents and some organic solvents may be important residues in herbal medicines,
- Mycotoxins comprise four main groups, namely, aflatoxins, ochratoxins, fumonisins and tricothecenes, all of which have toxic effects.

- The main agrochemical residues in herbal medicines are derived from pesticides and fumigants.
- A classification based on the chemical composition or structure of the pesticide is the most useful for analytical chemists.

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REVIEW QUESTIONS

1. Discuss the Classification of major contaminants and residues in herbal medicines.
2. Discuss chemical contaminants in herbal medicines.
3. Discuss mycotoxins and endotoxins.
4. Discuss biological contaminants
5. Discuss agrochemical residues.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

UNIT III: GUIDING PRINCIPLES FOR ASSESSING SAFETY OF HERBAL MEDICINES WITH REFERENCE TO CONTAMINANTS AND RESIDUES

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- describe the general approach with good practice guidelines.
- discuss about the foreign matter.
- know about the microbiological contaminants.
- define the acceptable residue level.

3.1 GENERAL APPROACH — COMPLIANCE WITH GOOD PRACTICE GUIDELINES

Compliance with GACP and GMP is crucial for the production of good quality herbal medicines. The entire production process, starting from cultivation and ending with the sale of the products, must adhere rigorously to these two sets of practices. The contents of these guidelines should therefore be read in conjunction with GACP and GMP in an effort to produce quality products for the local and international markets.

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3.2 FOREIGN MATTER

Foreign matter found in a sample of herbs and herbal materials should not exceed limits set in national, regional or international pharmacopoeias. Foreign matter includes insects and other animal contamination including animal excreta, as well as other species of plants. In general, any substance other than the acceptable sample of good quality medicinal plant material is regarded as foreign matter. A pure sample is seldom found and there is always some foreign matter present. However no poisonous, dangerous or otherwise harmful foreign matter should be allowed. Thus following the GACP should help to ensure that contamination is kept to a minimum.

Removal of larger pieces of foreign matter from whole and cut plants is often done by hand-sorting after macroscopic examination. Finished products should also be examined for foreign materials.

3.3 CONTAMINANTS

Arsenic and Toxic Metals

The maximum amounts of toxic metals and non-metals in medicinal plant materials can be given based on the Provisional Tolerable Intake (PTI) values. These values should be established on a regional or national basis.

The use of herbal medicinal products is not generally expected to contribute significantly to the exposure of the population to heavy metal contaminants. However, it should be understood that the heavy metal content of herbal medicines adds to the burden originating from food so it is recommended that heavy metal contamination is minimized. Guiding principles for assessing safety of herbal medicines with reference to con-taminants and residues. In general it would be desirable to harmonize limits for toxic metals and standards, as this would have many benefits including the facilitation of global trade.

Persistent Organic Pollutants

POPs comprise hundreds of chemicals that are not soluble in water and are persistent or stable in the environment. They are often transported globally because of their resistance to breakdown and they have the potential to cause harm to humans and wildlife that ingest them. They will not disappear from our environments in the short term because some are still being produced and used in many countries.

Internationally, through the Stockholm Convention on Persistent Organic Pollutants, efforts are being made to control their production and emission, and to substitute them with other less problematic pesticides.

Radioactive Contaminants

The amount of exposure to radiation depends on the intake of radionuclides and its significance depends in turn on other variables such as the age, metabolic kinetics and weight of the individual who ingests them (also known as the dose conversion factor).

The level of contamination might be reduced during the manufacturing process. Therefore, no limits for radioactive contamination are proposed in these guidelines

and herbal materials should be tested on a case-by-case basis according to national and regional standards if there are concerns. In such a process, national regulation on the limits could be set based on risk management, but no risk assessment.

Microbial Toxins

Mycotoxins and, when appropriate, endotoxins should be tested for using an appropriately validated and sensitive method, and amounts should be below the limits set in national or regional standards. It is recommended that in performing analyses for mycotoxins particular care should be taken, in line with good practice guidelines such as WHO good practices for national pharmaceutical control laboratories.

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3.4 MICROBIOLOGICAL CONTAMINANTS

Bacteria

Salmonella and *Shigella* species must not be present in herbal medicines intended for internal use, at any stage. Other microorganisms should be tested for and should comply with limits set out in regional, national or international pharmacopoeias.

Different pharmacopoeias have different testing requirements and these should be consulted when making the appropriate choice for the selected herbal materials and herbal product.

Office for Official Publications of the European Communities, Council Regulation (EEC). No. 737/90 of 22 March 1990 on the conditions governing import of agricultural products originating in third countries following the accident at the Chernobyl nuclear power station.

3.5 RESIDUES

Pesticide Residues

Limits for pesticide residues should be established following the recommendations of the JMPR which have already been established for food and animal feed. These recommendations include an ADI and the analytical methodology for the assessment of specific pesticide residues. Currently however there are no standard procedures for assignment of these MRLs in the medicinal plant area and thus the methods used for foods could probably be used for the preparation of a model.

This approach would apply in the case where a botanically identical medicinal plant is used as food. Historically the FAO and WHO have established MRLs, based on supervised trials and establishment of GAP (21) for the use of pesticides for a variety of food commodities and combinations of pesticides and food commodities. If the listed food commodity (name of the original plant and the part of it used as food) is botanically identical to the medicinal plant part concerned and has an established MRL for a specific pesticide, the relevant MRL could be regarded as the MRL for the specific raw medicinal plant material. In this case, the MRL for the medicinal plant material in question could be further elaborated by establishing an appropriate formula to factor in drying of the plant material. The Codex Alimentarius Commission has adopted a list of approved pesticides for spices and their MRLs.

NOTES

If the medicinal plant in question is identical to the plant, but the part concerned is different to that listed for the food commodity, or it is not identical to the plant on the food commodity list, elaboration of MRLs for medicinal plant materials could be attempted by similar approaches to those described below.

Maximum Limit of Pesticide Residues for Herbal Materials

The toxicological evaluation of pesticide residues in herbal materials should be based on the likely intake of the material by patients. In the absence of a full risk assessment and for practical reasons, it is recommended that, in general, the intake of residues from herbal materials should account for no more than 1% of total intake from all sources, including food and drinking-water (2). Since the level of pesticide residues may change during the production process, it is vital to determine the actual quantity of residues consumed in the final dosage form. Because herbal medicines may be used for treatment of chronic diseases or for prophylactic reasons, it is suggested that the approach of the FAO in determining MRLs should be followed.

Acceptable Residue Level

An ARL (in mg of pesticide per kg of medicinal plant material) can be calculated on the basis of the maximum ADI of the pesticide for humans, as, recommended by FAO and WHO, and the Mean Daily Intake (MDI) of the medicinal plant material. Some countries and/or regions have established national requirements for residue limits in medicinal plant materials. If no such requirements exist, other references can be consulted, such as other pharmacopoeias or published documents. The appropriateness of risk assessment using the ARL needs further investigation and research. Where such requirements do not exist, the following formula that is based on the Acceptable Daily Intake (ADI) determined by FAO and WHO, may be used:

$$ARL = (ADI \times E \times 60)/(MDI \times 100)$$

where:

ADI = Maximum acceptable daily intake of pesticide (mg/kg of body weight).

E = Extraction factor, determined experimentally, which determines the transfer rate of the pesticide from the medicinal plant material into the dosage form.

MDI = Mean daily intake in kilograms of medicinal plant material.

60: This number represents a mean adult body weight of 60 kg; it may need to be adjusted for certain patient groups, nationalities, etc.

100: This number is a consumption factor of 100 reflecting the requirement that no more than 1% of the total pesticide residue consumed should be derived from medicinal plant material.

Extraneous Pesticide Residues

According to the Codex Commission Committee on Pesticide Residues, residues of DDT and BHC have been found in some spices. It is suggested that for these compounds, EMRLs be established instead of MRLs in the same manner as for other pesticides for food commodities.

Residual Solvents

The term "permitted daily exposure" (PDE) is proposed as defining a pharmaceutically acceptable intake of residual solvents to avoid confusion resulting from differing values for ADIS of the same substance.

NOTES

SUMMARY

- Foreign matter found in a sample of herbs and herbal materials should not exceed limits set in national, regional or international pharmacopoeias.
- The maximum amounts of toxic metals and non-metals in medicinal plant materials can be given based on the Provisional Tolerable Intake (PTI) values.
- The toxicological evaluation of pesticide residues in herbal materials should be based on the likely intake of the material by patients.

REVIEW QUESTIONS

1. Discuss contaminants in herbals.
2. Discuss microbiological contaminants.
3. Know about the foreign matter.
4. Define the acceptable residue level.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

NOTES

UNIT IV: DETERMINATION OF PHYSICAL AND CHEMICAL CONSTANT: RECOMMENDED ANALYTICAL METHODS

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- discuss about the arsenic and toxic metals.
- define the radioactive contaminants.
- describe the determination of aflatoxins.
- explain the determination of pesticide residues.

4.1 INTRODUCTION

The test methods described here are presented as examples of suitable methods for the detection of selected contaminants and residues in herbal medicines, mainly for herbal materials. Where available, test methods applicable to different products and stages of herbal medicines, such as extracts and finished herbal products, are also described. In addition to the test methods, some examples of national experience regarding general limits for contaminants and residues are included, where applicable.

NOTES

Both should be considered as the basis for establishing national and regional requirements for limits and methodologies. WHO is currently not able to recommend limits for contaminants and residues because they are too diverse and there is a lack of consensus. Also the test procedures cannot take into account all possible impurities, but where impurities are known to occur, validated methods should be developed.

The test methods should be used where appropriate, only at certain stages, and on a case-by-case basis. The analysis of herbal medicines is not restricted to those methods discussed or recommended here and other techniques are also available. Details of analytical methods, such as volumetric analysis, are described in international pharmacopoeias.

When considering the choice of method, the level of detection and the plant product matrix used for the testing, *e.g.*, seeds containing oils or finished products, must be taken into account, and the method modified if required. The method of determination should be validated for the relevant matrix.

Although selected methods are described in detail in the annexes to this document, they may not necessarily be the most modern or state-of-the-art methods. They do offer some options and guidance, but the available technology and resources, including human and financial, may influence their use in particular countries.

In the event of limitations precluding the required analytical services for herbal products in a particular district(s) in a country, it is recommended that at least other national or regional official laboratories be made available for such purposes.

The guidance of good practices for national pharmaceutical control laboratories, including quality assurance measures, should be followed when methods are chosen for all analyses. All methods chosen should be properly validated in accordance with these good practices.

4.2 ARSENIC AND TOXIC METALS

In general, quantitative tests and limit tests accurately determine the concentrations of toxic metals in the form of impurities and contaminants. The latter are unavoidably present in the samples being tested *i.e.*, herbal medicines and their herbal products.

Member States can elect to use either quantitative tests or limit tests and their choices will be influenced by the nature of the sample and the contaminants or residues, assessed on a case-by-case basis. Another factor would be that the method(s) identified, and chosen to be applied to control heavy metals, should be relevant and should meet the requirements at a regional and national level.

Some examples of proposed national limits for arsenic and toxic metals in various types of herbal products are shown in Table 4.1. Country figures are based on information provided by national health authorities.

The metals are widely distributed throughout nature and occur freely in soil and water and are often components of certain pesticides. In general, during analysis of metals, one should always aim to use the best and latest methods whenever possible. However it is crucial to ensure that all methods are fully validated, not forgetting the need to validate the integrity of the starting matrix of plant product. This imperative should apply both to governments and companies/applicants submitting these methods as part of their applications to the national regulatory authority for market authorization.

Table 4.1 Examples of national limits for arsenic and toxic metals in herbal medicines and products

		Arsenic (As)	*Lead (Pb)*	*Cadmium (Cd)*	*Chromium (Cr)*	*Mercury (Hg)*	*Copper (Cu)*	*Total toxic metals as*
For herbal medicines								
Canada	raw herbal materials	5 ppm	10 ppm	0.3 ppm	2 ppm	0.2 ppm		
	finished herbal products	0.01 mg/day	0.02 mg/day	0.006 mg/day	0.02 mg/day	0.02 mg/day		
China	herbal materials	2 ppm	10 ppm	1 ppm		0.5 ppm		20 ppm
Malaysia	finished herbal products	5 mg/kg	10 mg/kg			0.5 mg/kg		
Republic of Korea	herbal materials							30 ppm
Singapore	finished herbal products	5 ppm	20 ppm			0.5 ppm	150 ppm	
Thailand	herbal material, finished herbal products	4 ppm	10 ppm	0.3 ppm				
WHO recommendations (2)			10 mg/kg	0.3 mg/kg				
For other herbal products								
National Sanitation Foundation draft proposal (Raw Dietary supplement)[a]		5 ppm	10 ppm	0.3 ppm	2 ppm			
National Sanitation Foundation draft proposal (Finished Dietary Supplement)[a]		0.01 mg/day	0.02 mg/day	0.006 mg/day	0.02 mg/day	0.02 mg/day		

[a] Dietary Supplement – for further information see ref. (32).

Limit tests find wide application in the area of pharmaceutical medicines where it is common to test for substances such as chlorides, sulfates, arsenic and heavy metals. Thus they will be very useful in the testing of herbal medicines and their products. Limit tests can also be modified in many instances to function as true limit tests where the actual amount of toxic metal can be estimated with great accuracy.

The need for the inclusion of tests for toxic metals and acceptance criteria should be studied at the various developmental stages of the plant and based on knowledge of the medicinal plant species, its growth and or cultivation and the manufacturing process. The choice of procedures for control should take account of this information: the choice between a limit test and a specific quantitative method will depend on the level of control required for a particular material, for example starting material, and should be justified.

In general, if the heavy metals burden of the herbal material is unknown, it is suggested that it be determined qualitatively and quantitatively on several batches, preferably collected over several years. These data should be used to establish acceptance limits that should be checked by appropriate limit tests.

4.3 RADIOACTIVE CONTAMINANTS

Method of Measurement

Following a severe nuclear accident, the environment may be contaminated with airborne radioactive materials. These may deposit on the leaves of medicinal plants. Their activity concentration and the type of radioactive contamination can be measured by the radiation monitoring laboratories of most of the WHO Member States. The activity concentration of radioisotopes in herbs should be assessed by the competent national radiohygiene laboratories taking into account the relevant recommendations of international organizations, such as Codex Alimentarius, the International Atomic Energy Agency (IAEA), FAO and WHO. Since radionuclides from accidental discharges vary with the type of facility involved, a generalized method of measurement is not yet available. However, should such contamination be a concern, suspect samples can be analysed by a competent laboratory. Details of laboratory techniques are available from the IAEA.

4.4 AFLATOXINS

Determination of aflatoxins should take place after using a suitable clean-up procedure, during which great care should be taken not to become exposed or to expose the working or general environment to these dangerous and toxic substances. Thus Member States should adapt their good practices for national pharmaceutical control laboratories and GMP accordingly. Only products that have a history of aflatoxin contamination need to be tested.

There are specific sampling problems especially of aflatoxins due to the way in which contamination spreads, as described for some food commodities, such as nuts and corn. This may need to be taken into consideration when sampling, for example in terms of sample selection and sample size, and when the analysis is made.

Tests for aflatoxins are designed to detect the possible presence of aflatoxins B_1, B_2, G_1 and G_2, which are highly toxic contaminants in any material of plant origin.

NOTES

Some examples of proposed national limits for aflatoxin in various types of herbal products are presented in Box 1 below. Country figures are based on information provided by national health authorities.

Box 1 Herbal materials, preparations and products

Argentina[a]

Determination method: HPLC-based technique using a monoclonal antibody immunoaffinity column.

- For herbs, herbal materials and herbal preparations used for herbal tea infusions

  20 µg/kg for aflatoxins $B_1 + B_2 + G_1 + G_2$ with the condition that aflatoxin $B_1 \leq 5$ µg/kg
- For finished herbal products for internal or topical use

  Absence per 1 gram

Germany[b]

Any materials used in manufacture of medicinal products (including medicinal herbal products)

- 2 µg/kg[c] for aflatoxin B_1 or 4 µg/kg[c] for total sum of aflatoxins B_1, B_2, G_1 and G_2.

  (The determination of the level of aflatoxin content has to be based on sampling procedures that take into account a potential heterogeneous distribution in the material.)

[a] *Farmacopea Argentina*, Vol. 1, 7th ed. Buenos Aires, Ministry of Health, 2003.

[b] Verordnung uber das Verbot der Verwendung von mil Aflatoxinen kontaminierten Stoffen bei der Herstellung von Arzneimitteln (Aflatoxin VerboteV) Vom 19 Juli 2000. *Bundesgesetzblatt*, Teil I Nr. 33. Bonn, 25 Juli 2000.

[c] Calculated on at feast 88% of dry weight.

4.5 MICROBIOLOGICAL CONTAMINANTS

Microbial Contamination Limits in Herbal Materials, Preparations and Finished Products

Different limits are set according to the intended use of the herbal material and the medicines themselves. Some examples are given here:

Raw medicinal plant and herbal materials intended for further processing

For contamination of *raw medicinal plant, and herbal materials intended for further processing (including additional decontamination by a physical or chemical process)* the limits, adapted from the provisional guidelines established by an international consultative group (35), are given for untreated herbal material harvested under acceptable hygienic conditions:

- *Escherichia coli*, maximum 10^4 per gram
- mould propagules, maximum 10^5 per gram
- shigella, absence per gram or ml.

NOTES

Herbal materials that have been pretreated

For *herbal materials that have been pretreated (e.g., with boiling water as used for herbal teas and infusions) or that are used as topical dosage forms*, the limits are:

- aerobic bacteria, maximum 10^7 per gram
- yeasts and moulds, maximum 10^4 per gram
- *Escherichia coli*, maximum 10^2 per gram
- other enterobacteria, maximum 10^4 per gram
- clostridia, absence per 1 gram
- salmonellae, absence per 1 gram
- shigella, absence per 1 gram.

Other herbal materials for internal use

For *other herbal materials for internal use*, the limits are:

- aerobic bacteria, maximum 10^5 per gram
- yeasts and moulds, maximum 10^3 per gram
- *Escherichia coli*, maximum 10 per gram
- other enterobacteria, maximum 10^3 per gram
- clostridia, absence per 1 gram
- salmonellae, absence per 1 gram
- shigella, absence per 1 gram.

Herbal medicines to which boiling water is added before use

For *herbal medicines to which boiling water is added before use*, the limits are:

- aerobic bacteria, maximum 10^7 per gram
- yeasts and moulds, maximum 10^4 per gram
- *Escherichia coli*, maximum 10 per gram
- other enterobacteria, maximum 10^3 per gram
- clostridia, absence per 1 gram
- salmonellae, absence per 1 gram
- shigella, absence per 1 gram.

Other herbal medicines

For *other herbal medicines*, the limits are:

- aerobic bacteria, maximum 10^5 per gram
- yeasts and moulds, maximum 10^3 per gram
- *Escherichia coli*, absence per 1 gram
- other enterobacteria, maximum 10^3 per gram
- clostridia, absence per 1 gram
- salmonellae, absence per 1 gram
- shigella, absence per 1 gram.

4.6 PESTICIDE RESIDUES

Some examples of national and regional limits set for various types of pesticide residues are shown in Table 4.2. As a reference, the list of approved pesticides for spices and their MRLs adopted by the Codex Alimentarius Commission is given in Table 4.3.

NOTES

Table 4.2 Examples of national limits set for various pesticide residues

Substances	Limit		
	EP[a] (mg/kg)	Japan[b] (ppm)	USP[c] and FA[c] (mg/kg)
acephate	0.1		
alachlor	0.05		0.02
aldrin and dieldrin (sum of)	0.05		0.05
azinphos-methyl	1		1.0
bromine	50		
bromophos-ethyl	0.05		
bromophos-methyl	0.05		
bromopropylate	3		3.0
chlordane (sum of cis-, trans- and oxythlordane)	0.05		0.05
chlorfenvinphos	0.5		0.5
chlorpyrifos			0.2
chlorpyrifos (ethyl)	0.2		
chlorpyrifos-methyl	0.1		0.1
chlorthal-dimethyl	0.01		
cyfluthrim, sum	0.1		
ramda-cyhalothrin	1		
cypermethrin (and isomers)	1		1.0
DDT (sum of *o, p'*-DDE, *p, p'*-DDE, *o, p'*-DDT, *p, p'*-DDT, *o, p'*-TDE and *p, p'*-TDE)	1.0		
DDT (sum of *p, p'*-DDT *o, p'*-DDT *p, p'*-DDD and *p, p'*-DDE)		0.2	
DDT (sum of *p, p'*-DDT, *o, p'*-DDT, *p, p'*-DDE, and *p, p'*-TDE)			1.0
deltamethrin	0.5		
diazinon	0.5		0.5
dichlofluanid	0.1		
dichlorvos	1		1.0
dicofol	0.5		
dimethoate and omethoate (sum of)	0.1		
dithiocarbamate (as CS_2)	2		2.0
endosulfan (sum of isomers and endosulfan sulfate)	3		3.0
endrin	0.05		0.05
ethion	2		2.0
fenchlorophos (sum of fenchlorophos and fenchlorophos-oxon)	0.1		
fenitrothion	0.5		0.5
fenpropathrin	0.03		
fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxonsulfon and fensulfothion-sulfon)	0.05		
fenvalerate	1.5		1.5
flucytrinate	0.05		

NOTES

τ-fluvalinate	0.05		
fonofos	0.05		0.05
heptachlor (sum of heptachlor, cis-heptachlorepoxide and trans-heptachlorepoxide)	0.05		
heptachlor (sum of heptachlor and heptachlorepoxide)			0.05
hexachlorobenzene	0.1		0.1
hexachlorocyclohexane (sum of isomers, α–, π–, γ–, and ε– hexachlorocyclohexane)	0.3		
hexachlorocyclohexane isomers (sum of α, β, γ and δ)		0.2	
hexachlorocyclohexane isomers (other than γ)			0.3
lindane (γ-hexachlorocyclohexane)	0.6		0.6
malathion			1.0
malathion and malaoxon (sum of)	1		
mecarbam	0.05		
methacriphos	0.05		
methamidophos	0.05		
methidathion	0.2		0.2
methoxychlor	0.05		
mirex	0.01		
monocrotophos	0.1		
parathion			0.5
paratlion-ethyl and paraoxon-ethyl (sum of)	0.5		
parathion-methyl			0.2
parathion-methyl and paraoxon-methyl (sum of)	0.2		
pendimethalin	0.1		
pentachloranisol	0.01		
permethrin (and isomers) (sum of)	1		
permethrin			1.0
phosalone	0.1		0.1
phosmet	0.05		
piperonyl butoxide	3		3.0
pirimiphos-ethyl	0.05		
pirimiphos-methyl (sum of pirimiphos-methyl and N-desethyl-pirimiphos-methyl)	4		
pirimiphos-methyl			4.0
procymidone	0.1		
profenophos	0.1		
prothiophos	0.05		
pyrethrins (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I and pyrethrin II)	3		
pyrethrins (sum of)			3.0
quinalphos	0.05		
quintozene (sum of quitozene, pentachloroaniline and methyl pentachlorophenyl sulfide)	1		1.0

s-421	0.02		
tecnazene	0.05		
tetradifon	0.3		
vinclozolin	0.4		

Ep, *European pharmacopoeia*; FA, *Farmacopea Argentina*; USP, *United States Pharmacopeia*.

[a] Applicable to medicinal plant material included in the *European pharmacopoeia*, 5th ed, unless otherwise indicated in the applicable monograph. Reference: *PHARMEUROPA* Volume 18, No. 4, October 2006.

[b] Currently applicable only to five medicinal plant materials (ginseng root, powdered ginseng root, red ginseng root, senna leaf and powdered senna leaf) included in *Japanese pharmacopoeia*, XIV.

[c] Values in *United States pharmacopeia* 28 and *Argentina pharmacopoeia*, Vol. 1.

NOTES

Table 4.3 The list of approved pesticides for spicesa and their maximum residue limits (MRLs)

Pesticide (CCPR-number)	*Group or sub-group of spices*	*MRL (mg/kg)*
Arephate (095)	Entire group 028[c]	0.2 (*)
Azinphos-methyl (002)	Entire group 028[c]	0.5 (*)
Chlorpyrifos (017)	Seeds	5
	Fruits or berries	1
	Roots or rhizomes	1
Chlorpyrifos-methyl (090)	Seeds	1
	Fruits	0.3
	Roots or rhizomes	6
Cypermethrin (118)	Fruits or berries	0.1
	Roots or rhizomes	0.2
Diazinon (22)	Seeds	5
	Fruits	0.1 (*)
	Roots or rhizomes	0.5
Dichlorvos (025)	Entire group 028[c]	0.1 (*)
Dicofol (026)	Seeds	0.05 (*)
	Fruits or berries	0.1
	Roots or rhizomes	0.1
Dimethoate (027)	Seeds	5
	Fruits or berries	0.5
	Roots or rhizomes	0.1 (*)
Disulfoton (074)	Entire group 028[c]	0.05 (*)
Endosulfan (032) (total)	Seeds	1
	Fruits or berries	5
	Roots or rhizomes	0.5
Ethion (034)	Seeds	3
	Fruits or berries	5
	Roots or rhizomes	0.3
Fenitrothion (037)	Seeds	7
	Fruits or berries	1
	Roots or rhizomes	0.1 (*)

NOTES

Iprodion (111)	Seeds	0.05 (*)
Malathion (049)	Roots or rhizomes	0.1 (*)
	Seeds	2
	Fruits or berries	1
	Roots or rhizomes	0.5
Metalaxyl (138)	Seeds	5
Methamidophos (100)	Entire group 028[c]	0.1 (*)
Parathion (058)	Seeds	0.1 (*)
	Fruits or berries	0.2
	Roots or rhizomes	0.2
Parathion-methyl (059)	Seeds	5
	Fruits or berries	5
	Roots or rhizomes	3
Permethrin (120)	Entire group 028[c]	0.05 (*)
Phenthoate (128)	Seeds	7
Phorate (112)	Seeds sub-group	0.5
	Fruits or berries	0.1 (*)
	Roots or rhizomes	0.1 (*)
Phosalone (060)	Seeds	2
	Fruits	2
	Roots or rhizomes	3
Pirimicarb (101)	Seeds	5
Pirimiphos-methyl (086)	Seeds sub-group	3
	Fruits sub-group	0.5
Quintozene (064)	Seeds sub-group	0.1
	Fruits or berries	0.02
	Roots or rhizomes	2
Vinclozolin (159)	Entire spice group[c]	0.05 (*)

(*) At or about the limit of determination.

[a] The residue definitions remain the same as those recommended for the given pesticide in other plant commodities (http://www.codexalimentarius.net/mrls/pestdes/pest_ref/MRLs_Spices_e.pdf).

[b] Report of Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, 28th Session, July, 2005 (http://www.codexalimentarius.net/download/report/644/al28_41e.pdf).

[c] The Group of A28 as modified by the 36th Session of CCPR.

SUMMARY

- In general, quantitative tests and limit tests accurately determine the concentrations of toxic metals in the form of impurities and contaminants.
- The metals are widely distributed throughout nature and occur freely in soil and water and are often components of certain pesticides.
- Following a severe nuclear accident, the environment may be contaminated with airborne radioactive materials.

NOTES

REVIEW QUESTIONS

1. Discuss the determination of arsenic and toxic metals.
2. Discuss determination of radioactive contaminants in the herbals.
3. Discuss determination of aflatoxins in herbals.
4. Discuss determination of microbiological contaminants.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. ***Standardization of Botanicals: Testing and Extraction Methods of Medicinal* Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

UNIT V: DETERMINATION OF PHYSICAL AND CHEMICAL CONSTANT: ARSENIC AND TOXIC METALS

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- describe the limit test for arsenic.
- discuss about the limit test for cadmium and lead.
- explain the preparation of sample solution and blank solution.
- define the detection of arsenic and mercury.

5.1 LIMIT TESTS

Limit Test for Arsenic

Arsenic is abundant in nature and its presence in herbal medicines should be no different to its wide occurrence in foods. A popular test method relies on the digestion of the plant matrix followed by subjection of the digestate to a comparative colorimetric test in a special apparatus.

The test method described below uses colorimetry and does not use toxic mercuric bromide paper. The method uses *N*-*N*-diethylmethyldithiocarbamate in pyridine

and it reacts with hydrogen arsenide to afford a red-purple complex. The limit is expressed in terms of arsenic (III) trioxide (As_2O_3).

Apparatus

Use the apparatus illustrated in Fig. 5.1.

NOTES

(A, generator bottle (capacity up to the shoulder: approximately 70 ml), B, exit tube C, glass tube (inside diameter: 5.6 mm, the tip of the part to be inserted in the absorber tube D is drawn out to 1 mm in diameter), D, absorber tube (inside diameter: 10 mm), E, small perforation, F, glass wool (about 0.2 g), G, mark at 5 ml, H and J, rubber stopper).

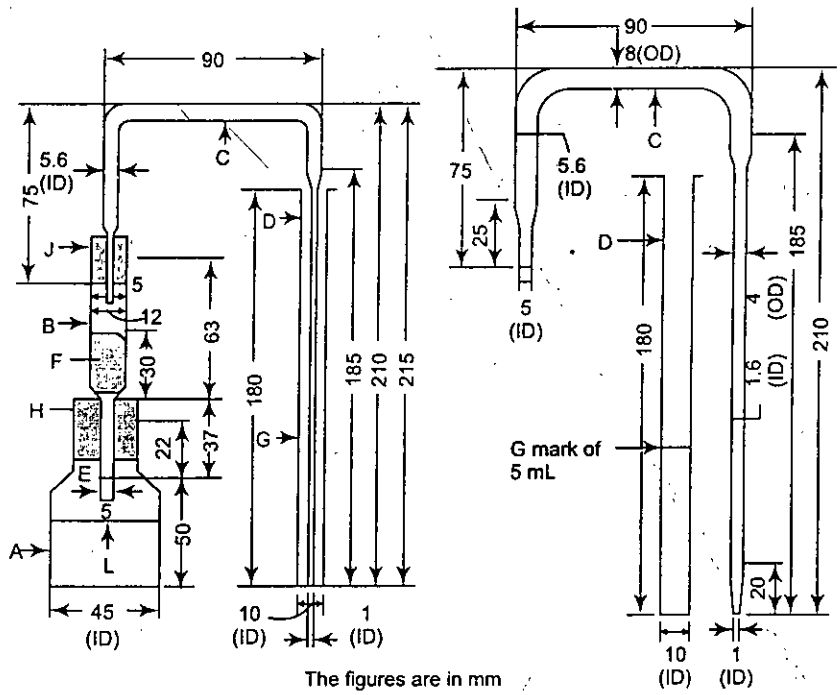

Fig. 5.1 Apparatus for arsenic limit test

Place glass wool F in the exit tube B up to about 30 mm in height, moisten the glass wool uniformly with a mixture of an equal volume of *lead (II) acetate TS* and water, and apply gentle suction to the lower end to remove the excess mixture. Insert the tube vertically into the centre of the rubber stopper H, and attach the tube to the generator bottle A so that the small perforation E in the lower end of B extends slightly below the stopper. At the upper end of B, attach the rubber stopper J to hold the tube C vertically. Make the lower end of the exit tube of C level with that of the rubber stopper J.

Preparation of the Test Solution

Unless otherwise specified, proceed as follows:

Examples for :g, *powdered ginseng and red ginseng*. Prepare the test solution with 1.0 g of pulverized ginseng (or red ginseng) according to the method described below, and perform the test using the apparatus described above.

Method

Weigh the amount of the sample as directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 ml of a solution of magnesium

nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, heat gradually, and ignite to incinerate. If carbonized material still remains after this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate in the same manner. After cooling, add 3 ml of hydrochloric acid, heat in a water bath to dissolve the residue, and designate it as the test solution.

NOTES

Standard Solutions

- *Absorbing solution for hydrogen arsenide.* Dissolve 0.50 g of silver *N, N*-diethyldithiocarbamate in pyridine to make 100 ml. Preserve this solution in a glassstoppered bottle protected from light, in a cold place.
- *Standard arsenic stock solution.* Weigh accurately 0.100 g of finely powdered arsenic (III) trioxide standard reagent dried at 105 °C for 4 hours, and add 5 ml of sodium hydroxide solution (1 in 5) to dissolve. Add dilute sulfuric acid to neutralize, add a further 10 ml of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 ml.
- *Standard arsenic solution.* Pipette 10 ml of standard arsenic stock solution, add 10 ml of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 ml. Each ml of the solution contains 1 μg of arsenic (III) trioxide (As_2O_3). Prepare standard arsenic solution just before use and preserve in a glass-stoppered bottle.

Procedure

Unless otherwise specified, proceed using the above-mentioned apparatus. Carry out the preparation of the standard colour at the same time. Place the test solution in the generator bottle A and, if necessary, wash down the solution in the bottle with a small quantity of water. Add 1 drop of *methyl orange TS*, and after neutralizing with *ammonia TS*, ammonia solution (NH_4OH), or diluted hydrochloric acid, add 5 ml of diluted hydrochloric acid (1 in 2) add 5 ml of *potassium iodide TS*, and allow to stand for 2 to 3 minutes. Add 5 ml of *acidic tin (II) chloride TS*, and allow to stand for 10 minutes. Then add water to make 40 ml, add 2 g of zinc for arsenic analysis, and immediately connect the rubber stopper H fitted with B and C with the generator bottle A. Transfer 5 ml of the absorbing solution for hydrogen arsenide to the absorber tube D, insert the tip of C to the bottom of the absorber tube D, then immerse the generator bottle A up to the shoulder in water maintained at 25 °C, and allow to stand for 1 hour. Disconnect the absorber tube, add pyridine to make 5 ml, if necessary, and observe the colour of the absorbing solution: the colour produced is not more intense than the standard colour.

Preparation of standard colour

Measure accurately 2 ml of Standard Arsenic Solution into the generator bottle A. Add 5 ml of diluted hydrochloric acid (1 in 2) and 5 ml of *potassium iodide TS*, and allow to stand for 2 to 3 minutes. Add 5 ml of *acidic tin (II) chloride TS*, allow to stand at room temperature for 10 minutes, and then proceed as directed above. The colour produced corresponds to 2 μg of arsenic (III) trioxide (As_2O_3) and is used as the standard.

Note: Apparatus, reagents and test solutions used in the test should contain little or no arsenic. If necessary, perform a blank determination.

NOTES

5.2 LIMIT TEST FOR CADMIUM AND LEAD

Procedure

Apparatus

The equipment comprises a digestion vessel, consisting of a vitreous silica crucible (DIN 12904), "tall form", height 62 mm, diameter 50 mm, capacity 75 ml, with a vitreous silica cover. The materials used are:

- *digestion mixture*: 2 parts by weight of *nitric acid (~1000 g/l) TS* and 1 part by weight of *perchloric acid (~1170 g/l) TS*.
- *reference materials*: olive leaves (*Olea europaea*)1 and hay powder. Clean scrupulously with *nitric acid (~1000 g/l) TS* the digestion vessel and all other equipment to be used for the determination, rinse thoroughly several times with water and dry at 120 °C.

Preparation of the sample

For the wet digestion method in an open system, place 200-250 mg of air-dried medicinal plant material, accurately weighed, finely cut and homogeneously mixed, into a cleaned silica crucible. Add 1.0 ml of the digestion mixture, cover the crucible without exerting pressure and place it in an oven with a controlled temperature and time regulator (computer-controlled, if available). Heat slowly to 100 °C and maintain at this temperature for up to 3 hours; then heat to 120 °C and maintain at this temperature for 2 hours. Raise the temperature very slowly to 240 °C, avoiding losses due to possible violent reactions, especially in the temperature range of 160-200 °C, and maintain at this temperature for 4 hours.

Dissolve the remaining dry inorganic residue in 2.5 ml of *nitric acid (~1000 g/l) TS* and use for the determination of heavy metals. Every sample should be tested in parallel with a blank.

Method

The contents of lead and cadmium may be determined by inverse voltammetry or by atomic absorption spectrophotometry.

5.3 LIMIT TEST FOR TOTAL TOXIC METALS AS LEAD

In this method, the heavy metals are the metallic inclusions that are darkened with *sodium sulfide TS* in acidic solution; their quantity is expressed in terms of the quantity of lead (Pb).

Preparation of Sample Solution and Blank Solution

Test solution

Place an amount of the sample, as directed in the monograph, in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition. After cooling, add 2 ml of nitric acid and 5 drops of sulfuric acid, heat cautiously until white fumes are evolved, and incinerate by ignition between 500 °C and 600 °C. Cool, add 2 ml of hydrochloric acid, evaporate to dryness in a water-bath, moisten the residue with 3 drops of hydrochloric acid, add 10 ml of hot water, and warm for 2 minutes.

Then add 1 drop of *phenolphthalein TS*, add *ammonia TS* drop by drop until the solution develops a pale red colour, add 2 ml of dilute acetic acid, filter, if necessary, and wash with 10 ml of water. Transfer the filtrate and washings to a Nessler tube, and add water to make 50 ml. Designate this as the test solution.

Control solution

NOTES

Evaporate a mixture of 2 ml of nitric acid, 5 drops of sulfuric acid, and 2 ml of hydrochloric acid on a water-bath, further evaporate to dryness on a sand-bath, and moisten the residue with 3 drops of hydrochloric acid. Hereinafter, proceed as directed above for the test solution, and then add the volume of standard lead solution as directed in the monograph and sufficient water to make 50 ml.

Procedure

Add 1 drop of *sodium sulfide TS* both to the test solution and to the control solution, mix thoroughly, and allow to stand for 5 minutes. Then compare the colours of the two solutions by viewing the tubes downwards or transversely against a white background. The test solution has no more colour than the control solution.

5.4 LIMIT TEST FOR TOTAL TOXIC METALS AS LEAD IN EXTRACTS

Test solution

Ignite 0.3 g of extracts to ash, warm with 3 ml of dilute hydrochloric acid, and filter. Wash the residue with two 5 ml portions of water. Neutralize the combined filtrate and washings by adding *ammonia TS*, filter, if necessary, and add 2 ml of dilute acetic acid and water to make 50 ml. Perform the heavy metals limit test using this solution as the test solution.

Control solution

Proceed with 3 ml of dilute hydrochloric acid in the same manner as directed above for the preparation of the test solution, and add 3 ml of standard lead solution 1 ppm, and water to make 50 ml.

Procedure

Add 1 drop of *sodium sulfide TS* to both the test solution and to the control solution, mix thoroughly, and allow to stand for 5 minutes. Then compare the colours of the two solutions by viewing the tubes downwards or transversely against a white background. The test solution has no more colour than the control solution.

Determination of Specific Toxic Metals

Atomic absorption spectrometry (AA) is used for the determination of the amount or concentration of specific heavy metals. AA uses the phenomenon that atoms in the ground state absorb light of a specific wavelength, characteristic of the particular atom, when the light passes through an atomic vapour layer of the element to be determined.

Caution must be exercised when using the recommended closed high-pressure digestion vessels and microwave laboratory equipment, and the operators should be fully familiar with the safety and operating instructions given by the manufacturer.

NOTES

Procedure

Apparatus

Usually the apparatus consists of a light source, a sample atomizer, a spectroscope, a photometer and a recording system, together with the following:

- *A digestion flask*: a polytetrafluoroethylene flask with a volume of about 120 ml, fitted with an airtight closure, a valve to adjust the pressure inside the container and a polytetrafluoroethylene tube to allow release of gas. A good example is the Digestion Vessel Assembly P/N ZZ 1000.
- *A system for making flasks airtight*, using the same torsional force. One example is the CEM Capping station calibration.
- *A microwave oven*, with a magnetron frequency of 2450 MHz, with a selectable output from 0 to 630 ± 70 W in 1% increments, a programmable digital computer, a polytetrafluoroethylene-coated microwave cavity with a variable speed exhaust fan, a rotating turntable drive system and exhaust tubing to vent fumes.
- *An atomic absorption spectrometer*, equipped with an appropriate lamp for each element as source of radiation and a deuterium lamp as background corrector; the system is fitted with a sample atomiser of which there are three types: the flame type, the electrochemical type and the cold-vapour type.
- A graphite furnace (electrochemical type) is used as an atomisation device for cadmium, copper, iron, lead, nickel and zinc. One example is the Vapour Generation Accessory.
- An automated continuous-flow hydride vapour generation system is used for arsenic and mercury.

Method

Clean all the glassware and laboratory equipment with a 10 g/l solution of *nitric acid R* in *water, carbondioxide free R* before use.

Test solution. In a digestion flask, place the prescribed quantity of the substance to be examined (about 0.5 g of powdered drug or 0.5 g of fatty oil). Add 3 ml of *nitric acid R*, 1 ml *hydrogen peroxide R* and 1 ml of *hydrochloric acid R*. Seal the flask so that it is airtight. Place the digestion flasks in the microwave oven. Carry out the digestion in 3 steps according to the following procedure, for 7 flasks each containing the test solution: 80% power for 15 min; 100% power for 5 min; then 80% power for 20 min. At the end of the cycle allow the flasks to cool in air and to each add 4 ml of *sulfuric acid* R. Repeat the digestion programme. After cooling in air, open each digestion flask and introduce the clear, colourless solution obtained into a 50-ml volumetric flask. Rinse each digestion flask with 2 quantities, each of 15 ml, of *water R* and collect the rinsings in the volumetric flask. Add 1 ml of 10 g/l solution of *magnesium nitrate* R and 1 ml of 100 g/l solution of *ammonium dihydrogen phosphate* R and dilute to 50 ml with *water R*.

Blank solution. Mix 3 ml of *nitric acid* R, 1 ml *hydrogen peroxide R* (30%) and 1 ml of *hydrochloric acid* R in a digestion flask. Carry out the digestion in the same manner as for the test solution.

5.5 DETECTION OF CADMIUM, COPPER, IRON, LEAD, NICKEL AND ZINC

NOTES

Measure the content of (Cd), copper (Cu), iron (Fe), lead (Pb), nickel (Ni) and zinc (Zn) by the standard additions method using reference solutions of each heavy metal. Suitable instrumental parameters are listed in Table 5.1.

The absorbance value of the compensation liquid (*blank solution*) is subtracted from the value obtained with the test solution.

Table 5.1 Instrumental parameters for heavy metals

	Cd	Cu	Fc	Ni	Pb	Zn	
Wavelength	nm	228.8	324.8	248.3	232	283.5	213.9
Slit width	nm	0.5	0.5	0.2	0.2	0.5	0.5
Hollow-cathode lamp current	mA	6	7	5	10	5	7
Ignition temperature	°C	800	800	800	800	800	800
Atomizatiori temperature	°C	1800	2300	2300	2500	2200	2000
Background corrector		on	on	on	on	on	on
Nitrogen flow	Litre/min	3	3	3	3	3	3

5.6 DETECTION OF ARSENIC AND MERCURY

Measure the content of arsenic (As) and mercury (Hg) in comparison with reference solutions containing these elements at a known concentration by direct calibration using an automated continuous-flow hydride vapour generation system. The absor-bance value of the compensation liquid (blank solution) is automatically subtracted from the value obtained with the test solution.

Arsenic

Sample solution: To 19 ml of the test solution or of the blank solution as described above, add 1 ml of a 200 g/l solution of *potassium iodide* R. Allow the test solution to stand at room temperature for about 50 min or at 70 °C for about 4 min.

Acid reagent: Heavy metal-free hydrochloric acid R.

Reducing reagent: A 6 g/l solution of *sodium tetrahydroborate* R in a 5 g/l solution of *sodium hydroxide* R.

The instrumental parameters in Table 5.2 may be used.

Mercury

Sample solution: Test solution or blank solution, as described above.

Acid reagent: A 515 g/l solution of heavy metal-free *hydrochloric acid* R.

Reducing reagent: A 10 g/l solution of *stannous chloride* R or sodium tetrahydroborate in dilute *hydrochloric acid* R.

NOTES

The instrumental parameters in Table 5.2 may be used.

Table 5.2 Instrumental parameters for determination of arsenic and mercury

		As	*Hg*
Wavelength	nm	193.7	253.7
Slit width	nm	0.2	0.5
Hollow-cathode lamp current	mA	10	4
Acid reagent flow rate	ml/min	1.0	1.0
Reducing reagent flow rate	ml/min	1.0	1.0
Sample solution flow rate	ml/min	7.0	7.0
Absorption cell		Quartz (heated)	Quartz (unheated)
Background corrector		on	on
Nitrogen flow rate	litre/min	0.1	0.1
Heating		800°C	100°C

SUMMARY

- Arsenic is abundant in nature and its presence in herbal medicines should be no different to its wide occurrence in foods.
- Atomic absorption spectrometry (AA) is used for the determination of the amount or concentration of specific heavy metals.
- The absorbance value of the compensation liquid (blank solution) is automatically subtracted from the value obtained with the test solution.

REVIEW QUESTIONS

1. What is limit test for arsenic? Discuss in detail.
2. What is limit test for cadmium and lead? Discuss in detail.
3. Discuss the detection of cadmium, copper, iron, lead, nickel and zinc.
4. Discuss the detection of arsenic and mercury.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

UNIT VI: DETERMINATION OF PHYSICAL AND CHEMICAL CONSTANT: AFLATOXINS

NOTES

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- describe the tests for aflatoxins.
- define the preparation of sample.
- discuss about the interpretation of results.

6.1 INTRODUCTION

Whenever testing for aflatoxins is required this should be done after using a suitable clean-up procedure during which great care should be taken not to expose any personnel or the working or general environment to these dangerous and toxic substances. Thus Member States should adapt their good practices for national pharmaceutical control laboratories and GMP accordingly. Only products that have a history of aflatoxin contamination need to be tested.

6.2 TESTS FOR AFLATOXINS

These tests are designed to detect the possible presence of aflatoxins B_1, B_2, G_1 and G_2, which are highly toxic contaminants in any material of plant origin.

Test Method

The method described below does not require the use of toxic solvents, such as chloroform and dichloromethane. It uses a multifunctional column, which contains

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lipophilic and charged active sites, and high-performance liquid chromatography (HPLC) using fluorescence detection to determine aflatoxins B_1, B_2, G_1 and G_2. The advantages of employing a multifunctional column are:

- high total recoveries of aflatoxins B_1, B_2, G_1 and G_2 (more than 85%); and
- the column can be kept (stocked) at room temperature and for a fairly long time prior to use.

Standard Solutions of Aflatoxin B_1, B_2, G_1 and G_2 (2.5 ng/ml)

Stock standard solution: Weigh exactly 1.0 mg each of crystalline material of aflatoxins B_1, B_2, G_1 and G_2 and dissolve in 50 ml of toluene-acetonitrile (9:1) solution by shaking vigorously in a glass flask to obtain a standard stock solution (20 µg/ml). This standard solution should be kept in a tightly sealed container, covered with aluminium foil, and kept in a refrigerator at 4°C in the dark.

Working standard solution: 0.5 ml of stock standard solution is added to toluene:acetonitrile (9:1) solution to give 200 ml (working standard solution (50 ng/ml)).

Standard solution: Take 1.0 ml of working standard solution and add to toluene:acetonitrile (9:1) solution to give 20 ml (final standard solution (2.5 ng/ml)).

Standard solution for liquid chromatography analysis: Transfer 0.25 ml of the final standard solution (as described above) into a glass centrifuge tube and evaporate to dryness at 40°C or by using a nitrogen air stream. To derivatize 1 aflatoxins B_1 and G_1 (precolumn derivatization), add 0.1 ml Annex 4 of trifluoroacetic acid (TFA) solution to the residue in the tube, tightly seal the tube and shake vigorously. Allow the tube to stand at room temperature for 15 min in the dark. Add 0.4 ml of acetonitrile:water (1:9) solution to the tube. A 20-µl portion of the sample solution in the tube is subjected to liquid chromatography analysis.

Preparation of Sample

Grind the medicinal plant material for testing to a uniform consistency using a coffee mill, and extract a 50-g test sample with 400 ml of acetonitrile-water (9:1) by shaking vigorously in a glass flask fitted with a stopper for 30 min or by using a mechanical blender for 5 min. Filter the solution through a filter paper or centrifuge. Transfer a 5-ml portion of the filtrate, or the top clean layer, to a multifunctional column (such as a MultiSep #228 cartridge column (Romer Labs) or an Autoprep MF-A (Showa-denko)) and pass through at a flow rate of 1 ml/min. The aflatoxins present in a sample are passed through the column as the first eluate. Obtain the first 1-ml of the eluate as the test solution. Evaporate 0.5 ml of the test solution in a glass centrifuge tube to dryness at 40°C or by using a nitrogen air stream to remove solvent.

To derivatize aflatoxins B_1 and G_1 (precolumn derivatization), add 0.1 ml of trifluoroacetic acid (TFA) solution to the residue in the tube, tightly seal the tube and shake vigorously. Allow the tube to stand at room temperature for 15 min in the dark. Add 0.4 ml of acetonitrile-water (1:9) solution to the tube. Subject a 20-µl portion of the sample solution in the tube to liquid chromatography analysis.

Method

Liquid chromatography conditions: The mobile phase is acetonitrile-methanol-water (1:3:6). De-gas the mobile phase by sonication. Connect an octadecyl-silica gel (ODS)

column (4.6 mm inner diameter (ID) 250 mm, 3–5 µm), such as Inertsil ODS-3 (4.6 mm ID 250 mm, 3 µm) as the liquid chromatography column. Maintain the column at 40°C with a flow rate of 1 ml/min. The aflatoxin and its derivatives are detected at the excitation and emission wavelengths of 365 nm and 450 nm, respectively. The injection volume is 20 µl.

If an impurity peak overlaps the peaks corresponding to aflatoxins, the alternative liquid chromatography conditions, described below, are recommended.

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Alternative liquid chromatography conditions: The mobile phase is methanol-water (3:7). De-gas the mobile phase by sonication. Connect a fluorocarbonated column, such as Wako-pack Fluofix 120E (4.6 mm ID × 250 mm, 5 µm) as the liquid chromatography column. Maintain the column at 40°C with a flow rate of 1 ml/min. The aflatoxin and its derivatives are detected at the excitation and emission wavelengths of 365 nm and 450 nm, respectively. The injection volume is 20 µl.

Interpretation of the Results

Compare the retention time of peak area or peak heights of the aflatoxin under study in the chromatograms: if they are bigger or higher than those obtained in a standard solution of the aflatoxin under investigation, it should be regarded as a positive result for the presence of aflatoxin in the sample solution.

SUMMARY

- Only products that have a history of aflatoxin contamination need to be tested.
- The method described below does not require the use of toxic solvents, such as chloroform and dichloromethane.

REVIEW QUESTIONS

1. Discuss the tests for aflatoxins.
2. Describe the standard solutions of aflatoxin.
3. Know about the interpretation of the results.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

NOTES

UNIT VII: MICROBIAL COUNT, BIOBURDEN AND MICROBIALS ARRAY: DETERMINATION OF MICROORGANISMS

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- define the pretreatment of the test herbal material.
- discuss about the membrane filtration.
- describe the effectiveness of the culture medium.
- explain the validation of specific microorganisms.

7.1 TOTAL VIABLE AEROBIC COUNT

The total viable aerobic count (TVC) of the herbal material being examined is determined, as specified in the test procedure below, using one of the following methods: membrane-filtration, plate count or serial dilution. Aerobic bacteria and fungi (moulds and yeasts) are determined by the TVC.

Usually a maximum permitted level is set for certain products, but when the TVC exceeds this level then it is unnecessary to proceed with determination of specific organisms; the material should be rejected without being subjected to further testing.

7.2 PRETREATMENT OF THE TEST HERBAL MATERIAL

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Depending on the nature of the crude medicinal plant material, grind, dissolve, dilute, suspend or emulsify it using a suitable method and eliminate any antimicrobial properties by dilution, neutralization or filtration. Either phosphate buffer pH 7.2; buffered sodium chloride-peptone solution, pH 7.0; or fluid medium, used for the test, is used to suspend or dilute the test specimen.

Some materials have special requirements, which have to be met for acceptable pretreatment to be performed. Some examples are as follows:

Materials containing tannins, antimicrobial substances: Some herbal preparations present difficulties in determining levels of microbes, *e.g.*, those containing high contents of tannins or essential oils. When test specimens have antimicrobial activity or contain antimicrobial substances, any such antimicrobial properties are removed as mentioned above. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If the test specimens are diluted with fluid medium, the test should be performed quickly.

Water-soluble materials: Dissolve or dilute 10 g or 10 ml of plant material, unless otherwise specified in the test procedure for the material concerned, in lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test. Adjust the volume to 100 ml with the same medium. (Note that some materials may require the use of larger volumes.) If necessary, adjust the pH of the suspension to about 7.

Non-fatty materials insoluble in water: Suspend 10 g or 10 ml of the plant material, unless otherwise specified in the test procedure for the material concerned, in lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test; dilute to 100 ml with the same medium. (Note that some materials may require the use of a larger volume.) If necessary, divide the material and homogenize the suspension mechanically. A suitable surfactant, such as a solution of *polysorbate 20 R* or *80 R* containing 1 mg per ml may be added to aid dissolution. If necessary, adjust the pH of the suspension to about 7.

Fatty materials: Homogenize 10 g or 10 ml of material, unless otherwise specified in the test procedure for the material concerned, with 5 g of *polysorbate 20 R* or *80 R*. If necessary, heat to a temperature not exceeding 40°C. (Occasionally, it may be necessary to heat to a temperature of up to 45°C, for the shortest possible time.) Mix carefully while maintaining the temperature in a water-bath or oven. Add 85 ml of lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test, if necessary heated to a temperature not exceeding 40°C. Maintain this temperature for the shortest time necessary until an emulsion is formed and, in any case, for not more than 30 minutes. If necessary, adjust the pH of the emulsion to about 7.

7.3 TEST PROCEDURES

Plate Count

For bacteria use Petri dishes 9–10 cm in diameter. To one dish add a mixture of 1 ml of the pre-treated plant material and about 15 ml of liquefied casein-soybean

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digest agar at a temperature not exceeding 45°C. Alternatively, spread the material on the surface of the solidified medium in a Petri dish. If necessary, dilute the material to obtain an expected colony count of not more than 300. Prepare at least two dishes using the same dilution, invert them and incubate them at 30–35°C for 48–72 hours, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the plate with the largest number of colonies, up to a maximum of 300.

For fungi use Petri dishes 9-10 cm in diameter. To one dish add a mixture of 1 ml of the pretreated material and about 15 ml of liquefied *Sabouraud glucose agar with antibiotics* (also used is potato dextrose agar with antibiotics) at a temperature not exceeding 45°C. Alternatively, spread the pretreated material on the surface of the solidified medium in a Petri dish. If necessary, dilute the material as described above to obtain an expected colony count of not more than 100. Prepare at least two dishes using the same dilution and incubate them upright at 20–25°C for 5 days, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the dish with not more than 100 colonies.

Membrane Filtration

Use membrane filters with a nominal pore size of not greater than 0.45 ìm, and with a proven effectiveness at retaining bacteria, *e.g.*, cellulose nitrate filters are used for aqueous, oily and weakly alcoholic solutions, whereas cellulose acetate filters are better for strongly alcoholic solutions. The technique below uses filter discs of about 50 mm in diameter. Where filters of a different diameter are used, adjust the volumes of the dilutions and washings accordingly. Sterilize, by appropriate means, the filtration apparatus and the membrane, as the solution is introduced, filtered and examined under aseptic conditions, and the membrane is then transferred to the culture medium.

The Detailed Method

Transfer 10 ml or a solution containing 1g of the material to each of two membrane filter apparatuses and filter immediately. If necessary, dilute the pretreated material to obtain an expected colony count of 10–100. Wash each membrane, filtering three or more successive quantities of approximately 100 ml of a suitable liquid such as buffered sodium chloride-peptone solution at pH 7.0. For fatty materials, a suitable surfactant may be added, such as *Polysorbate 20 R* or *80 R*. Transfer one of the membrane filters, intended primarily for the enumeration of bacteria, to the surface of a plate with soybean-casein digest agar and the other, intended primarily for the enumeration of fungi, to the surface of a plate with *Sabouraud glucose agar with antibiotics*. Incubate the plates for 5 days, unless a more reliable count can be obtained otherwise, at 30–35°C for the detection of bacteria and at 20–25°C for the detection of fungi. Count the number of colonies formed. Calculate the number of microorganisms per gram or per ml of the material tested, if necessary counting bacteria and fungi separately.

Serial Dilution

Prepare a series of 12 tubes each containing 9–10 ml of *soybean-casein digest medium*. To each of the:

- *first group of three tubes,* add 1 ml of the 1:10 dilution of dissolved, homogenized material (containing 0.1 g or 0.1 ml of specimen) prepared as described later in these guidelines (see section B.2);
- *second group of three tubes,* add 1 ml of a 1:100 dilution of the material;
- *third group of three tubes,* add 1 ml of a 1:1000 dilution of the material; and to the
- *last three tubes,* add 1 ml of the diluent.

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Incubate the tubes at 30-35°C for at least 5 days. No microbial growth should appear in the last three tubes. If the reading of the results is difficult or uncertain, owing to the nature of the material being examined, prepare a subculture in a liquid or a solid medium, and evaluate the results after a further period of incubation. Determine the most probable number of microorganisms per gram or per ml of the material using Table 7.1.

If, for the first column, the number of tubes showing microbial growth is two or less, the most probable number of microorganisms per g or per ml is less than 100 (Table 7.1).

Table 7.1 Determination of total viable aerobic count

Number of tubes with microbial growth[a]			*Most probable number of microorganisms per g or ml*
100 mg or 0.1 ml per tube	*10 mg or 0.01 ml per tube*	*1 mg or 0.001 ml per tube*	
3	3	3	>1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

[a] Amounts in mg or ml are quantities of original plant material.

7.4 EFFECTIVENESS OF THE CULTURE MEDIUM, CONFIRMATION OF ANTIMICROBIAL SUBSTANCES AND VALIDITY OF THE COUNTING METHOD

The following strains are normally used:

- *Staphylococcus aureus:* NCIMB 8625 (ATCC 6538-P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83, IFO 13276)
- *Bacillus subtili:* NCIMB 8054 (ATCC 6633, CIP 52.62, IFO 3134)

- *Escherichia coli* NCIMB 8545 (ATCC 8739, CIP 53.126, IFO 3972)
- *Candida albicans* ATCC 2091 (CIP 1180.79, IFO 1393) or ATCC 10 231 (NCPF 3179, CIP 48.72, IFO 1594)
- *Clostridia botulinum* ATCC 19297 (NCTC 7272)
- *Clostridium perfringens* ATCC 13124 (NCTC 8239)
- *Clostridium tetani* ATCC e19406 (NCTC 279).

NOTES

Allow the test strains to grow separately in tubes containing soybean-casein digest medium at 30–35°C for 18–24 hours for aerobic bacteria and between 20–25°C for *Candida albicans*, for 48 hours. (Antibiotics are often added to the culture medium to attain a particular selectivity.)

Dilute portions of each of the cultures using buffered sodium chloride-peptone solution, pH 7.0 or phosphate buffer, pH 7.2 to prepare test suspensions containing 50–200 viable Colony Forming Units (CFU) (microorganisms) per ml. Growthpromoting qualities are tested by inoculating 1 ml of each microorganism into each medium. The test media are satisfactory if clear evidence of growth appears in all the inoculated media after incubation at the indicated temperature for 5 days.

When a count of test organisms with a test specimen is less than one-fifth of that without the test specimen, any such effect must be eliminated by dilution, filtration, neutralization or inactivation.

To confirm the sterility of the medium and of the diluent and the aseptic performance of the test, follow the TVC method using sterile buffered sodium chloride-peptone solution, pH 7.0, or phosphate buffer, pH 7.2, as a control. There should be no growth of microorganisms.

To validate the method, a count for the test organism should be obtained differing by not more than a factor of 10 from the calculated value for the inoculum.

Tests for Specific Microorganisms

Microbial tests should be applied to starting plant materials, intermediate and finished products where necessary. Enterobacteria and certain other Gram-negative bacteria *Escherichia coli, Salmonella* and *Staphylococcus aureus* are included as target strains of the test.

The conditions of the tests for microbial contamination are designed to minimize accidental contamination of the materials being examined and the precautions taken must not adversely affect any microorganisms that could be revealed.

Pretreatment of the Material Being Examined

Refer to the sampling and preparation of the test solution in TVC, including the elimination of any antimicrobial substances which may be present.

7.5 TEST PROCEDURE FOR THE ENTEROBACTERIACEAE AND CERTAIN OTHER GRAM-NEGATIVE BACTERIA

Detection of Bacteria

Homogenize the pretreated material appropriately and incubate at 30–37°C for a length of time sufficient for revivification of the bacteria, but not sufficient for

multiplication of the organisms (usually 2–5 hours). Shake the container, transfer aliquots equivalent to 1 g or 1 ml of the homogenized material to 100 ml of *Enterobacteriaceae enrichment broth Mossel* and incubate at 35–37°C for 18–48 hours. Prepare a subculture on a plate with *violet-red bile agar with glucose and lactose.*

Incubate at 35–37°C for 18–48 hours. The material passes the test if no growth of colonies of Gram-negative bacteria is detected on the plate.

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Quantitative Evaluation

Inoculate a suitable amount of *Enterobacteriaceae enrichment broth Mossel* with quantities of homogenized material prepared as described under the above section on "Detection of bacteria", appropriately diluted as necessary, to contain 1 g, 0.1 g and 10 µg, or 1 ml, 0.1 ml and 10 µl, of the material being examined. Incubate at 35–37°C for 24–48 hours. Prepare a subculture of each of the cultures on a plate with *violet-red bile agar with glucose and lactose* in order to obtain selective isolation.

Incubate at 35–37°C for 18–24 hours. The growth of well-developed colonies, generally red or reddish in colour, of Gram-negative bacteria constitutes a positive result. Note the smallest quantity of material that gives a positive result. Determine the probable number of bacteria using Table 7.2.

Table 7.2 Determination of Enterobacteriaceae and certain other Gram-negative bacteria

Result for each quantity or volume			*Probable number of bacteria per g of material*
1.0 g or 1.0 ml	*0.1 g or 0.1 ml*	*0.01 g or 0.01 ml*	
+	+	+	More than 10^2
+	+	–	Less than 10^2 but more than 10
+	–	–	Less than 10 but more than 1
–	–	–	Less titan 1

Escherichia Coli

Transfer a quantity of the homogenized material in *lactose broth,* prepared and incubated as described above, and containing 1 g or 1 ml of the material being examined, to 100 ml of *MacConkey broth* and incubate at 43–45°C for 18–24 hours.

Prepare a subculture on a plate with *MacConkey agar* and incubate at 43–45°C for 18–24 hours. Growth of red, generally non-mucoid colonies of Gram-negative rods, sometimes surrounded by a reddish zone of precipitation, indicates the possible presence of *E. coli.* This may be confirmed by the formation of indole at 43.5–44.5°C or by other biochemical reactions. The material passes the test if no such colonies are detected or if the confirmatory biochemical reactions are negative.

Salmonella Spp

Incubate the solution, suspension or emulsion of the pretreated material prepared as described above at 35–37°C for 5–24 hours, as appropriate for enrichment.

Primary Test

Transfer 10 ml of the enrichment culture to 100 ml of *tetrathionate bile brilliant green broth* and incubate at 42–43°C for 18–24 hours. Prepare subcultures on at least two of the following three agar media: *deoxycholate citrate agar; xylose, lysine, deoxycholate*

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agar; and *brilliant green agar*. Incubate at 35–37°C for 24–48 hours. Carry out the secondary test if any colonies are produced that conform to the description given in Table 7.3.

Secondary Test

Prepare a subculture of any colonies showing the characteristics described in Table 7.3 on the surface of *triple sugar iron agar* using the deep inoculation technique. This is done by first inoculating the inclined surface of the culture medium, followed by a stab culture with the same inoculating needle and then, incubating at 35–37°C for 18–24 hours. The test is positive for the presence of Salmonella spp. if a change of colour from red to yellow is observed in the deep culture (but not in the surface culture), usually with the formation of gas with or without production of hydrogen sulfide in the agar. Confirmation is obtained by appropriate biochemical and serological tests.

The material being examined passes the test if cultures of the type described do not appear in the primary test, or if the confirmatory biochemical and serological tests are negative.

Table 7.3 Description of Salmonella colonies appearing on different culture media

Medium	*Description of colony*
Deoxycholate citrate agar	Well-developed, colourless
Xylose, lysine, deoxycholate agar	Well-developed, red, with or without black centres
Brilliant green agar	Small, transparent and colourless, or opaque, pink or white (frequently surrounded by a pink to red zone)

Pseudomonas Aeruginosa

Pretreat the material being examined as described under A.1, but using buffered sodium chloride-peptone solution, pH 7.0, or another suitable medium shown not to have antimicrobial activity under the conditions of the test, in place of lactose broth. Inoculate 100 ml of *soybean-casein digest medium* with a quantity of the solution, suspension or emulsion thus obtained containing 1 g or 1 ml of the material being examined. Mix and incubate at 35–37°C for 24–48 hours. Prepare a subculture on a plate of *cetrimide agar* and incubate at 35–37°C for 24–48 hours. If no growth of microorganisms is detected, the material passes the test. If growth of colonies of Gram-negative rods occurs, usually with a greenish fluorescence, apply an oxidase test and test the growth in *soybean-casein digest medium* at 42°C. The following method may be used. Place 2 or 3 drops of a freshly prepared 0.01 g/ml solution of *N, N, N', N'*-tetramethyl-*p*-phenylenediamine dihydrochloride R on a filter paper and apply a smear of the suspected colony; the test is positive if a purple colour is produced within 5-10 seconds. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative.

Staphylococcus Aureus

Prepare an enrichment culture as described for *Pseudomonas aeruginosa*. Prepare a subculture on a suitable medium such as *Baird-Parker agar*. Incubate at 35–37°C for 24–48 hours. The material passes the test if no growth of microorganisms is detected. Black colonies of Gram-positive cocci often surrounded by clear zones may

indicate the presence of *Staphylococcus aureus*. For catalase-positive cocci, confirmation may be obtained, for example, by coagulase and deoxyribonuclease tests. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative.

Clostridium Spp

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Add 10 g (10 ml) of the herbal materials, preparation or product to be examined to two suitable vessels, each containing 100 ml of *cooked-meat medium*, heated just prior to use, to 100°C for a few minutes and cooled to 37°C. To distinguish between sporing and non-sporing organisms, immediately seal one vessel with a layer of sterile paraffin or agar, heat the other vessel at 65°C for 30 minutes, and then similarly seal.

Incubate both vessels at 35–37°C and examine every 24 hours for up to 4 days. Growth of sporing organisms occurs in the vessel which was heated after inoculation. If no growth occurs in either of the vessels, the sample passes the test for absence of *Clostridia* and other anaerobic bacteria.

If sporing anaerobic organisms (Table 7.4) are found, inoculate the cultures, each in duplicate, on one half of the surface of plates containing *5% defibrinated sheep blood agar medium*. Incubate at 37°C for 48 hours, one plate anaerobically and the other aerobically, to check that the organisms will not grow under aerobic conditions.

Table 7.4 Characteristics of Clostridium species on cooked-meat medium

Clostridium botulinum	*Clostridium perfringens*	*Clostridium tetani*
No digestion of meat; much gas, white sediment	No digestion of meat; meat turns pink colour	No digestion of meat; burnt organic smell

After 24 and 48 hours examine the appearance of the colonies together with the type and extent of haemolysis, and also examine microscopically for spore formation using Gram stain or spore stain techniques. Match the result with the description in Table 7.5, for further identification of specified clostridia.

Table 7.5 Characteristics of Clostridium species on 5% defibrinated sheep blood agar medium

	Clostridium botuimum	*Clostridium perfringens*	*Clostridium tetani*
Colonies	Irregular, translucent with a granular surface and indefinite fimbriated spreading edge	Large, circular, convex, semitranslucent, smooth with an entire edge	Transparent with long feathery spreading projections
Haemolysis	+	Double zone	+
Spores	Oval, central, subterminal distended bacilli	Absent	Spherical and terminal (drum stick)

Shigella

The method described below is adapted from the WHO guidelines for the control of epidemics due to *Shigella dysenteriae* type 1.

Direct inoculation of agar plates: Use 2 or 3 loopfuls of the herbal materials, preparations or products to be tested. Incubate plates at 35–37°C for 18–24 hours. Inoculate a general purpose plating medium of low selectivity and one of moderate or high

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selectivity. *MacConkey agar* is recommended as a medium of low selectivity. *MacConkey agar* with 1 mcg/ml of potassium tellurite has been reported to be particularly useful for *S. dysenteriae* type 1 (Sd1). Use a small inoculum. Incubate at 35–37°C for 18–24 hours.

Xylose-lysine-desoxycholate (XLD) agar is recommended as a medium of moderate or high selectivity for isolation of *Shigella*. *Desoxycholate citrate agar* (DCA) is a suitable alternative.

Note: Do not use salmonella-shigella (SS) agar, as it often inhibits growth of Sd1. Each new batch of medium should be controlled for quality before routine use by inoculating it with known reference strains and observing their growth and colony characteristics.

Identif cation of colonies on plating media: Colonies suspicious for *Shigella* will appear as follows:

- MacConkey agar: convex, colourless, 2–3 mm
- XLD agar: red, smooth, 1–2 mm
- DCA agar: colourless, translucent, 2–3 mm.

Identify well-separated colonies of typical appearance to be transferred from each of the plating media for further testing by making a mark on the bottom of the Petri dish. Whenever possible a person experienced in the identification of *Shigella* should train laboratory workers who are unfamiliar with its identification.

Inoculation of Kligler iron agar : Pick three characteristic colonies from the plating media and inoculate into *Kligler iron agar* (KIA) as follows: stab the butt and then streak the slant with a zigzag configuration. Pay attention to proper labelling of the tubes. If screw-cap KIA tubes are used, make sure that the caps are loose. Incubate overnight. On the following morning, examine the reactions in the KIA tubes. Tubes suspicious for *Shigella* will have an acid (yellow) butt and an alkaline (red) slant. They will not produce gas (no bubbles or cracks in the agar) and will not produce hydrogen sulfide (no black along the stab line).

Triple sugar iron agar (TSI) can also be used for the identification of *Shigella*. It will give the same reactions as KIA.

7.7 VALIDATION OF THE TESTS FOR SPECIFIC MICROORGANISMS

If necessary, grow separately the test strains on the culture media indicated, at 30–35°C for 18–24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 so that the test suspensions contain about 103 microorganisms per ml. Mix equal volumes of each suspension and use 0.4 ml (approximately 102 microorganisms of each strain) as an inoculum in tests for *Escherichia coli, Salmonella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus,* in the presence of the material being examined, if necessary. The test method should give a positive result for the respective strain of microorganism.

SUMMARY

- Aerobic bacteria and fungi (moulds and yeasts) are determined by the TVC.
- Microbial tests should be applied to starting plant materials, intermediate and finished products where necessary.

- Prepare an enrichment culture as described for *Pseudomonas aeruginosa.*
- The test method should give a positive result for the respective strain of microorganism.

REVIEW QUESTIONS

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1. Discuss the total viable aerobic count in herbals.
2. Discuss Pretreatment of the test herbal material.
3. Discuss Effectiveness of the culture medium, confirmation of antimicrobial substances and validity of the counting method.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

NOTES

UNIT VIII: DETERMINATION OF PESTICIDE RESIDUES

CONTENTS

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- describe the general aspects of analytical methodology.
- discuss about the determination of chlorine and phosphorus.
- explain the qualitative and quantitative determination.
- define the analysis of esters of organophorus compounds.

8.1 GENERAL METHODS OF PESTICIDE RESIDUES

Chromatography (mostly column and gas) is recommended as the principal method for the determination of pesticide residues. These methods may be coupled with Mass Spectrometry (MS). Samples are extracted by a standard procedure, impurities are removed by partition and/or adsorption, and the presence of a moderately broad spectrum of pesticides is measured in a single determination. However, these techniques are not universally applicable. Some pesticides are satisfactorily carried through the extraction and clean-up procedures, others are recovered with a poor yield, and some are lost entirely. Following chromatography, the separations may not always be complete, pesticides may decompose or metabolize, and many of the metabolic products are still unknown. Consequently, as a result of limitations in the analytical

technique and incomplete knowledge of pesticide interactions with the environment, it is not yet possible to apply an integrated set of methods that will be satisfactory in all situations.

Generally the methodology should be adapted to the type of herbal material being tested and modifications may be necessary for different samples including seeds, leaves, oils, extracts, finished products and for samples containing different quantities of moisture. Also the spectrum of pesticides to be tested for is dependent on the specific pesticides used on the plant material and the history of use of persistent pesticides in the region.

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It is, therefore, desirable to test plant materials of unknown history for broad groups of compounds rather than for individual pesticides. A variety of methods are suitable for this purpose. Pesticides containing chlorine in the molecule, for example, can be detected by the measurement of total organic chlorine; insecticides containing phosphate can be measured by analysis for total organic phosphorus, whereas pesticides containing arsenic and lead can be detected by measurement of total arsenic or total lead, respectively. Similarly, the measurement of total bound carbon disulfide in a sample will provide information on whether residues of the dithiocarbamate family of fungicides are present.

Importantly, where such general methods are employed, care must be taken to ensure that results are not adversely affected by contributions from certain plant constituents containing the targeted elements. If the pesticide to which the plant material has been exposed is known or can be identified by suitable means, an established method for the determination of that particular pesticide residue should be employed.

8.2 GENERAL ASPECTS OF ANALYTICAL METHODOLOGY

The samples should be tested as quickly as possible after collection, before any physical or chemical changes occur. If prolonged storage is envisaged, the samples should preferably be stored in airtight containers under refrigeration.

The water content of samples can also be problematic and in some official pharmacopoeias, content is limited to 15% and below for the determination of organochlorine and pyrethroid insecticides. Light can cause degradation of many pesticides, and it is therefore advisable to protect the samples and any extracts or solutions from undue exposure to light. The type of container or wrapping material used should not interfere with the sample or affect the analytical results.

Solvents and reagents used in the analytical method should be free from substances that may interfere with the reaction, alter the results or provoke degradation of the pesticide residue in the sample. It is usually necessary to employ specially purified solvents or to distil them freshly in an all-glass apparatus. Blank determinations with the solvents should be carried out, concentrating and testing them as specified in the test procedure for the plant material concerned.

The simplest and quickest procedure should be used to separate unwanted material from the sample (clean-up procedure) in order to save time when many samples have to be tested. The process of concentrating solutions should be undertaken with great care, especially during the evaporation of the last traces of solvent, to avoid losses of pesticide residues. For this reason, it is often not advisable to remove the last traces of solvent. Agents, such as mineral oil or other oils of low volatility

that may help to preserve the solution could be added to retard the loss of the relatively volatile pesticides, especially when the last traces of solvent are being evaporated.

However, these agents, while satisfactory in colorimetric procedures, are usually not desirable in gas chromatographic methods. It may be necessary to evaporate heat-labile compounds using a rotary vacuum apparatus.

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8.3 TOTAL CHLORINE AND PHOSPHORUS

Most pesticides contain organically bound chlorine or phosphorus.

Procedure

Preparation of samples

Reduce the plant material to a fine powder, and extract with a mixture of water and *acetonitrile R*. Most pesticides are soluble in this mixture, while most cellular constituents (*e.g.*, cellulose, proteins, amino acids, starch, fats and related compounds) are sparingly soluble and are thus removed. A number of polar and moderately polar compounds may also be dissolved; it is therefore necessary to transfer the pesticides to *light petroleum R*. For pesticides containing chlorine, further purification is seldom required, but for those containing phosphorus, further purification by column chromatography may be necessary, eluting with mixtures of *light petroleum R* and *ether R*.

Preparation of the column

Use Florisil R grade 60/100 PR (or equivalent), activated at 650°C, as the support. If this material is obtained in bulk, transfer it immediately after opening to a 500 ml glass jar or bottle with a glass stopper or foil-lined, screw-top lid. Store in the dark. Before use, heat at not less than 130°C, cool in a desiccator to room temperature and heat once again to 130°C after 2 days.

Prepare a Florisil column (external diameter, 22 mm), which contains, after settling, 10 cm of activated Florisil topped with about 1 cm of *anhydrous sodium sulfate R*. Prewet the column with 40–50 ml of *light petroleum R*. Place a graduated flask under the column to receive the eluate.

Method

Grind the material to allow it to pass through a sieve no. 710 or 840 and mix thoroughly. Place 20-50 g of the ground sample into a blender, add 350 ml of *acetonitrile R* with a water content of 35% (to 350 ml of water add sufficient *acetonitrile R* to produce 1000 ml). Blend for 5 minutes at a high speed. Filter under vacuum through an appropriate funnel, diameter 12 cm, fitted with filter paper, into a 500 ml suction flask. Transfer the filtrate to a 250-ml measuring cylinder and record the volume.

Transfer the measured filtrate to a 1-litre separating funnel and carefully add 100 ml of *light petroleum R*. Shake vigorously for 1–2 minutes, add 10 ml of *sodium chloride (400 g/l) TS* and 600 ml of water. Hold the separating funnel in a horizontal position and mix vigorously for 30–45 seconds. Allow to separate, discard the aqueous layer and gently wash the solvent layer with two 100 ml portions of water. Discard the washings, transfer the solvent layer to a 100 ml glass-stoppered cylinder, and record

the volume. Add about 15 g of *anhydrous sodium sulfate R* and shake vigorously. The extract must not remain in contact with this reagent for longer than 1 hour. Transfer the extract directly to a Florisil column; if necessary, reduce the volume first to 5–10 ml. Allow it to pass through the column at a rate of not more than 5 ml per minute. Carefully rinse the cylinder with two portions, each of 5 ml, of *light petroleum R*, transfer them to the column, rinse with further small portions of *light petroleum R* if necessary, and then elute at the same rate with 200 ml of *ether/light petroleum TS1*. Change the receiver and elute with 200 ml of *ether/light petroleum TS2*. Again change the receiver and elute with 200 ml of *ether/light petroleum TS3*. Evaporate each eluate to a suitable volume, as required, for further testing.

- The first eluate contains chlorinated pesticides (aldrin, DDE, TDE (DDD), *o, p-* and *p, p*-DDT, HCH, heptachlor, heptachlor epoxide, lindane, methoxychlor), polychlorinated biphenyls (PCB), and phosphated pesticides (carbophenothion, ethion and fenchlorphos).
- The second eluate contains chlorinated pesticides (dieldrin and endrin) and phosphated pesticides (methyl parathion and parathion).
- The third eluate contains phosphated pesticide (malathion).

Combustion of the organic matter

Combustion of the organic matter in oxygen is the preparatory step for the determination of chlorine and phosphorus. The pesticide is extracted from the sample and purified if necessary. The extract is concentrated, evaporated to dryness, transferred to a sample holder, and burned in a suitable conical flask flushed with oxygen. The gases produced during combustion are then absorbed in a suitable solution. The absorbed chlorine is determined as chloride and the absorbed phosphorus as orthophosphate, both using colorimetry.

Apparatus: The combustion is carried out in a 1-litre conical flask made of borosilicate glass, into the stopper of which is fused one end of a piece of platinum wire about 1 mm in diameter. To the free end of the wire is attached a piece of platinum gauze measuring about 1.5 × 2 cm to provide a means of holding the sample clear of the absorbing liquid during combustion.

Sample holder for chlorine-containing residues: For a small quantity of solid material, use a sample holder made from a piece of halide-free filter-paper about 5 cm long and 3 cm wide; for a small volume of liquid, it is preferable to use a sample holder in the form of a cone made from cellulose acetate film. Prepare the cone as follows: wearing cloth gloves and using a suitable cardboard template, cut from the film a circle of radius 4 cm.

Manually pin the two edges together to form a cone. Seal the joined edges using heat to form a seam about 5 mm wide. Immerse the seam in *acetone R* to about one half of its width for 10 seconds. Remove and dry it immediately in a stream of hot air. Using forceps, wash the cone by dipping in a 1-litre beaker containing warm *sodium hydroxide (~240 g/l) TS* for 10 seconds at a temperature of about 60°C. Rinse the cone thoroughly with water and allow to drain dry on a piece of aluminium foil. Place each cone in a clean funnel (diameter 65 mm).

Sample holder for phosphorus-containing residues: Use a piece of halide-free filter-paper about 4 cm square as the sample holder.

Combustion of chlorine-containing residues: Transfer an aliquot of the extract as prepared above onto the sample holder, which is placed in a funnel using a solvent that

will not dissolve the sample holder. Allow the solvent to evaporate. Wearing rubber gloves, remove the sample holder and its dry contents from the funnel, and fold it over and up to form a small packet, about 1 cm^2 in area, and secure it in the centre of the platinum gauze. Insert a narrow strip of filter paper, about 1 – 3 cm, as a fuse into the top of the holder, between the folds of the packet. Add 30 ml of water to the combustion flask. Moisten the neck of the flask with water. Fill the flask thoroughly with oxygen by means of a tube with its end just above the liquid. Ignite the free end of the paper strip and immediately insert the stopper. Hold the stopper firmly in place. When vigorous burning has begun, tilt the flask to prevent incompletely burned material from falling into the liquid. Immediately after combustion is completed, shake the flask vigorously for 10 minutes to dissolve the combustion products. Place a small quantity of water around the rim of the flask, and carefully withdraw the stopper. Rinse the stopper, platinum wire, platinum gauze and sides of the flask with water. Transfer the liquid and liquids used for rinsing to a 50-ml volumetric flask and dilute to volume with water.

Combustion of phosphorus-containing residues: Dip the sample holder made from filter paper into *methanolic sodium hydroxide TS*, and then suspend it in a current of heated air. Immediately transfer about 0.2 ml of an aliquot of the extract as prepared above to the sample holder with the aid of 0.2 ml portions of *chloroform R* using a micropipette. Allow the solvent to evaporate from the paper, fold it to form a small packet about 1 cm^2 in area and place it in the centre of the platinum gauze. Insert a strip of filter paper, about 1 × 3 cm, as a fuse into the top of the holder, between the folds of the packet. Add 10 ml of *sulfuric acid (~37 g/l) TS* to the combustion flask and continue with the combustion as described above. Transfer the solution and the liquid used for rinsing to a 25 ml volumetric flask and dilute to volume with water.

8.4 CHLORIDES

Apparatus

The determination is made with a spectrophotometer capable of measuring absorbance at 460 nm using absorption cells with path-lengths of 2 cm and 10 cm.

Method

Place 15 ml of the solution obtained after combustion in a 50 ml conical flask together with 1 ml of *ferric ammonium sulfate (0.25 mol/l) VS* and 3 ml of *mercuric thiocyanate TS*. Swirl the contents of the flask and allow to stand for 10 minutes. Transfer a portion of the solution to a 2-cm cell and measure the absorbance at 460 nm using water in the reference cell. The reading should be made promptly to minimize absorption of chloride from the air.

Prepare a standard solution of *sodium chloride R* containing 5 µg of chloride per ml. Transfer aliquots of this solution (0 ml, 2 ml, 4 ml, 6 ml, 8 ml and 10 ml) into a series of 50 ml conical flasks and dilute to 15 ml with water. Develop the colour and measure the absorbances as described above. Plot the absorbances against the chloride content of the dilutions in µg per ml and interpolate the chloride content of the solutions of the material tested.

8.5 PHOSPHATES

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The phosphomolybdate method is based on the reaction of phosphate ions with ammonium molybdate to form a molybdophosphate complex, which is subsequently reduced to form a strongly blue-coloured molybdenum complex.

The intensity of the blue colour is measured spectrophotometrically. This method is applicable for the determination of any phosphates that have undergone a prior separation procedure.

Naturally occurring phosphates are present in most samples, and are often not removed during the clean-up procedure. In order to obtain background values, therefore, it is necessary to proceed with the determination for all samples, even those with no phosphate-containing pesticides. These background values should be subtracted from the results obtained on testing pesticide residues. Extracts of most uncontaminated materials contain about 0.05-0.1 mg/kg of phosphorus.

Therefore, no contamination with organophosphate pesticides can be assumed for results in this range.

Apparatus

The determination is made with a spectrophotometer capable of measuring absorbance at 820 nm using an absorption cell with a path-length of 1 cm.

Method

Place 7 ml of the solution obtained after combustion in a calibrated 10-ml testtube. Add 2.2 ml of *sulfuric acid (300 g/l) TS* and mix the solution well. Add 0.4 ml of *ammonium molybdate (40 g/l) TS* and swirl the mixture. Then add 0.4 ml of *aminonaphtholsulfonic acid TS* and swirl again. Heat the solution to 100 °C for 12 minutes (± 2 minutes), cool, and transfer a portion to a 1 cm cell. Measure the absorbance at 820 nm using water in the reference cell.

Prepare standard dilutions with a known content of phosphate and measure the absorbance as described above. Plot the absorbances against the phosphate content of the dilutions in µg per ml and interpolate the phosphate content of the solutions of the material tested.

8.6 QUALITATIVE AND QUANTITATIVE DETERMINATION OF ORGANOCHLORINE PESTICIDES

Preparation of Sample

Place 20 g of powdered plant material (sieve no. 180), accurately weighed, in a 500 ml beaker (tall form), mix with 98 ml of water and allow to macerate for at least 30 minutes. Add 200 ml of *acetone R;* the resulting volume of extraction solvent will be 295 ml. Extract for 5 minutes, while cooling, using a high-speed mixer. Filter the homogenized mixture through a porcelain filter (Bhner funnel, diameter 70 mm) fitted with a filter paper, using a slight vacuum, into a 250 ml graduated cylinder, allowing the process to last no longer than 1 minute, and then measure the volume (*V*) of the filtrate in ml.

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Method

Transfer the filtrate prepared as above to a 500 ml separating funnel. Add a quantity of *sodium chloride R* equivalent in grams to one-tenth of the volume of the filtrate, then add 100 ml of *dichloromethane R*. Shake vigorously for 5 minutes, allow the phases to separate and discard the lower (aqueous) layer. Dry the acetone-dichloromethane phase, transfer it to a 500 ml conical flask, add 25 g of *anhydrous sodium sulfate R* and swirl occasionally. Next, filter the solution into a 500 ml flask with a ground-glass stopper using a glass funnel (diameter 100 mm) containing purified glass-wool and *anhydrous sodium sulfate R*. Rinse the separating funnel, the conical flask and the glass funnel twice with 10 ml of *ethyl acetate R*. Add 5 ml of 2, 2, 4-*trimethylpentane R*, and concentrate the crude extract to about 2 ml in a rotary vacuum evaporator in a water-bath at 30–40°C. Expel the remaining solvent in a gentle stream of air.

To purify by gel chromatography, macerate 50 g of suitable beads (*e.g.*, S-X3 biobeads) in an elution mixture of *cyclohexane R* and *ethyl acetate R* (1:1) and pour them into a chromatographic column (length 600 mm, diameter 25 mm) adapted for use with a vacuum pump. Rinse the gel bed with the elution mixture under air-free conditions. Dissolve the extract in the flask with 5 ml of *ethyl acetate R*. Add 2 g of *anhydrous sodium sulfate R*, swirl gently and add 5 ml of *cyclohexane R*. Filter the completely dissolved crude extract through a rapid filter into a 10 ml test tube with a ground-glass stopper and close the tube immediately. Then transfer 5 ml of the filtrate on to the gel column. Elute with the elution mixture at an average rate of 5 ml/minute.

Plant material components leave the gel column first, followed by the active ingredients of pesticides. Fractionation must be determined for each column, using appropriate reference substances. Discard the first fraction (about 100 ml) containing the impurities. Collect the organochlorine pesticides appearing in the next eluate (about 70 ml) in a flask with a ground-glass stopper. Add 10 ml of 2, 2, 4-*trimethylpentane R* and concentrate the solution to about 5 ml in a rotary vacuum evaporator and a water-bath at 30–40°C. Pipette another 5 ml of 2, 2, 4-*trimethylpentane R* into the flask and carefully evaporate the solution to about 1 ml (do not allow to become completely dry).

Determination by Gas Chromatography

A capillary gas chromatograph with an ECD is used for the measurement. *Helium R* is used as the carrier gas and a mixture of *argon R* and *methane R* (95:5) as an auxiliary gas for the detection.

First separation system: Use a vitreous silica column, 30 m long with an internal diameter of 0.25 mm, packed with a chemically bound phase of 5% phenyl, 95% methyl-polysiloxane. Use the following temperature programme:

- heat at 60°C for 0.5 minutes;
- increase the temperature at a rate of 30°C per minute to 160°C and maintain this temperature for 2 minutes;
- increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use a "split/split-free" injector to inject the sample solution and maintain the injection port at a temperature of 240°C. Inject a volume of 1 µl at a rate of 30 seconds ("split-free"). The detector temperature should be 300°C.

Second separation system: Use a vitreous silica column, 15 m long with an internal diameter of 0.25 mm, packed with a chemically bound phase of 7% cyanopropyl, 7% phenyl, 86% methylpolysiloxane. Use the following temperature programme:

- heat at 60°C for 12 seconds;
- increase the temperature at a rate of 30°C per minute to 180°C and maintain this temperature for 1 minute;
- increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use an on-column injector to inject a volume of 1 µl of the sample solution. The detector temperature should be 300°C. Use the "external standard" method for the qualitative and quantitative evaluation of the organochlorine pesticides in the test solutions with reference solutions of the following pesticides: α-, β-, γ- and δ-hexachlorocyclohexane (HCH); hexachlorobenzene; quintozene; aldrin; dieldrin; endrin; α- and β-endosulfan; endosulfan sulfate; heptachlor, heptachlorepoxide; camphechlor; TDE, DDE and DDT (both *o, p*- and *p, p*-isomers); methoxychlor.

8.7 ANALYSIS OF ESTERS OF ORGANOPHOSPHORUS COMPOUNDS

Although most organophosphorus compounds undergo rapid decomposition, Member States may elect to test for them because of their harmful nature if present in significant concentrations. Testing may be more relevant in the case of herbal medicines used at high concentrations and frequency.

The extraction and the clean-up procedures can be performed as described above, but the detection requires a phosphorus flame ionization detector (P-FID).

8.8 SPECIFIC PESTICIDE RESIDUES IN MEDICINAL PLANT MATERIAL: GENERAL RECOMMENDATIONS

For the total determination, mix thoroughly 1 kg of plant material. In order to obtain reliable chromatographic results, do one or more of the following:

- repeat the separation using another column;
- use a different separation system;
- use a different detector system;
- apply a coupling technique;
- prepare a derivative;
- perform chromatography with a mixture of the sample and a reference substance;
- change the sample preparation;
- use a fractionated elution during the column-chromatography clean-up procedure of the plant extract and test every fraction by chromatography; and
- compare the distribution coefficient of the material with that of a reference substance.

Prior to the quantitative determination of the material to be tested, check whether there is a linear relationship between the values obtained for the reference substance and its concentration over the range 0.1-2 times the standard concentration.

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Otherwise, prepare another concentration range or evaluate the results using a reference curve. Use any suitable mechanical or manual technique for the chromatographic determination.

Store the reference solutions protected from light to prevent decomposition. Use glass vessels closed with glass stoppers and keep them in a container saturated with the solvent employed to avoid any increase in concentration due to evaporation. Check the loss by evaporation by interim weighing of the vessels.

Rate of Recovery

The rate of recovery (R) is the percentage of the reference material originally added to the plant material that is determined using the method described below.

8.9 DESMETRYN, PROMETRYN AND SIMAZINE RESIDUES

Preparation of the Plant Material Extract

Place 10 g of powdered plant material in a 500 ml conical flask and add 125 ml of *chloroform R*.1 Shake the mixture for 60 minutes and filter under reduced pressure through a filter paper (medium grade) into a round-bottomed flask. Wash the residue with 3 successive volumes each of 25 ml of *chloroform R*.

Method

Concentrate the combined filtrates to a volume of 3-5 ml using a rotary vacuum evaporator and a water-bath at 40°C. Transfer the extract to a chromatographic column as prepared below, rinsing the round-bottomed flask twice with 5 ml of *chloroform R*.

Preparation of chromatographic column: Use a glass tube (internal diameter 20–22 mm) with a restricted orifice and protected with a sintered-glass plate (*e.g.*, P10 or P16, glass filter G4; or P40, glass filter G3).

Fill the column with *chloroform R*, and then pour purified *aluminium oxide R* into it to form a 100-mm thick layer. The support material should remain covered with *chloroform R*. After transferring the extract and the rinsing liquids to the column, elute with 150 ml of *chloroform R*, at a rate of 1-2 drops per second, collecting the eluate in a round-bottomed flask. The first purifying process is completed when no further eluate drips from the column.

Evaporate the eluate to dryness using a rotary vacuum evaporator and a waterbath at 40°C. To the residue add 10 ml of *light petroleum R* and transfer the mixture to a chromatographic column containing a layer of purified *aluminium oxide R*, 50 mm thick, in *light petroleum R*. Elute the mixture with 90 ml of *light petroleum R*, using this to rinse the round-bottomed flask, at a rate of 1–2 drops per second. Discard the eluate. Dissolve any remaining residue, which has not dissolved in *light petroleum R* in 10 ml of a mixture composed of 60 volumes of *chloroform R*, and 40 volumes of *light petroleum R* and transfer the solution to the column. Rinse the round-bottomed flask twice more with 10 ml of the solvent mixture. Transfer the liquid used for rinsing to the column. Elute with 120 ml of the same solvent mixture, at a rate of 1–2 drops per second and collect the eluate in a round-bottomed flask. The second purifying process is completed when no further eluate drips from the column.

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Evaporate the eluate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. To prepare a purified extract for the determination by gas chromatography, dissolve the residue in sufficient *acetone R* to produce a volume of 10 ml. If an especially purified extract is required, proceed as described below. To the residue add 10 ml of *light petroleum R* and 10 ml of *dimethyl sulfoxide R*. Shake the mixture and transfer it to a separating funnel. Extract the dimethyl sulfoxide layer twice with 10 ml of *light petroleum R*. Discard the petroleum ether extract. Then add 100 ml of water to the dimethyl sulfoxide layer and extract 3 times, each with 20 ml of *chloroform R*. Extract the combined chloroform extracts twice with 20 ml of water and evaporate them to dryness using a rotary vacuum evaporator and a water-bath at 40°C. Transfer the residue together with a mixture of 10 ml of *light petroleum R* and 10 ml of *hydrochloric acid (1 mol/l) VS* to a separating funnel and extract the mixture first with 10 ml and then with 5 ml of *hydrochloric acid (1 mol/l) VS*. Discard the petroleum ether layer and adjust the pH of the combined aqueous solutions to a value between 7 and 8 using *sodium hydroxide (1 mol/l) VS*. Extract the solution 3 times, each with 20 ml of *chloroform R*. Dry the combined chloroform extracts with *anhydrous sodium sulfate R* and filter into a round-bottomed flask, rinsing the funnel 3 times with 10.0-ml portions of *chloroform R*. Evaporate the filtrate to dryness using a rotary vacuum evaporator and a water-bath at 40°C.

Dissolve the residue in sufficient *acetone R* to produce 10 ml of especially purified extract to be used for the determination by gas chromatography. Use the extracts as indicated in Table 8.1 for the following plant materials.

Table 8.1 Extract to be used for specific herbal materials

No.	*Material*	*No.*	*Material*
1	Flores Calendulae	10	Fructus Foeniculi
2	Flores Chamomillae	11	Herba Millefolii
3	Folia Melissae	12	Herba Plantaginis lanceolatae
4	Folia Menthae piperitae	13	Radix Althaeae
5	Folia Salviae	14	Radix Angelicae
6	Folia Thymi	15	Radix Levistici
7	Fructus Carvi	16	Radix Petroselini
8	Fructus Coriandri	17	Radix Valerianae
9	Fructus Cynobasti		

For materials no. 1 and 2, use an especially purified extract (see above); for materials no. 3–17, use a purified extract.

SUMMARY

- Chromatography (mostly column and gas) is recommended as the principal method for the determination of pesticide residues.
- The type of container or wrapping material used should not interfere with the sample or affect the analytical results.
- Most pesticides contain organically bound chlorine or phosphorus.
- Combustion of the organic matter in oxygen is the preparatory step for the determination of chlorine and phosphorus.

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REVIEW QUESTIONS

1. Discuss the general methods for the determination of pesticide residues.
2. Discuss determination of total chlorine and phosphorus.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

PART 2: STANDARDIZATION

UNIT I: FOOD ADDITIVES STANDARDIZATION OF FOOD PRODUCT

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★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- describe the concept of neutritional requirement.
- discuss about the safety and types of additives.
- know about the myths and fallacies.
- define the clean labels.

1.1 INTRODUCTION

The role of food additives in food manufacture has been much maligned and misunderstood in recent years. Additives fell victim to bad press to the extent that, at the height of the anti-"E" numbers campaign in the 1980s, the word "additive" became almost synonymous with "adulteration", and foods containing additives were as much to be avoided as foods containing genetically modified ingredients have become since their introduction in the late 1990s. Authors whose main objective appeared to be the denigration of the food manufacturing industry, particularly the major multinationals, found this easy meat in an atmosphere of consumer ignorance, and were guaranteed support for their cause by scaring their audience into believing that additives were responsible for a wide range of ill effects from intolerance and hyperactivity to long-term chronic diseases.

Constantly prefacing the words "food additive" with "chemical" was sufficiently emotive to result in the perception of "nasty". Alongside this was the implication that ready-prepared, processed food was inherently inferior to, and less wholesome than food prepared in the home. The catalyst for the 1980s focus on additives was a change in labeling legislation in 1986, which required the detailing of each individual

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additive in the ingredients list of most pre-packed products. Until that time, the use of additives had been indicated by reference to a generic functional group, such as "preservatives", "antioxidants" and "colours". The new labelling requirements resulted in the appearance on some food labels of some very long lists of additives, including some lengthy chemical names. Some products looked as though they were nothing more than a couple of simple ingredients held together by a dictionary of chemical substances. The "E" number system, intended to assist as a short code for some of the lengthier chemical names and to indicate common European safety approval, became the butt of the criticism against the use of additives, and consumers voted with their feet by leaving products containing long lists of "E" numbers on the shelf.

The interest in, and fear of, what was being put into food spawned a number of books on additives, their use in food, potential (harmful) effects and protocols for their safety approval, along with the author's specific treatise on the subject. Some were informative, intended to assist the consumer in understanding what additives were, how they were produced, why they were used and how to avoid them, if desired. Others were more politically motivated and used the fashionable attack on additives as an illustration of all that was bad about the food industry and the allegedly secretive systems of safety assessment of all chemicals and processes used in food production. The implication was that any chemicals added to food, either as pesticides in primary production or additives in processing, were suspect.

A generation brought up on convenience foods, removed from the messy business of primary food production, fell easy prey to this suggestion, apparently oblivious to the substances and techniques employed by their grandmothers, when no self-respecting household would have been without baking powder, bicarbonate of soda, cream of tartar, a selection of flavourings and a bottle of cochineal - some of the most common everyday "food additives". These everyday ingredients might well be frowned upon by many a modern shopper uninitiated in the art of cookery, if spotted on the ingredients label of a manufactured product in the form of an "E number" or prefaced, as legislation requires, by its additive class. How many people think of additives when they buy a lemon or a bottle of vinegar? Yet these too are authorised additives (as citric and acetic acid, respectively) and widely used in food manufacture for their preservation properties, as well as their acidic taste, precisely as they are used in everyday cooking. The use of saltpetre as a preservative can be traced back to Roman times, and the controversy over additives use goes back to at least 1925, when the use of boric acid in food was banned under the Preservatives Regulations. However, in recent years the use of boric acid has been accepted under the Miscellaneous Food Additives Regulations 1995 as amended, but only for the treatment of caviar.

Whilst its complexity and scale do not lend modern food manufacture entirely to direct comparison with the traditional kitchen, it is often forgotten that the overall purpose is the same – to prepare, preserve, process and, as the case may be, cook basic raw ingredients to convert them into wholesome, attractive, better tasting and nutritious foods ready to be consumed. Every cook has his or her own techniques, and knows many a trick to prevent peeled vegetables and apples from browning, thicken sauces, brown the gravy, and transform an everyday dish into something special; he or she will also ease dinner party preparations by preparing in advance and storing the part-ready dishes for lastminute completion. Food manufacturers do much the same, and, over years of product development, first on the basis of

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trial and error and now underpinned by research programmes, have developed the most effective and economical methods of producing a wide range of foods to suit every taste and pocket. In order to achieve this, they need at their disposal a wide range of additives to perform a number of tasks in the process, from cleaning and refining the raw materials, to preserving them in optimal condition throughout further processing or distribution, combining them with other ingredients and ensuring that they appear attractive to the consumer. The types of additive used and some of the functions they perform are explored in greater detail below.

The anti-additives campaign and consequent consumer pressure to remove or minimise the use of additives inevitably led to changes in manufacturing practice and marketing. In addition, trends towards more "fresh" foods and the growth in market share of chilled foods, together with changes in legislation following completion of the European harmonisation exercise, all had an impact on the use of additives. It is therefore timely to review the place and use of additives in the food supply, whilst bearing in mind that they will always be essential to food preparation, quality and preservation.

1.2 CONCEPT OF NEUTRITIONAL REQUIREMENT

The use of food additives is nothing new. Preserving food is an age-old necessity. Many of the techniques that we now take for granted, such as canning, refrigeration and freezing, are relatively new. Even the overwintering of farm animals was rare until the 17th century, when feeding and husbandry techniques became better understood. Any old or weakly livestock such as oxen, cows, sheep, pigs and poultry had to be slaughtered in the autumn, and the meat was dried, salted or pickled to preserve it for the winter months. When food shortage ceases to be a problem, greater emphasis is placed on making food look and taste good, and we look beyond food as a survival necessity to food as a pleasure and a treat.

Food additives are used either to facilitate or complement a wide variety of production methods in the modern food supply. Their two most basic functions are that they either make food safer by preserving it from bacteria and preventing oxidation and other chemical changes, or they make food look or taste better or feel more pleasing in the mouth.

The use of additives in food preservation is, not surprisingly, one of the oldest traditions. Our forbears may not have thought of saltpetre, used as a curing agent, or vinegar (acetic acid) as additives, but they would have been the mainstay for ensuring a longer-term supply of precious perishable foods. Salt, though not an additive by the modern definition, was the other essential. Food additives are defined in European legislation as "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to a food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods". Known as the additives "framework" Directive, this Directive also defines processing aids as "any substance not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods or their ingredients, to fulfil a certain technological purpose during treatment or processing, and which may result in

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the unintentional but technically unavoidable presence of residues of the substance or its derivatives in the final product, provided that these residues do not present any health risk and do not have any technological effect on the finished product."

Processing Aids

Whilst many of the substances used as additives may also be used as processing aids, the latter function is outside the scope of additives legislation. The differentiating criterion, and the question that any manufacturer must ask in terms of regulatory requirements, is "does it continue to function in the final food?" So, for example, sulphur dioxide (E220) may be used to prevent discoloration of fruit destined for pie making, but would have no effect in the fruit pie itself, and indeed would be cooked off during processing. Thus, in this application, it is a processing aid used in the making of a fruit pie, not an additive performing a function in the pie itself. Many of us will be used to similar techniques in the kitchen, such as using lemon juice to prevent discoloration. In the complex world of food manufacture, where production is increasingly specialised and expertise focused at specific sites, it is not unusual for the manufacturer of an end product to buy in many of his supplies as part-processed proprietary ingredients. So additives may be needed at the "intermediate" stage, but would have no function in the final product, and would therefore not appear on the label, unless considered to have the potential to cause an allergenic reaction. Thus, anti-caking agents may be required in dry ingredients to prevent them from turning lumpy before being made into a fancy cake, but will have no effect once the cake is baked and decorated, so the anti-caking agent functions as an additive in the dry mix, but is a processing aid as far as the cake is concerned. Other examples of processing aids are release agents used to prevent food from sticking to a mould or, perhaps, slicing equipment. Again, this is part of the process of production, not the composition of the food, even though there may be traces of the "processing aid" left on the product, as there would be on a cake from greasing the cake tin.

This, then, is the essential technical difference between a processing aid and an additive. The "framework" Directive identifies a number of classes of additives, *e.g.*, sweeteners, colours and "miscellaneous" additives (including additive categories such as preservatives, antioxidants, emulsifiers, stabilisers, thickeners, flavour enhancers etc.), for which more detailed legislation was eventually developed, and lays down general criteria for their use, notably that technological need must be demonstrated that cannot be achieved by other means; that their presence presents no hazard to the consumer; and that they do not mislead the consumer. Their use may be considered only where there is demonstrable benefit to the consumer, namely to preserve the nutritional quality of the food; to provide necessary ingredients or constituents for foods manufactured for groups of consumers with special dietary needs, or to enhance the keeping quality or stability of a food or to improve its organoleptic properties, provided that, in doing so, it does not deceive the consumer; and to assist in manufacture, processing, preparation, treatment, packing, transport or storage of food, provided that the additive is not used to disguise the effects of the use of faulty raw materials or of undesirable (including unhygienic) practices or techniques during the course of any of these activities. These are similar to the principles enshrined in the Codex Alimentarius, the joint FAO/WHO body responsible for international standards in food.

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The harmonisation of European legislation was a prerequisite for trade in the Single Market as differences in national legislation constituted barriers to trade. This is explored in greater detail in a later chapter, but it is important to appreciate that the development of a new raft of additives legislation in the late 1980s and through the 1990s was not indicative of an absence of controls before that time, but a recognition that differences in national approaches throughout the Member States were not conducive to the free movement of goods within a single economic entity. The new legislation reinforced the requirement for justification of a case of need in the use of additives and of the importance of not deceiving the consumer.

The primary aim of the food-manufacturing industry is to provide a wide range of safe, wholesome, nutritious and attractive products at affordable prices all year round in order to meet consumer requirements for quality, convenience and variety. It would be impossible to do this without the use of food additives. They are essential in the battery of tools used by the food manufacturer to convert agricultural raw materials into products that are safe, stable, of consistent quality and readily prepared and consumed.

Different types of additive are used for different purposes, though many individual additives perform more than one function. For the purposes of both classification and regulation, they are grouped according to their primary function. The main groupings, or classes, of additives are explained below, together with their functions and some examples of their use.

Preservatives

Preservatives are probably the single most important class of additives, as they play an important role in the safety of the food supply. Despite this fact, any chemical used to counteract the perishability of food raw materials has often become perceived as suspect, and any food containing a preservative has been considered inferior or unsafe. Yet the use of chemical preservatives, such as sulphur dioxide and sulphites, is but a continuation of the age-old practices of using salt, sulphite and spices to preserve perishable foods in the days before refrigeration and modern processing techniques. All food raw materials are subject to biochemical processes and micro-biological action, which limit their keeping qualities. Preservatives are used to extend the shelf-life of certain products and ensure their safety through that extended period. Most importantly, they retard bacterial degradation, which can lead to the production of toxins and cause food poisoning. Thus they offer a clear consumer benefit in keeping food safe over the shelf-life of the product, which itself may be extended by their use and thus meet the demands of modern lifestyles, including infrequent bulk shopping expeditions. The continued perception of preservatives as undesirable, to which the many labels protesting "no artificial preservatives" 1, is therefore an unfortunate consumer misapprehension.

Antioxidants

Antioxidants reduce the oxidative deterioration that leads to rancidity, loss of flavour, colour and nutritive value of foodstuffs. Fats, oils, flavouring substances, vitamins and colours can all oxidise spontaneously with oxygen when exposed to air. The rate of deterioration can vary considerably and is influenced by the presence of natural antioxidants and other components, availability of oxygen, and sensitivity of the substance to oxidation, temperature and light, for example. Oxidation can be avoided, or retarded, by a number of means, such as replacing air

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by inert packaging gases, removal of oxygen with glucose oxidase, incorporation of UV-absorbing substances in transparent packaging materials, cooling, and use of sequestering agents. These may not be possible in all cases, or sufficient for an adequate shelf-life for some foods. Thus antioxidants are used to retard oxidative deterioration and extend shelf-life. Some antioxidants actually remove oxygen by self-oxidation, *e.g.*, ascorbic acid, whilst others interfere in the mechanism of oxidation, *e.g.*, tocopherols, gallic acid esters, BHA and BHT. All have specific properties, making them more effective in some applications than in others. Often a combination of two or more antioxidants is more effective than any one used simply because of their synergistic effects. The presence of sequestering agents, such as citric acid, may also have a synergistic effect, by reducing the availability of metallic ions that may catalyse oxidation reactions. The use of the powerful synthetic antioxidants BHA, BHT and the gallic acid esters is very restricted. Tocopherols, which can be either natural or synthetic, are less restricted but are less effective in the protection of processed foods. Antioxidants cannot restore oxidised food; they can only retard the oxidation process. As oxidation is a chain reaction process, it needs to be retarded as early as possible. The most effective use of antioxidants is therefore in the fats and oils used in the manufacturing process.

Emulsifiers and Stabilisers

The purpose of emulsifiers and stabilisers is to facilitate the mixing together of ingredients that normally would not mix, namely fat and water. This mixing of the aqueous and lipid phases is then maintained by stabilisers. These additives are essential in the production of mayonnaise, chocolate products and fat spreads, for example. The manufacture of fat spreads (reduced-fat substitutes for butter and margarine), has made a significant contribution to consumer choice and dietary change, and would not be possible without the use of emulsifiers and stabilisers. Other reduced- and low-fat versions of a number of products are similarly dependent on this technology. Anyone who has ever made an emulsified sauce, such as mayonnaise or hollandaise, will appreciate the benefits of this technology - still more so those who have failed miserably in the technique and ended up with an expensive mess of curdled ingredients!

In addition to this function, the term stabiliser is also used for substances that can stabilise, retain or intensify an existing colour of a foodstuff and substances that increase the binding capacity of the food to allow the binding of food pieces into reconstituted food.

The increasing awareness of problems with food allergy and intolerance has led to the requirement to state the source of certain emulsifiers on food labelling. For example, lecithin derived from soya is not suitable for an individual with an allergy to soya, therefore clear labelling of the source of the ingredient is vital to aid in consumer choice of products safe for individuals with specific dietary requirements.

Colours

Colours are used to enhance the visual properties of foods. Their use is particularly controversial, partly because colour is perceived by some as a means of deceiving the consumer about the nature of the food, but also because some of the most brightly coloured products are those aimed at children. As with all additives, their use is strictly controlled and permitted only where a case of need is proven, e.g. to restore colour that is lost in processing, such as in canning or heat treatment; to ensure

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consistency of colour; and for visual decoration. The use of colour in food has a long and noble tradition in the UK. Medieval cooks were particularly fond of it. The brilliant yellow of saffron (from which Saffron Walden derives its name) and the reddish hue of saunders (powdered sandalwood) were used along with green spinach and parsley juice to colour soups in stripes or to give marbleised effects. So, whilst adding colour to food may appear to some to be an unnecessary cosmetic, which is not in the consumer's interests, there can be no doubt that the judicious use of colour enhances the attractiveness of many foods. Some retailers tried introducing ranges of canned vegetables and fruits such as strawberries and peas without adding back the colour leached out by heat processing. They were still trying to dispose of the unsold returns several years later! Colour is important in consumer perception of food and often denotes a specific flavour. Thus, strawberry flavour is expected to be red and orange flavour orange-coloured. Consumer expectation is therefore a legitimate reason for adding colour.

Food colourings, in particular, have long been the scapegoat in the popular press for behaviour problems in children. It has been over 30 years since Feingold suggested that artificial food colours and preservatives had a detrimental effect on the behaviour of children. Since then, research into the effect of colours and preservatives in foods on children's behaviour has added fuel to the fire of negative consumer perception of these additives, particularly in products aimed specifically at this age group. Significant changes were found in the hyperactivity behaviour of children by removing colorants and preservatives from the diet. There was no gender difference in this result and the reduction of hyperactivity was independent of whether the child was initially extremely hyperactive, or not hyperactive at all. More recently in 2007, a study on the effect of two mixtures of certain artificial food colours together with the preservative sodium benzoate showed an adverse effect on the hyperactive behaviour of children in some age groups in comparison with a placebo, although the increases in the levels of children's hyperactive behaviour were not consistently significant for the 2 mixtures or in the 2 age groups. The findings of this new study replicate and extend the findings from an earlier study in preschool children in 2004 (6). The colours used in this study are already included in work of the European Food Safety Authority (EFSA) on the re-evaluation of colours.

Colouring Foodstuffs

The term 'colouring foodstuffs' has been adopted for colourings that are derived from recognised foods and processed in such a way that the essential characteristics of the food from which they have been derived are maintained. This is a different situation to natural colours that are regarded as additives where the pigment is selectively extracted and concentrated. A colouring foodstuff can be declared as an ingredient on the label without a requirement for its function to be listed, as legislation only requires this of additives.

These colouring foodstuffs include bright yellow colours derived from turmeric, oleoresin and safflower; golden yellow to natural orange colours from carrots and paprika; toffee brown colour from caramelised sugar syrup; green colours from spinach leaves and stinging nettles, both rich in chlorophylls; and red, blue and purple colour from concentrates of red and blue fruits, red cabbage and beetroot, rich in anthocyanins. It is clear that the full spectrum of colour shades is achievable using colouring foodstuffs, although developers should ensure that the colouring

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foodstuff exhibits the same stability and vibrancy of colour in the final application as a conventional food colouring would.

Sweeteners

Sweeteners perform an obvious function. They come in two basic types – "bulk" and "intense", and are permitted in foods that are either energy-reduced or have no added sugar. They are also sold direct to consumers as "table-top" sweeteners – well-known to dieters and diabetics. For example the table top sweetener Sunette contains acesulfame-K while Splenda contains sucralose. Intense sweeteners, such as aspartame, saccharin, acesulfame-K and sucralose have, as their name suggests, a very high sweetening property, variable from type to type but generally several magnitudes greater than that of sucrose. (For example, aspartame is approximately 200 times sweeter than sugar, weight for weight; saccharin 300–500 times; and acesulfame-K 130–200 times.) Bulk sweeteners, where the majority are polyols, including erythritol, sorbitol, isomalt and lactitol are less sweet, but provide volume and hence mouthfeel. Amongst the polyols, maltitol is one of the sweetest and xylitol, which is the sweetest, has the same sweetness intensity as sucrose. Due to the reduced sweetness characteristics of the majority of polyols, it is possible to blend them with other polyols or with intense sweeteners to improve the sweetness and taste quality. This property is known as sweetness synergy. Another benefit is the ability to mask the undesired bitter metallic aftertaste of some intense sweeteners. Commonly used combinations include, saccharin with cyclamate, acesulfame-K with aspartame, erythritol with acesulfame-K and there are many more. Both types of sweetener (bulk or intense) are useful in low-calorie products, and are increasingly sought after by many consumers, and for special dietary products such as for diabetics. The absence of sucrose also lowers the cariogenic properties of the product.

Flavour Enhancers

This is a group of additives that has attracted adverse attention, in particular monosodium glutamate (MSG:E621), which is widely blamed for an intolerance reaction that became known as "Chinese Restaurant Syndrome". Flavour enhancers are substances that have no pronounced flavour or taste of their own but which bring out and improve the flavours in the foods to which they are added. Although salt has a distinctive taste of its own and is not classed as a food additive, it is in fact the most widely used flavour enhancer. The next best known is glutamic acid and its salts, most commonly found in the form of monosodium glutamate, which has been used for several centuries in the Far East as a condiment in savoury products. It is a normal constituent of all proteins, an essential amino acid and present in the body. The alleged intolerance reaction was never confirmed in sound scientific studies. Anyone showing a reaction to MSG used as an additive would necessarily also react to foods that contain it naturally in high quantities, such as tomatoes and cheese.

Some sweeteners have also been found to have flavour-enhancing properties and have been authorised for use as such. For example, neohesperidine DC (E959) can enhance the flavour of meat products and margarine, and acesulfame K, aspartame and thaumatin are used to enhance the flavour of chewing gum and desserts.

Flavourings

Although flavour enhancers are categorised as additives, flavourings are technologically different and regulated separately, even though they are often considered by the general public to be the same thing. Flavourings are defined as imparting odour and/or taste to foods and are generally used in the form of mixtures of a number of flavouring preparations and defined chemical substances.

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These do not include edible substances and products intended to be consumed as such, or substances that have exclusively a sweet, sour or salty taste, *i.e.*, ordinary food ingredients such as sugar, lemon juice, vinegar or salt. The latest draft of the proposed new EC Regulation on Flavourings would also exclude from the definition of flavourings raw foods and non-compound foods, and mixtures of spices or herbs, mixtures of tea provided they are not used as food ingredients. In addition to the types of flavouring such as process flavours or smoke flavours, there are three distinct classes of flavouring substances: natural, *e.g.*, citral; natureidentical, *e.g.*, vanillin; and artificial, *e.g.*, ethyl vanillin. Some 2700 substances were identified and included in a European register following Commission Decision (EC) 1999/217/EC as amended. Then there are flavouring preparations, e.g. vanilla extract. Many flavourings are sold as a complex mixture of individual preparations and flavouring substances, generally confidential to the company that has produced the flavouring. Legislation has been designed to protect commercial confidentiality in registering on the EC list newly discovered flavouring substances. Because of the complexity of the flavouring used in a food, labels generally indicate simply "flavourings" in the ingredients list. This is all that is legally required, as to list every individual substance would often be extremely lengthy and virtually incomprehensible to the consumer, although the manufacturer may be more specific if he wishes. Any flavourings labelled as "natural" must meet the legal definition. The Food Standards Agency has issued criteria for the use of the term "natural" in product labelling.

The new proposal for an EC Regulation on flavourings and certain food ingredients with flavouring properties for use in and on foods means that in future there are likely to be stricter controls for the labelling of natural flavourings.

As with additives, some flavourings are sold direct to the consumer for domestic culinary use. Vanilla and peppermint are amongst the best known, as well as the popular brandy and rum essences. Anyone who has ever added too much flavouring to a home-made cake or a batch of peppermint creams will appreciate the minute quantities in which they are used. Similarly, in commercial manufacture, the quantity of flavouring used is extremely small in relation to that of other ingredients. Most flavourings are developed from substances naturally present in foods. Citrus and orange oils, for example, are amongst the most common natural source materials used in flavouring preparations and substances.

Other Additives

Colours and sweeteners are very specific, well-defined classes of additives and, because of the nature of their function, are subject to specific legislation. All other classes of additive now fall under the general heading of "miscellaneous". In addition to the larger groups mentioned above, there are other categories within this more general grouping – namely thickeners, acids, acidity regulators, anticaking agents, anti-foaming agents, bulking agents, carriers, glazing agents, humectants, raising agents and sequestrants.

The function of most of these is obvious from the name, with the possible exception of sequestrants. These are substances that form chemical complexes with metallic ions. They are not widely used and this is a class of additives rarely seen on a food label. Thickeners, on the other hand, are amongst the most commonly used additives, as they exert an effect on the texture and viscosity of food and drinks products. Much as various types of flour are used extensively in the kitchen to thicken sauces, soups, stews and other dishes with a high liquid content, most commercial thickeners are starch- or gum-based and serve much the same purpose.

One class of additive that has no domestic equivalent is that of packaging gases. These are the natural atmospheric gases now widely used in certain types of pre-packed products, such as meat, fish and seafood, fresh pastas and ready-prepared vegetables found on the chilled food counters in sealed containers. The "headspace" of the container is filled with one or a combination of the gases, depending on the product, to replace the air and modify the atmosphere within the pack to help retard bacteriological deterioration, which would occur under normal atmospheric conditions – hence the term "packaged in a protective atmosphere". Arguably, the gases do not have an additive function as they are not detectable in the food itself and function only to preserve the food for longer in its packaged state, but for regulatory purposes they were deemed to be additives and must therefore be labelled. Carbon dioxide will, of course, also be familiar as an ingredient in many fizzy drinks – an illustration of the many different functions and uses of additives.

Current EC legislation on additives does not cover the use of enzymes apart from invertase and lysozyme. However, in July 2006, the European Commission published a package of legislative proposals to introduce harmonised EU legislation on food enzymes for the first time and upgrade current rules for food flavourings and additives to bring them into line with the latest scientific and technological developments.

1.3 SAFETY AND TYPES OF ADDITIVES

The safety of all food additives, whether of natural origin or synthetically produced, is rigorously tested and periodically re-assessed. In the UK, the responsible authority is the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), a Government-appointed expert advisory committee, which provides advice to the Food Standards Agency, the Department of Health and other Government Departments and Agencies on matters concerning the toxicity of chemicals, including food additives. At European level, all additives approved for use in legislation have been evaluated by the Scientific Committee on Food (SCF) or, since May 2003, by its replacement the European Food Safety Authority (EFSA). Therefore, EFSA's Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC panel) is now responsible for the safety evaluation of new food additives.

Only additives evaluated in this way are given an "E" number; thus the "E" number is an indication of European safety approval, as well as a short code for the name of the additive. In evaluating an additive, EFSA allocates an "Acceptable Daily Intake" ADI), the amount of the substance that the panel considers may be safely consumed, daily, throughout a lifetime. This assessment is used to set the maximum amount of a particular additive (or chemically related group of additives) permitted in a specific food, either as a specified number of grams or milligrams per kilogram

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or litre of the food or, if the ADI is very high or "nonspecified", at *quantum satis*, *i.e.*, as much as is needed to achieve the required technological effect, according to Good Manufacturing Practice. In establishing the ADI, a safety factor is always built in, usually 100-fold, to ensure that intake of any additive is unlikely to exceed an amount that is anywhere near toxicologically harmful. To ensure that consumers are not exceeding the ADI by consuming too much of or too many products containing a particular additive, the EU legislation requires that intake studies be carried out to assess any changes in consumption patterns. The UK has carried out a number of intake surveys involving specific additives. None has culminated in results that have given cause for concern, except that in its 1994 survey of artificial sweeteners, consumption by some toddlers was considered to be excessive, given their high consumption of fruit squash. This potential problem was resolved by advice to add extra water to squash given to toddlers. It also raised questions about the establishment and application of the ADI, given that it is intended to cover changes in patterns of eating throughout a lifetime, from weaning to old age, but that is a separate scientific debate in itself.

At international level, there is a further level of evaluation of food additives, contaminants and residues of veterinary drugs in food by the Joint Expert Committee on Food Additives (JECFA), which advises the UN's Food and Agriculture Organization (FAO) and World Health Organization (WHO) Codex Alimentarius, which sets international standards. This has become increasingly important in recent years as World Trade Organisation (WTO) arrangements specify that Codex standards will apply in any dispute over sanitary and phytosanitary standards, *i.e.*, the safety and composition of foods. For this reason, the Codex General Standard for Food Additives (GSFA), was adopted to recommend usage levels of food additives in all products traded internationally.

As part of EFSA's role in the area of food additives, it is involved in the re-evaluation of all authorised food additives in the EU. In September 2004, EFSA issued an opinion on the safety of parabens (E214-219) used as preservatives in foods following a risk assessment of its use in foods. As a result, Directive 2006/52/EC amending Directive 95/2/EC on food additives other than colours and sweeteners and Directive 94/35/EC on sweeteners for use in foodstuffs, deleted the preservatives, E216 propyl phydroxybenzoate and E217 sodium propyl p-hydroxybenzoate from the list of permitted preservatives in Annex III.

In the area of sweeteners, the safety of aspartame was considered controversial, especially following a long-term study on its carcinogenicity in 2005. Hence, EFSA evaluated findings from this study, and, in this case, confirmed that there was no need to revise the previously established ADI. On the other hand, in re-evaluating the colour E128, Red 2G, in 2007, EFSA decided that there was a safety concern, and later the Commission suspended its use.

Intolerance

Additives have often been blamed for causing intolerance or allergic reactions, especially hyperactivity in children. Whilst there is no doubt that certain foods and food ingredients, including additives, are responsible for intolerance reactions, the prevalence of such reactions has often been greatly exaggerated. Genuine intolerance to food additives is extremely rare. It has been estimated that the true prevalence of intolerance to foods is about 2% in adults and up to 20% in children, and for food additives from 0.01 to 0.23%. The substantial overestimation of such reactions

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by the general public probably owes itself to the adverse media coverage and anti-additives campaigning of the 1980s, when popular belief was that additives were responsible for harmful behavioural effects and hyperactivity was attributed solely to the consumption of tartrazine (E102). The result was that tartrazine, an azo (synthetic) colour, was removed from a wide range of products, especially sweets and soft drinks that were likely to be consumed by children, as consumers in their droves ceased to buy anything that was labelled as containing it. Manufacturers are still reluctant to use this colour, unless there is nothing else in the palette of yellow colours authorised for the product. Such is the power of consumer choice, be it informed or otherwise. Food intolerance, and especially allergy, is again under the spotlight, not now because of alleged hyperactivity in children, but, far more seriously, because of the seemingly growing prevalence of severe allergic reactions, particularly to peanuts. Since the mid-1990s, there have been a number of widely reported incidents, including several tragic deaths as a result of anaphylactic shock, a severe allergic reaction to specific proteins, most commonly those found in tree nuts and peanuts and a small number of other foods, including milk, wheat, eggs, soya, fish and shellfish. The reasons for such reactions are not yet fully understood and are still under investigation, as are the causes of this apparently growing problem, but the need to address the issue and do everything possible to assist the small but significant number of people affected by this most severe form of allergy caused the European Commission to task its former Scientific Committee for Food (SCF) with identifying the scope of the problem and the foods and ingredients associated with it. This 1996 Report reaffirmed the SCF's earlier (1982) estimation of intolerance to additives as affecting from 0.01 to 0.02% of the European population (14). More specifically, the prevalence of intolerance to food additives in the population was put at 0.026%, or about 3 people per 10,000 of the population. This compares with the prevalence of adverse reactions to cows' milk of 1 to 3%. The most commonly observed reaction is now to sulphur dioxide (E220) and sulphites, especially in asthma sufferers, again growing in number or perhaps being more frequently reported. It must be understood that the incidence of genuine intolerance to additives is very low. Accurate labelling is the key to avoiding unnecessary suffering of an adverse reaction, such as urticaria, asthma or atopic symptoms, in the case of sensitised consumers, or adverse publicity in the case of food producers, and for this reason the EC Labelling Directive 2000/13/EC was amended in 2002, 2003, 2006 (to establish a list of potential allergens that must be declared by name on food labels) and in 2007.

Myths and Fallacies

Nothing is guaranteed to fill column inches and dominate the airwaves more than a good food scare. Additives have seen their share of these, though not on the scale of the 1996 BSE crisis or the more recent controversy over genetic modification: though equally long-running and bearing similarities to the latter issue, additives were never the butt of a concerted campaign by environmentalists and others dedicated to the downfall of a specific technology. Anti-additives campaigns would either target a specific additive or class of additives, for whatever reason, or cite the use of additives as part of a general thrust to disparage the modern food-manufacturing industry and seek to encourage a "back to basics" trend towards good old-fashioned home cooking and away from the purported less healthy foods produced by industrial processing for the UK's largely urbanised society.

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Hence the periodic targeting of preservatives, antioxidants, azo colours, sweeteners and monosodium glutamate. The evidence of such "scares" still abounds on the labels of countless products that claim to be free from "artificial" preservatives, colours and additives in general. This is indicative of the susceptibility of both marketing men and consumers to perceived adverse effects of particular additives. Such a response is unhelpful; whilst it is understandable that consumer concern in response to a media scare may result in a company removing an additive, or indeed any other ingredient, from a product for reasons of short-term expediency, the options and alternatives will inevitably become reduced every time something is removed from the range of ingredients, and the controversy left unresolved. It would be far better to address the issue through appropriate scientific investigation and seek to ensure that evidence of safety and absence of adverse effects are given at least some airing in the public domain to explode the myth engendered by the original controversy. This, of course, is not easy, as good news is, generally speaking, no news at all and certainly unlikely to make the headlines. The tabloid newspapers had a field day with the Food Commission's stories that "Cyclamates 'may cause testicular atrophy'" and "Aspartame 'may cause brain tumours'". Refuting such headlines is not easy; the full barrage of scientific evidence generally needs to be brought out in defence of any food ingredient or additive placed under the media spotlight and accused of causing some adverse effect. Often the "evidence" produced in support of the story needs to be pulled apart under the microscope and any deficiencies, such as in the research protocols or the way in which any experimentation was conducted, identified. The motivation for publishing such "research", and any exaggeration of the findings, also need to be examined.

All this takes time and will not protect any company using the additive or additives concerned from a barrage of enquiries from worried customers who, not unnaturally, seek reassurances that they have not already been harmed or will not be if they continue to consume the product. Again, a sense of proportion is important. The "problem" needs to be placed in context, given perspective against the wide range of risk factors to which all of us are exposed in daily life, and consumers assisted and encouraged to develop their own sense of risk assessment and risk management. This will become all the more important as communication becomes ever more global and instantaneous. The internet offers both threats and benefits: threats in that anyone can rapidly set off a scare by posting adverse information about, say, a specific sweetener. This may be a genuine concern that some possible risk to, perhaps, a certain sector of the population has been found, maybe to people suffering from a specific condition. It may also be that an unscrupulous company seeking to target that group with a new product decides to set off a scare shortly before launching its product, which is marketed as "free from" that additive or ingredient. The benefit lies in being able to expose such scares equally quickly, and the opportunity to post true and accurate information about food production for those who want to know.

Clean Labels

The growing demand from health-conscious consumers is for the replacement of artificial food additives with 'natural' ingredients, which perform similar technological functions. Thus, food processors are continuously seeking natural alternatives to food additives as, when these are listed on labels as the named ingredients rather by E-number, it gives the food product a 'clean label' declaration.

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Clean label declarations are not regulated; however, the Food Standards Agency in the UK has issued "Criteria for the use of the terms Fresh, Pure, Natural etc." which could be used as guidance. In addition, when incorporating new substances into foods one would also need to comply with the EC Regulation 258/97 concerning Novel Foods and Novel Food Ingredients. A number of ingredients are now being manufactured that claim to give foods a clean label status *e.g.*, emulsifiers such as lecithin and soya protein; antioxidants including grape seed, chestnut and olive leaf extracts; colours for example, lycopene, anthocyanin and chlorophyll; and preservatives including cinnamic acid, carvacol, chitosan, and lysozyme.

Some bacterial cultures, known as 'protective cultures', able to inhibit the growth of pathogenic bacteria and mycotoxin-producing mould are being used as inhibitors of foodborne microorganisms. These protective cultures produce antimicrobial metabolites like organic acids (lactic and acetic acid), and bacteriocins (nisin and natamycin), and are substitutes for conventional additives, helping manufacturers make the 'Clean Label' claim. It will be some time before we see a complete shift to clean label products, and in some situations this may not be possible due to a lack of suitable natural alternatives.

Conclusions

Much has happened to and in the food industry and the market for food since the great focus on additives in the 1980s. The popular books produced on the subject at that time focused largely on the potential adverse effects of additives; the potential misleading of consumers about the food they were eating; and the profitdriven nature of the industry motivated to use additives in their products. But not all of this criticism was without justification, and there were undoubtedly bad practices in place in some sectors of the industry, where unscrupulous traders saw opportunities for quick profit. The use of phosphates in reconstituted meat and fish products to make them appear as better-quality cuts and fillets or to add weight to a chicken was a dodge that trading standards officers rightly pursued with some zeal. This is not a criticism of the legitimate use of phosphates in meat products such as hams, but of the instances of false description of reconstituted products as prime cuts, and frozen "scampi" that disintegrated on defrosting. Any business will always have its unscrupulous operators, but strict regulation and enforcement now make this increasingly difficult in the food industry.

The 1990 Food Safety Act provided the framework of primary legislation for the food industry in the UK. The raft of legislation on food additives developed as part of the European Single Market, and explored in detail in a later chapter, strictly controls the use of all additives. The establishment of the Food Standards Agency, with its dual role of protecting and informing the consumer, may well influence both trends in the use of additives and public perceptions of their worth. Furthermore, the market has changed considerably in recent years, partly as a result of European integration and partly because consumers have become more sophisticated, more knowledgeable, and more affluent. Overseas travel has greatly broadened the British palate and increased demand for a wide range of exotic and adventurous foods that have been sampled overseas. Our increasingly cosmopolitan society has also led to the availability of more and more "ethnic" foods, both in restaurants and for domestic consumption, while busy lifestyles, and the increasing number of working women have led to more and more food being consumed outside the home. Never has the range and choice of foods been so great, in terms of availability

in the supermarkets and specialist food shops, or through the catering trade. This is not to say that additives are less widely used or less relevant – far from it. But those who wish to avoid them, either as manufacturers or consumers, should find it possible to do so, and those who do use them need have no concerns, except to obey the law in the case of manufacturers, and to understand the meaning of the ingredients list in the case of consumers. Astute consumers now notice that it is not only pre-packed foods that contain additives: foods sold "loose" at delicatessen counters are now also labelled to indicate the content of additives - or should be. And it has not escaped the notice of public health analysts that the greatest use of food colours is in ethnic restaurants. Public protection is ensured and additives cannot be used to deceive, but we would be deceiving ourselves if we thought that we could continue to enjoy the choice, ease and convenience of our food supply without them. Like them or not, they are a fact of life and their usefulness cannot be denied.

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SUMMARY

- Preservatives are probably the single most important class of additives, as they play an important role in the safety of the food supply.
- Antioxidants reduce the oxidative deterioration that leads to rancidity, loss of flavour, colour and nutritive value of foodstuffs.
- The purpose of emulsifiers and stabilisers is to facilitate the mixing together of ingredients that normally would not mix, namely fat and water.
- Flavourings are defined as imparting odour and/or taste to foods and are generally used in the form of mixtures of a number of flavouring preparations and defined chemical substances.
- Additives have often been blamed for causing intolerance or allergic reactions, especially hyperactivity in children.

REVIEW QUESTIONS

1. Discuss the need of food additives standardization of food product.
2. Discuss the concept of neutritional requirement according tom age, sex and working conditions.
3. Discuss safety and types of additives.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

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UNIT II: WHAT SHOULD BE DECLARED ON THE LABEL: ETHICAL AND NONETHICAL FOOD

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- discuss about the food safety act 1990.
- describe the trade descriptions Act 1968.
- know about the compound ingredients.
- define the exemptions from ingredient listing.

2.1 INTRODUCTION

The primary legislation that needs to be examined when looking at labelling of food additives is the Food Safety Act 1990, which stipulates that it is illegal to sell food that is 'injurious to health' or to falsely describe it. Similar provisions are set in the General Food Regulations 2004. Other legislation that also needs to be considered is the Trade Descriptions Act 1968, the Weights and Measures Act 1985, and the Food (Lot Marking) Regulations 1996. The main regulations that relate specifically to labelling of food additives are the Food Labelling Regulations 1996 (as amended) and the Food Additives Labelling Regulations 1992.

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This unit gives a basic outline of what should be on the label of a prepacked food, as specified in the Food Labelling Regulations. Details are given on what should be declared, including the name of the food, the list of ingredients, the appropriate durability indication, a quantitative ingredients declaration, storage conditions, place of origin and instructions for use. The requirements of the Food Additives Labelling Regulations are outlined and relevant areas that define food additives and prescribe requirements for labelling of food additives for business and consumer sale are highlighted. Please note that the actual legislation should be consulted when constructing or checking label copy.

2.2 FOOD SAFETY ACT 1990

It is worth noting that, although the Food Safety Act does not contain details of labelling requirements, it does set an overarching provision prohibiting labeling of food with a false description or a description that may mislead the consumer as to the nature, substance or quality of the food. The Act makes it an offence for anyone to sell, or possess for sale, food that:

- has been rendered injurious to health;
- is unfit or so contaminated that it would be unreasonable to expect it to be eaten;
- is falsely described, advertised or presented;
- is not of the nature, substance or quality demanded.

The General Food Regulations 2004 (S.I. 2004 No. 3279)

These regulations enforce certain provisions of Regulation (EC) No. 178/2002 laying down the general principles and requirements of food law.

- Article 14 specifies food safety requirements and prohibits the placing of unsafe food on the market.
- Article 16 states that the labelling/advertising/presentation of food should not mislead consumers.
- Article 18 states that the traceability of food/any other substance to be incorporated into a food shall be established at all stages of production, processing and distribution and sets obligations to food business operators.
- Article 19 places an obligation on food business operators to take responsibility and initiate the withdrawal of food if it does not comply with food safety requirements.

2.3 TRADE DESCRIPTIONS ACT 1968

The Trade Descriptions Act 1968 makes it an offence for a trader to:

- apply a false trade description to any goods;
- supply or offer to supply any goods to which a false trade description is applied.

Parts of the Trade Descriptions Act will soon be amended or repealed by virtue of the UK implementation of the Unfair Commercial Practices Directive 2005/29/EC. This Directive harmonises unfair trading laws in all EU member states and its provisions must have been applied in member states by December 2007. In the UK, it is

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expected that the regulation implementing this Directive, the Consumer Protection from Unfair Trading Regulations 2007, will come into force by April 2008.

Weights and Measures Act 1985

This Act provides for regulations to be drawn up on the expression of net quantityon prepacked food. The Act also provides for the 'average' system of quantity control for prepacked goods. Most foods and additives prepacked in quantities greater than 5 g or 5 ml need a quantity mark.

Food (Lot Marking) Regulations 1996 (S.I. 1996 No. 1502)

The aim of these Regulations is to establish a framework for a common batch or lot in order to facilitate the tracing and identification of product along the food chain.

Food Labelling Regulations 1996 (S.I. 1996 No. 1499 as amended by S.I. 1998 No. 1398, S.I. 1999 No. 747, S.I. 1999 No. 1483, S.I. 2003 No. 474, S.I. 2004 No. 1512, S.I. 2004 No. 2824, S.I. 2005 No. 899, S.I. 2005 No. 2057, S.I. 2005 No. 2969 and S.I. 2007 No. 3256.)

The general requirements laid down by the Food Labelling Regulations 1996 are set out below in more detail. The Regulations stipulate that all prepacked foods that will be supplied to the ultimate consumer or to a catering establishment must be labelled with:

Name of the food

If there is a name prescribed by law for a food it must be used as the name of the food. If there is no name, a customary name may be used. If there is neither a name prescribed by law nor a customary name, the name must inform the buyer of the true nature of the food and, if necessary, must include a description of use. Trademarks or brand names may be used but these may not substitute for the name of the food. If necessary, the name must include an indication of the physical condition of the food or any treatment that it has undergone.

List of ingredients

All labels must include a list of ingredients, and all ingredients in the food must be declared in the list, unless there are specific exemptions. The title 'Ingredients' must be contained in the heading for the list.

The names given to these ingredients must be the same as if they were being sold as a food. If the ingredient has been irradiated in any way, its name must be accompanied by the word 'irradiated' or the phrase 'treated with ionizing radiation'.

Ingredients must be listed in descending order of weight as used during the preparation of the food. The exception is water and volatiles, which should be listed in order of weight in the final product. Ingredients that are reconstituted during preparation may be included in the list of ingredients in the order of their weight before concentration or drying. However, if the food is a dehydrated or concentrated food, which will be reconstituted with water, then the ingredients may be listed as reconstituted.

If the food contains mixed fruit, vegetables, or mushrooms present in variable proportions but of similar weight, these ingredients may be grouped together in one

place in the list of ingredients by their designation of 'fruit', 'vegetables' or 'mushrooms' and labelled with the words 'in variable proportions' followed by a list naming all the fruit, vegetables or mushrooms present.

If the food contains a mixture of herbs and spices and these are in equal proportion, these ingredients may be listed in any order and labelled with the words 'in variable proportions'.

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Ingredients that constitute less than 2% of the finished product may be listed in a different order after the other ingredients. Similar or mutually substitutable ingredients and those used in the preparation of a food without altering its nature or its perceived value (excluding additives and allergenic ingredients) that make up less than 2% of the finished product may be referred to by the phrase 'contains... and/or...' where at least one of no more than two such ingredients is present in the finished product.

The Labelling Regulations include a list of permitted generic names that may be used to name an ingredient provided it meets the specified conditions, for example for oils the generic name 'vegetable oil' or 'animal oil' may be used rather than the specific source of the oil, provided an indication that the oil has been hydrogenated is given where appropriate. If water constitutes more than 5% of the finished product, it must be included on the ingredients list.

For a list of foods that are exempt from ingredients listing, see Appendix A. It is important to note that the allergen labelling requirements detailed later override these exemptions.

2.4 COMPOUND INGREDIENTS

If a compound ingredient (an ingredient composed of two or more ingredients, including additives) is used in the food, the names of the ingredients in the compound ingredient must be given in the list of ingredients either instead of the name of the compound ingredient or in addition to it.

If the name of the compound ingredient is given, its ingredients must immediately follow the name. The names of the ingredients of a compound ingredient do not need to be listed if the compound ingredient:

- is a food that if sold by itself would not require a list of ingredients;
- is an ingredient which is identified by a permitted generic name;
- constitutes less than 2% of the finished product and its composition is defined in Community legislation (e.g. that on chocolate, fruit juice, jam, fat spreads); or
- constitutes less than 2% of the finished product and consists of a mixture of spices and/or herbs.
- If they are exempt when the compound food is sold as such.

It is important to note that the allergen labelling requirements detailed later override these exemptions. If an ingredient of a compound ingredient has been irradiated, it must be listed and accompanied by the word 'irradiated' or words 'treated with ionizing radiation' except in the case of food prepared for patients needing sterile diets under medical supervision.

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2.5 ADDITIVES

Additives added to or used in a food to serve the function of one of the categories of additives listed below must be identified in the ingredients list by the name of the category followed by the specific name or serial number ('E number'). If an additive serves more than one function, it is only necessary to indicate the category that represents the principal function served by the additive in the food. If an additive serves none of these functions, it must be declared by its specific name in the ingredients list. The following list shows the categories of additives that must be identified in a list of ingredients by their category name (Schedule 4 Food Labelling Regulations).

Acid	Flour treatment agent
Acidity regulator	Gelling agent
Anti-caking agent	Glazing agent
Anti-foaming agent	Humectant
Antioxidant Modified	starch
Bulking agent	Preservative
Colour	Propellant gas
Emulsifier	Raising agent
Emulsifying Salts	Stabiliser
Firming agent	Sweetener
Flavour enhancer	Thickener

2.6 FLAVOURINGS

If a flavouring is added to or used in a food, it should be described in the ingredients list using the word 'flavouring' or, where more than one flavouring ingredient is used, the word 'flavourings'. A more specific name or description of the flavouring may be used.

Use of the Word 'Natural'

The word 'natural' or any word having substantially the same meaning, may be used for an ingredient being a flavouring only where the flavouring component of such an ingredient consists exclusively of:

- a flavouring substance (a defined chemical substance) that is obtained by physical (*e.g.*, distillation and solvent extraction), enzymatic or microbiological processes, from material of vegetable or animal origin, which is either raw or subjected only to a normal process used to prepare food for human consumption; or
- a flavouring preparation, i.e. other products, possibly concentrates, obtained by physical, enzymatic or microbiological processes from material of vegetable or animal origin.

Processes normally used in preparing food for human consumption include drying and fermentation. If the name of the flavouring refers to the vegetable or animal nature or origin of the material contained in it, 'natural' or similar words, may be used only if the flavouring components have been isolated solely or almost solely from that vegetable or animal source. The proposed regulation on flavourings and

certain food ingredients with flavouring properties for use in and on foods sets tighter controls for the use of the term 'natural' in the labelling of flavourings which is discussed later in this unit.

2.7 SWEETENERS

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Foods that contain sweeteners must be labelled with the indication 'with sweetener(s)', and those that contain sugars and sweeteners with the indication 'with sugar(s) and sweetener(s)'. These statements must accompany the product name.

Foods that contain aspartame must be labelled with the words 'contains a source of phenylalanine'. Foods that contain more than 10% added polyols must carry the indication 'excessive consumption may produce laxative effects'.

2.8 EXEMPTIONS FROM INGREDIENT LISTING

Ingredients which need not be named:

1. constituents of an ingredient which have become temporarily separated during the manufacturing process and are later re-introduced in their original proportions
2. any additive whose presence in the food is due only to the fact that it was contained in an ingredient of the food, provided it does not serve any significant technological function in the finished product
3. any additive that is used solely as a processing aid
4. any substance other than water that is used as a solvent or carrier for an additive and is used in an amount that is no more than that which is strictly necessary for that purpose.
5. Any substance which is not an additive but which is used in the same way and for the same purpose as a processing aid.

However, the allergen labelling requirements detailed later override the above exemptions.

Appropriate Durability Indication

All foods must be date marked unless specifically exempt from this requirement (see Appendix B for list of exemptions). Highly perishable foods with the potential to endanger human health must be labelled with a 'use by' date, for other foods a minimum durability date must be given.

The date and any storage conditions that need to be observed may be placed apart from the 'best before' or 'use by', as long as there is a reference to the place where the date appears, *e.g.*, 'best before end - see lid'.

Labelling of Minimum Durability

The words 'best before' must be used to indicate the minimum durability. It must be followed by the date, shown as the day, month and year. For foods that will keep for 3 months or less, the label may state 'best before' with the day and the month only. Foods that will keep for more than 3 months but not more than 18 months may be labelled with 'best before end' with the month and the year only. For foods that will last longer than 18 months, the label may state 'best before end' plus month

NOTES

and year only, or year only. Labelling of 'use by' date If 'use by' is required, it must be followed by the day and month or the day, month and year in that order.

Quantitative Ingredients Declaration (QUID)

The aim of QUID labelling is to help consumers differentiate between similar products and so be able to make a more informed choice. It is required that the quantity of an ingredient or category of ingredients used in the manufacture or preparation of a foodstuff is declared where the ingredient:

1. appears in the name under which the food is sold, or is usually associated with that name by the consumer; or
2. is emphasised in the labelling, either by words or by the use of pictorial representations; or
3. is essential to characterise the food and to distinguish it from products with which it could be confused because of its name or appearance; or
4. in other cases, as determined.

QUID is not required if:

1. the drained net weight of the food is indicated;
2. the quantity of the ingredient is already required to be given;
3. the ingredient is used in small quantities for the purpose of flavouring;
4. the name of the ingredient appears in the name under which the food is sold, but where the variation in its quantity does not distinguish the food from similar products.

The quantity must be expressed as a percentage and must correspond to the quantity of the ingredient at the time of use. The declaration must appear either in or immediately next to the name under which the food is sold or in an appropriate place in the list of ingredients.

QUID declarations are not triggered by:

- 'with sweeteners' or 'with sugars and sweeteners', in the name of the food;
- references to vitamins and minerals, as long as these are indicated in nutrition labelling.

QUID Calculations

QUID declarations should be calculated on the finished product for foodstuffs that have lost moisture following heat treatment or other treatment. If the resultant % exceeds 100%, then it is to be replaced by the weight of ingredient used in the preparation of 100 g of the finished product.

In the case of volatile ingredients, QUID should be calculated on the finished product. Dehydrated or concentrated ingredients, which are reconstituted during manufacture, may be declared on the basis of ingredient weight prior to concentration or dehydration. Alternatively, for concentrated or dehydrated foods that are intended to be reconstituted with water, QUID may be given on the basis of the reconstituted product.

Storage conditions: Any special storage conditions or conditions of use need to be included on the label.

Name and address: The business name and address of the manufacturer or packer, and/or seller established within the European Community needs to be included on the label.

Place of origin: Details of the place of origin of the food must be given if the failure to provide such information would mislead as to the true origin of the food.

Instructions for use: These need to be included if it would be difficult to use the food without instructions.

Additional labelling: There are additional labelling requirements for the following types of food:

NOTES

- Food sold from vending machines
- Prepacked alcoholic drinks
- Raw milk
- Products that contain skimmed milk with non-milk fat
- Foods packaged in certain gases.

Certain foods with compositional standards (*e.g.*, jams, chocolate, infant formulae) also have additional labelling requirements specified within the appropriate compositional regulation. There are labelling requirements for some cheese varieties, cream types, milk, ice cream and indication of specific flavours, which aim to prevent misleading descriptions set in Schedule 8 to the Food Labelling Regulations.

Allergens

EC Directive 2000/13/EC as amended states that foods containing allergenic ingredients or ingredients originating from an allergenic ingredient listed below must be marked with a declaration of these ingredients in the ingredients list, unless they have already been mentioned in the product name. This means that even if an ingredient meets the criteria for which it wouldn't usually need to be declared in an ingredients list, or could have been declared by a generic name, if it contains an allergenic ingredient or originated from one, the ingredient must be declared.

The current list of allergens is:

- Cereals containing gluten: wheat, rye, barley, oats, spelt, kamut and their hybridised strains
- Crustaceans
- Eggs
- Fish
- Peanuts
- Soybeans
- Milk
- Nuts - specific varieties
- Celery
- Mustard
- Sesame seeds
- Sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/l, expressed as SO_2.
- Lupin
- Molluscs.

Lupin and molluscs were added to the list of allergens under Commission Directive 2006/142/EC and member states were required to transpose this Directive into national legislation by 23 December 2007. For example, in the UK, this Directive is implemented through the Food Labelling (Declaration of Allergens) (England) Regulations 2007.

All products must comply with the requirements of this Directive by 23 December 2008. Directive 2007/68/EC (amending Annex IIIa to Directive 2000/13/EC) has been published following a review by EFSA of dossiers submitted for highly processed ingredients derived from the allergens listed in Annex IIIa, to allow exemption from labelling with reference to the allergen. The exemptions are set out below:

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(*a*) wheat-based glucose syrups including dextrose;
(*b*) wheat-based maltodextrins;
(*c*) glucose syrups based on barley;
(*d*) cereals used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages;
(*e*) fish gelatine used as carrier for vitamin or carotenoid preparations;
(*f*) fish gelatine or Isinglass used as fining agent in beer and wine;
(*g*) fully refined soybean oil and fat;
(*h*) natural mixed tocopherols (E306), natural D-alpha tocopherol, natural Dalpha tocopherol acetate, natural D-alpha tocopherol succinate from soybean sources;
(*i*) vegetable oils derived phytosterols and phytosterol esters from soybean sources;
(*j*) plant stanol ester produced from vegetable oil sterols from soybean sources;
(*k*) whey used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages;
(*l*) lactitol;
(*m*) nuts used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages.

And products thereof, insofar as the process that they have undergone is not likely to increase the level of allergenicity assessed by the EFSA for the relevant product from which they originated.

Alcoholic drinks which have an alcoholic strength by volume of more than 1.2% and contain any allergenic ingredient listed need to be labelled with the word 'contains' followed by the name of the allergenic ingredient.

Prescribed Nutrition Labelling

When a claim is made and/or food is fortified, prescribed nutrition labelling is triggered; otherwise nutritional labelling is voluntary. The only exceptions are natural mineral waters and food supplements, which are exempt from prescribed nutrition labelling as set in the Food Labelling Regulations but are subject to product specific controls. There are several different criteria for nutrition labelling, depending on the type of claim being made.

Prescribed nutrition labelling must include either Group 1 (*a*) or Group 2 (*b*):

(*a*) energy and the amounts of protein, carbohydrate and fat; or
(*b*) energy and the amounts of protein, carbohydrate, sugars, fat, saturates, fibre and sodium (this format should be used if a claim is being made for sugars, saturates, fibre or sodium).

Where a nutrition claim is made for polyols, starch, monounsaturates, polyunsaturates, cholesterol, vitamins or minerals, the amount/s must be included in the prescribed nutrition labelling. Where no claim is made, these nutrients may be optionally included.

The nutrients need to be listed in the following order and in the same style:

energy	[*x*] kJ and [*x*] kcal
protein	[*x*] g
carbohydrate	[*x*] g
of which:	

• sugars	[x] g
• polyols	[x] g
• starch	[x] g
fat	
of which:	
• saturates	[x] g
• monounsaturates	[x] g
• polyunsaturates	[x] g
• cholesterol	[x] mg
fibre	[x] g
sodium	[x] g
[vitamins]	[x units]
[minerals]	[x units]

NOTES

Where monounsaturates and/or polyunsaturates are included, saturates must also be included. All amounts must be expressed per 100 g or 100 ml of the food. In addition, they may be given per quantified serving of food or per portion of food. The use of nutrition claims is controlled through Regulation (EC) No.

1924/2006 on nutrition and health claims made on foods which was applied from 1 July 2007 and is directly applicable in England through the Nutrition and Health Claims Regulations 2007. Making a claim is voluntary and the regulation details this information to include conditions for their use. Any foods which do not meet the requirements stated in the regulations would be subject to transitional measures. A health claim triggers group 2 nutrition declaration and other additional labelling requirements.

As well as this, Regulation (EC) 1925/2006 on the Addition of Vitamins and Minerals and of certain other substances to foods requires that foods to which vitamins and minerals have been added (covered by Regulation (EC) 1925/2006) must contain nutrition labelling and be of the Group 2 format as described previously in this section.

At time of publication, EU food labelling legislation is being reviewed by the European Commission; it is likely that the current food labelling directive will be superseded by a directly applicable regulation.

The Commission published a draft proposal for a Regulation on the provision of food information to consumers at the end of 2007. The draft proposal includes a new requirement to provide mandatory nutrition labelling for the energy value and the amount of fats, saturated fats, sugars and salt, and requires this information to be given in the principal field of vision of a food label and in this order. It is anticipated that this regulation will be adopted by the European Parliament and Council by 2010.

The national provisions of the Food Labelling Regulations 1996 (as amended) are being reviewed by the Food Standards Agency with the intention of either removing or seeking to retain specific provisions for inclusion at European level.

Food Additives Labelling Regulations 1992 (S.I. 1992 No. 1978)

These Regulations relate to business and consumer sales of food additives sold as such. They define food additives, list excluded substances including processing

NOTES

aids, a definition of which is given, and prescribe requirements for labelling. These Regulations do not apply to:

(*a*) Processing aids

(*b*) Substances used in the protection of plants and plant products.

(*c*) Flavourings within the meaning of the Flavourings in Food Regulations 1992.

(*d*) Substances added to foods as nutrients.

Definition

A food additive must fall within a category or categories listed below:

- Colours
- Preservatives
- Emulsifying salts
- Gelling agents
- Flavour enhancers
- Acidity regulators
- Modified starch
- Raising agents
- Glazing agents.
- Antioxidants
- Emulsifiers
- Thickeners
- Stabilisers
- Acids
- Anti-caking agents
- Sweeteners
- Anti-foaming agents

Flour bleaching agents: Any substance primarily used to remove colour from flour.

Flour treatment agents: Any substance that is added to flour or dough to improve its baking quality:

- Firming agents
- Humectants.

Enzyme preparations: Any substance that contains a protein capable of catalyzing a specific chemical reaction:

- Sequestrants
- Propellants
- Carriers and carrier solvents.
- Bulking agents
- Packaging gas

An additive is normally neither consumed as a food in itself or used as a characteristic ingredient of food, whether or not it has nutritive value, and is intentionally added to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of that food, and results, or could result, in it or its by-products becoming directly or indirectly a component of the food.

A processing aid is defined as a substance that is not consumed as a food ingredient by itself; is intentionally used in the processing of raw materials, foods or their ingredients, to fulfil technological purposes during treatment or processing; and is capable of resulting in the unintended but technically unavoidable presence of its residues or its derivatives in the finished product, and the residues of which do not present any risk to human health and do not have any technological effect on finished products.

Labelling requirements for business sale of food additives

The container of the food additive must bear the information listed under 1 or 2 below, and it must be clearly legible, conspicuous and indelible.

(*a*) The label must have the correct EC name and number, or in the absence of such name, a description of the food additive that will distinguish it from any other that it could be confused with. If there is more than one food additive

NOTES

present, the information must be given in descending order of the proportion by weight.

(*b*) If there is any supplementary material, (substances to facilitate storage, sale, standardisation, dilution or dissolution of a food additive), each component of the supplementary material must be labelled in descending order of the proportion by weight of the components.

(*c*) The label must state that the food additives are 'for use in food' or 'restricted use in food' or a more specific reference to its intended food use.

(*d*) If there are any special storage conditions for the food additive, or if there are any special conditions of use, this needs to be labelled.

(*e*) Instructions for the use of the food additive must be given if it would be difficult to use the food additive without them.

(*f*) An identifying batch or lot mark.

(*g*) The name and address of the manufacturer or packer, or EC seller of the food additive must be stated on the label.

(*h*) If it is prohibited to exceed a specified quantity of the food additive in a food, the percentage of each component of the food additive must be stated.

Alternatively, enough information must be given to enable the purchaser to decide whether, and to what level, he could use such food additives in food sold by him. Or The label needs to include 1(*a*), (*c*), (*d*) and (*e*) (above) and in an obvious place the words 'intended for manufacture of foodstuffs and not for retail sale'. Relevant trade documents must be supplied to the purchaser and must include the remainder of the information given in section 1(*b*), (*f*), (*g*) and (*h*).

Labelling Requirements for Consumer Sale of Food Additives

The container must bear the following information, which must be clearly legible, conspicuous and indelible.

(*a*) The name of the product. A description of food additives specified in Community provisions and the EC number. If there is no EC name or EC number, a description must be given to identify it from any other product with which it may be confused.

(*b*) In addition, the label must include all the information stated in section 1. (*a*)–(*g*) in 'Labelling requirements for business sale', above.

(*c*) The minimum durability of the product must be stated.

Exemption: These regulations do not apply to any food additive that is part of another food.

Additive Numbers

Where the serial number of the additive is to be given in the ingredients list:

- The number used should be one that appears in the column headed 'EC No.' in the relevant schedule (*e.g.*, E150b, E420).

Additive Names

Where the specific name of the additive is to be given in the ingredients list:

- The name used should be one that appears in the column headed 'Colour' or 'Permitted sweetener' or 'Name' in the relevant schedule (*e.g.*, Cochineal, Aspartame).

NOTES

- A summary name that appears in the column headed 'Colour' or 'Permitted sweetener' or 'Name' in the relevant schedule may be used in place of a more specific name, provided that the latter does not have its own serial number (*e.g.*, carotene may be used for 'mixed carotenes', 'sorbitol' may be used for 'sorbitol syrup').
- If the name in the column headed 'Colour' or 'Permitted sweetener' or 'Name' in the relevant schedule is preceded by a bracketed letter or Roman numeral (*e.g.*, (*ii*) Beta carotene), this need not be given as part of the name.
- In the case of miscellaneous additives, where an alternative to the specific name is given in brackets in the column headed 'Name' in the relevant schedule, this may be used in place of the specific name (*e.g.*, 'polysorbate 20' instead of 'polyoxyethylene sorbitan monolaurate').
- In the case of miscellaneous additives being phosphates, the names, 'diphosphates', 'triphosphates' and 'polyphosphates' are acceptable as specific names for the phosphates covered by the serial numbers E450, E451 and E452, respectively. They should not be used for the phosphates covered by serial numbers E338, E339, E340 and E341.
- Synonyms or acronyms that are not included in the relevant schedule should not be used as alternatives to the specific name.

What Should be Declared on the Label

Relevant schedules

Schedule 1 to the Sweeteners in Food Regulations 1995 as amended by the Sweeteners in Food (Amendment) Regulations 1997

Schedule 1 to the Colours in Food Regulations 1995

Schedules 1, 2, 3, and 4 of the Miscellaneous Food Additives Regulations 1995, as amended.

Flavourings in Food Regulations 1992 (S.I. 1992 No. 1971, as Amended by S.I. 1994 No. 1486)

Definition

A flavouring is a material used or intended for use in or on food to impart odour, taste or both.

Labelling requirements for business sale of relevant flavourings

The container must be labelled with the following information:

(*a*) The name and business name and address of the manufacturer or the packer, or of the EC seller.

(*b*) The word 'flavouring' or more specific names or descriptions of the relevant flavourings.

(*c*) The words 'for foodstuffs' or a more specific reference to the food for which the relevant flavouring is intended.

(*d*) A list, in descending order of weight, using the following classifications:

- 'natural flavouring substances' – for flavouring substances obtained by physical, enzymatic or microbiological processes from appropriate material of vegetable or animal origin;

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- 'flavouring substances identical to natural substances' – for flavouring substances obtained from chemical synthesis or isolated by chemical processes and chemically identical to a substance naturally present in appropriate material of vegetable or animal origin;
- 'artifical flavouring substances' – for flavouring substances obtained by chemical synthesis;
- 'flavouring preparations' – for flavouring preparations;
- 'process flavourings' – for process flavourings;
- 'smoke flavourings' – for smoke flavourings.

In the case of other substances or materials, their names or EC numbers.

(e) The quantity of any material in or on the relevant flavourings where the sale of food containing excess of such quantity would be prohibited by the Food Safety Act.

The information must be visible, legible, and indelible, and must be expressed interms easily understood by the purchaser.

2.9 USE OF THE WORD 'NATURAL'

The word 'natural' or any similar word may not be used to describe the relevant flavouring unless it is used in compliance with the labelling requirements above; or the flavouring components of the relevant flavouring comprise flavouring substances obtained by physical, enzymatic or microbiological processes from appropriate material of vegetable or animal origin or flavouring preparations or both.

The word 'natural' or any similar word shall not be used to qualify any substance used in its preparation unless the relevant flavouring is a permitted flavouring, the flavouring component of which has been isolated solely, or almost solely, from that substance by physical processes, enzymatic or microbiological processes, or processes normally used in preparing food for human consumption. The conditions governing the use of the word 'natural flavouring' in labelling will be amended by the proposed regulation on flavourings and certain food ingredients with flavouring properties for use in and on foods as discussed later in this chapter. According to this proposed regulation, the term 'natural' may only be used for the description of flavouring if the flavouring component comprises only flavouring preparations and/or natural flavouring substances. A 'natural flavouring substance' shall mean a flavouring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes as listed in the regulation. They are:

- Chopping
- Coating
- Cooking, baking, frying (up to 240 °C)
- Cooling
- Cutting
- Distillation/rectification
- Drying
- Emulsification
- Evaporation
- Extraction, including solvent extraction
- Fermentation
- Filtration
- Grinding
- Heating
- Infusion
- Maceration

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- Microbiological processes
- Peeling
- Pressing
- Roasting/grilling
- Steeping.
- Mixing
- Percolation
- Refrigeration/freezing
- Squeezing

For 'flavouring preparation' it is natural under the conditions that it is a product, other than a flavouring substance which is obtained from food by appropriate physical, enzymatic or microbiological processes either in the raw state of the material or after processing for human consumption by one or more of the traditional food preparation processes listed above and/or appropriate physical processes.The term 'natural' may only be used in combination with a reference to a food, food category or a vegetable or animal flavouring source, if at least 95% (by w/w) of the flavouring component has been obtained from the source material referred to.

The flavouring component may contain flavouring preparations and/or natural flavouring substances.

Labelling requirements for consumer sale of relevant flavourings

The container must include the following information:

(*a*) The name and business name and address of the manufacturer or the packer, or of the EC seller.

(*b*) The word 'flavouring' or more specific names or descriptions of the relevant flavourings.

(*c*) The words 'for foodstuffs' or a more specific reference to the food for which the relevant flavouring is intended.

(*d*) An indication of minimum durability.

(*e*) Any special storage conditions or conditions of use.

(*f*) Instructions for use, where omission would prevent appropriate use of the flavouring.

(*g*) Where the relevant flavouring contains other substances or materials, a list in descending order of weight:

- in respect of components of the relevant flavouring, the word 'flavouring' or more specific names or descriptions of the relevant flavourings;
- in respect of each other substance or material, its name or, where appropriate, its E number.

The information must be visible, legible and indelible, and must be expressed in terms easily understood by the purchaser.

Sale of food containing flavourings:

Generally no food shall be sold which has in it or on it any added relevant flavouring other than a permitted flavouring (complying with general purity criteria).

Relevant schedule

Schedule 1: General purity criteria applicable to permitted flavourings. Likewise no food sold which has in it or on it any relevant flavouring shall have in it or on it any specified substance which has been added as such. These specified substances may be present in a food either naturally or as a result of the inclusion of the relevant flavouring which has been made from natural raw materials.

The presence of the specified substance in foods that are ready for consumption should not exceed specified limits.

Relevant schedule

Schedule 2: Specified substances.

Smoke Flavourings Regulations 2005 (S.I. 2005 No. 464)

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Definition

'Smoke flavouring' means a smoke extract used in traditional foodstuffs smoking processes.The following definitions are also given:

- 'primary smoke condensate' shall refer to the purified water-based part of condensed smoke and shall fall within the definition of 'smoke flavourings';
- 'primary tar fraction' shall refer to the purified fraction of the water-insoluble high-density tar phase of condensed smoke and shall fall within the definition of 'smoke flavourings';
- 'primary products' shall refer to primary smoke condensates and primary tar fractions;
- 'derived smoke flavourings' shall refer to flavourings produced as a result of the further processing of primary products and which are used or intended to be used in or on foods in order to impart smoke flavour to those foods.

Smoke flavourings need to be indicated as such, see the previous section on Flavourings in food.

Colours in Food Regulations 1995 (S.I. 1995 No. 3124, as amended by S.I. 2000 No. 481, S.I. 2001 No. 3442, S.I. 2005 No. 519 and S.I. 2007 No. 453)

Definition

A food colour is a food additive used or intended to be used primarily for adding or restoring colour to a food. This includes:

- any natural constituent of food and any natural source not normally consumed as food as such and not normally used as a food ingredient; and
- any preparation obtained from food or any other natural source material by physical and/or chemical extraction resulting in selective extraction of the pigment relative to the nutritive or aromatic constituent.

For labelling of colours in foods see the Food Labelling Regulations – Additives section. See section on Food Additives Labelling Regulations, for business/consumer sale of food additives.

Sweeteners in Food Regulations 1995 (S.I. 1995 No. 3123, as amended by S.I. 1996 No. 1477, S.I. 1997 No. 814, S.I. 1999 No. 982, S.I. 2001 No. 2294, S.I. 2002 No. 379, S.I. 2003 No. 1182, S.I. 2004 No. 3348 and S.I. 2007 No. 1778)

Definition

A sweetener is a food additive used or intended to be used to impart a sweet taste to food, or as a table-top sweetener. For labelling of sweeteners, see sections on:

(*a*) Food Additives Labelling Regulations, for business/consumer sale of food additives.

(*b*) The Food Labelling Regulations – both Additives and Sweeteners.

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Miscellaneous Food Additives Regulations 1995 (S.I. 1995 No. 3187, as amended by S.I. 1997 No. 1413, S.I. 1999 No. 1136, S.I. 2001 No. 60, S.I. 2001 No. 3775, S.I. 2003 No. 1008, S.I. 2003 No. 3295, S.I. 2004 No. 2601, S.I. 2005 No. 1099 and S.I. 2007 No. 1778)

Definition

The term 'miscellaneous additive' refers to any food additive that is used or intended to be used primarily as an acid, acidity regulator, anti-caking agent, antifoaming agent, antioxidant, bulking agent, carrier, carrier solvent, emulsifier, emulsifying salt, firming agent, flavour enhancer, flour treatment agent, foaming agent, gelling agent, glazing agent, humectant, modified starch, packaging gas, preservative, propellant, raising agent, sequestrant, stabiliser or thickener; but does not include use as a processing aid or any enzyme except invertase or lysozyme.

Relevant schedules

The Regulations contain nine schedules:

Schedule 1: Miscellaneous additives generally permitted for use in foods

Schedule 2: Conditionally permitted preservatives and antioxidants

Schedule 3: Other permitted miscellaneous additives

Schedule 4: Permitted carriers and carrier solvents

Schedule 5: Purity criteria

Schedule 6: Foods in which miscellaneous additives listed in Schedule 1 are generally prohibited

Schedule 7: Foods in which a limited number of miscellaneous additives listed in Schedule 1 may be used

Schedule 8: Miscellaneous additives permitted in foods for infants and young children

Schedule 9: Revocations.

The Regulations function in the form of positives lists, as detailed in the schedules above.

Extraction Solvents in Food Regulations 1993 (S.I. 1993 No. 1658, as amended by S.I. 1995 No. 1440 and S.I. 1998 No. 2257)

Definition

An extraction solvent is any solvent used or intended to be used in an extraction procedure, including, in any particular case further to its use in such a procedure, any substance other than such a solvent derived exclusively from such a solvent. See Appendix C for the list of Permitted Extraction Solvents.

Labelling of permitted extraction solvents sold as such

The following information must be provided with any of the extraction solvents listed in Appendix C.

(*a*) The name of the permitted extraction solvent that is stated in the list of Permitted Extraction Solvents.

(*b*) A clear statement that the permitted extraction solvent is of suitable quality for use in an extraction procedure.

(c) An identifying batch or lot mark.

(d) The name or business name and address of the manufacturer or packer, or of an established EC seller.

(e) The net quantity or volume, in metric units, of the permitted extraction solvent in any container or other packaging in which it is to be sold or imported.

(f) Any special storage conditions or conditions of use.

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The information must be easily visible, clearly legible and indelible. The information must be given on the packaging, container or label of the permitted extraction solvent to which it relates; alternatively, statements c - f may be specified on relevant trade documents that accompany or precede the delivery.

The quantity may also be accompanied with other units of measurement, provided the metric indication is predominant and expressed in characters that are no smaller than the other units.

The Genetically Modified Food (England) Regulations 2004 (S.I. 2004 No. 2335)

This Regulation makes provisions for the enforcement of EC Regulation No. 1829/2003 on genetically modified food and feed which harmonises procedures for the scientific assessment and authorisation of genetically modified organisms (GMOs) and genetically modified food and feed and lays down labeling requirements.

The EC Regulation applies to the whole of the UK although the S.I. 2004 No. 2335 applies only in England. Similar legislation has been made in Scotland, Wales and Northern Ireland as follows:

- The Genetically Modified Food Regulations (Northern Ireland) 2004 (Statutory Rule 2004 No. 385)
- The Genetically Modified Food (Scotland) Regulations 2004 (Scottish Statutory Instrument 2004 No. 432)
- The Genetically Modified Food (Wales) Regulations 2004 No. 3220 (W.276)

The Genetically Modified and Novel Foods (Labelling) (England) Regulations 2000 have been revoked. The Food Standards Agency was designated as the national competent authority to receive applications for the authorisation of:

- new genetically modified organisms for food use
- food containing or consisting of GMOs or
- food produced from or containing ingredients produced from GMOs.

The Genetically Modified food (England) Regulations sets labelling requirements for:

- food containing or consisting of genetically modified organisms (GMOs) or
- food produced from or containing ingredients produced from GMOs.

The labelling requirements apply regardless of whether or not the final product contains DNA or protein resulting from genetic modification. The labelling requirements of this regulation do not apply to foods containing material which contains, consists of or is produced from GMOs that have an EU authorisation in a proportion <0.9% of the food ingredients, where the presence is adventitious or technically unavoidable. This unintentional presence is subject to the operator being able to supply evidence to satisfy the competent authorities that they have taken appropriate steps to avoid the presence of such material.

GM material that has not been authorised in the EU cannot be present at any level in food products. The Regulation requires that:

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- If the food consists of more than one ingredient, the words 'genetically modified' or 'produced from genetically modified X', are to appear in the ingredient list in parentheses immediately after the ingredient name (or in a prominent footnote linked to indicate this) in the ingredients list.
- If the ingredient is designated by a category name, the words 'contains genetically modified Y', or 'contains X produced from genetically modified Y', are to appear in the ingredients list.
- For a food without an ingredient list, the words 'genetically modified' or 'produced from genetically modified Y' are to appear on the label. where, X = name of ingredient, where Y = name of organism In the case of non-prepackaged products or pre-packaged food in small containers of which the largest surface has an area of less than 10 cm^2 the information required under this paragraph must be permanently and visibly displayed either on the food dis-play or immediately next to it, or on the packaging material, in a font sufficiently large for it to be easily identified and read. In addition to the labelling requirements given above, the labeling should also mention any characteristic or property, as specified in the authorisation where a food or ingredient has changed in respect to its composition, nutritional value/nutritional effects, intended use, implications for the health of certain sections of the population as well as any ethical/religious concerns.

Also, genetically modified food must not:

- have adverse effects on human health, animal health or the environment
- mislead the consumer
- differ from the food which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for the consumer.

Note: Legally there are no controls for 'GM free' labelling for food ingredients other than rules on misleading claims. Consumers should check with the company/retailer as to the criteria that are being employed in using the term.

The Genetically Modified Organisms (Traceability and Labelling) (England) Regulations 2004 (S. I. 2004 No. 2412)

This Regulation provides for the enforcement in England of EC Regulation No. 1830/2003 on the traceability and labelling of GMOs and GM food and feed. It requires the identification of GM products throughout the supply chain in order to facilitate accurate labelling in accordance with Regulation (EC) 1829/2003 for:

- food consisting or containing GMOs
- food produced from GMOs.

Unique identifier codes on GMOs can be found in a register and are used in traceability documentation. These codes must be used for the traceability of products consisting of or containing GMOs (*e.g.*, maize) but not of foods produced from GMOs (*e.g.*, maize gluten). The EC Regulation applies to the whole of the UK and similar legisl n has been made in Scotland, Wales and Northern Ireland.

Package of Proposals for New Legislation on Food Additives, Flavourings and Enzymes

In July 2006, The European Commission published a package of legislative proposals to introduce harmonised EU legislation on food enzymes for the first time and

upgrade current rules for food flavourings and additives to bring them into line with the latest scientific and technological developments. The proposals were amended in October 2007. The package includes four proposals on food improvement agents as follows:

(*a*) Establishing a common authorisation procedure for food additives, food enzymes and food flavourings

(*b*) Food additives

(*c*) Food enzymes

(*d*) Flavourings and certain food ingredients with flavouring properties for use in and on foods.

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The safety of additives, enzymes and flavourings used in foodstuffs for human consumption must be assessed before they can be placed on the community market. Currently, the general criteria for the use of food additives is given in the Framework Directive 89/107/EEC concerning food additives authorised for use in foodstuffs intended for human consumption, which is discussed further in the next chapter. The authorisation procedure for a food additive at Community level currently involves a two-step procedure. Therefore, firstly the additive is included in the relevant Directive, and then the Commission would adopt a specification for that additive after this is agreed by the Standing Committee on the Food Chain and Animal Health.

The proposed Regulation lays down a common assessment and authorisation procedure for food additives, food enzymes, food flavourings and sources of food flavourings used or intended for use in or on foodstuffs. Under Regulation (EC) 178/2002 laying down procedures in matters of food safety, the placing of substances on the market must be authorised only after an independent scientific assessment by the European Food Safety Authority of the risks that they pose to human health. This is followed by a risk management decision taken by the Commission. Food additives, food enzymes, food and flavourings must be included in the positive list for each respective regulation in order for them to be marketed for human consumption. These positive lists will be created, maintained and published by the Commission. Currently, food additives are governed by the following:

- Council Directive 89/107/EEC of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorised for use in foodstuffs intended for human consumption. Official Journal of the European Communities L40, 11.02.89,27- 33, as amended.
- European Parliament and Council Directive 94/35/EC of 30 June 1994 on sweeteners for use in foodstuffs. Official Journal of the European Communities. L237, 10.9.94, 3-12, as last amended.
- European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. Official Journal of the European Communities. L237, 10.9.94, 13-29.
- European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. Official Journal of the European Communities. L61, 18.3.95, 1-40, as last amended.
- Decision No 292/97/EC of the European Parliament and of the Council of 19 December 1997 on the maintenance of national laws prohibiting the use of certain additives in the production of certain specific foodstuffs.

At present, the authorisation of a food additive at community level is based on a co-decision procedure. If the new proposal is adopted, the provisions on additives

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in the different existing Directives will be brought together in one regulation. This single Regulation will harmonise the use of food additives in foods in the Community.

The regulation will include the principles for the use of food additives, and a positive list of approved food additives, as well as covering the use of food additives in food additives and food enzymes, and carriers for nutrients. The regulation will be based on a comitology approach since the package was adopted around the time of entry into force of Decision 2006/512/EC, amending Decision 1999/468/EC laying down the procedures for the exercise of implementing powers conferred on the Commission.

The inclusion of food additives onto a positive list will be based on their safety when used, a technological need and their usage must be of benefit to the consumer. Their use must not mislead the consumer and this would include issues related to the quality of ingredients used, naturalness, nutritional quality of the product or its fruit and vegetable content. The European Food Safety Authority (EFSA) will be responsible for carrying out all safety evaluations.

The additives present in the positive list will have specifications including purity criteria and origin. Producers or users of additives should provide the Commission with information on their use which may affect the assessment of the safety of the food additive. When a food additive is already included in a Community list but there is a significant change in the production methods or the starting materials, the food additive prepared by these new methods or materials shall be considered as a different additive and a new entry in the Community lists or change in the specifications shall be required before it can be placed on the market.

Additionally, any GM-containing additives must be authorised following Regulation (EC) No 1829/2003 on genetically modified food and feed. Food Additives currently included in Directives 95/2/EC, 94/35/EC and 94/36/EC will be entered into Annex II of the proposal following a review undertaken by the Standing Committee on Food Chain and Animal Health (SCFCAH). The SCFCAH will evaluate the compliance of existing authorizations for food additives and their conditions of use with general criteria *i.e.*, technological needs, and consumer aspects. However, Annex III will be completed with other food additives used in food additives and food enzymes as well as carriers for nutrients and their conditions for use as follows:

Part I: Carriers in food additives (transferred from Annex V of Directive 95/2/EC on food additives authorised for use in food additives as permitted carriers/carrier solvents).

Part 2: Additives other than carriers in food additives.

Part 3: Additives including carriers in food enzymes.

Part 4: Additives including carriers in food flavourings (transferred from Directive 95/2/EC on food additives authorised for use in food flavourings).

Part 5: Carriers in nutrients.

Currently, Council Directive 95/2/EC on food additives other than colours and sweeteners allows two enzymes to be used as food additives. (In addition, Council Directive 2001/112/EC relating to fruit juices and certain similar products intended for human consumption, Council Directive 83/417/EEC relating to certain lactoproteins intended for human consumption and Council Regulation 1493/1999/EC on the common organisation of the market in wine, regulate the use of certain food enzymes in these specific foods.) Other uses of enzymes aren't regulated at all or are regulated as processing aids under the national legislation of the Member States; however, requirements differ significantly between each Member State.

The proposed regulation on enzymes would establish a positive list of approved enzymes, conditions for their use in foods and rules on labelling of food enzymes sold as such.

Hence, the regulation will apply to all enzymes including enzymes used as processing aids and miscellaneous additives, although the regulation will not apply to enzymes for nutritional or digestive purposes. Likewise, microbial cultures traditionally used in the production of food (*e.g.*, cheese) that may contain enzymes but aren't specifically used to make them will not be considered as food enzymes.

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The proposed regulation also defines the terms 'enzyme', 'food enzyme' and 'food enzyme preparation'. In the positive list, the entry of a food enzyme shall specify:

- The description of the food enzyme (including its common name)
- Specification (including origin, purity criteria etc)
- Foods in which it may be used
- Conditions for its use
- If there any restrictions for the enzyme when sold directly to consumers
- Any specific labelling requirements (in the food where the enzyme has been used to ensure the physical condition of the food and specific treatment is indicated if necessary).

The proposed regulation lays down labelling requirements of food enzymes and food enzyme preparations whether or not they are intended for sale to the final consumer. Enzymes that are already on the market can be transferred onto the positive list if EFSA accepts the previous safety assessment done at community level. The proposal states there is an initial two-year authorisation period during which EFSA must evaluate all applications for food enzymes.

Novel foods falling within the scope of Regulation (EC) No 258/97 concerning novel foods and novel food ingredients should be excluded from the scope of this proposed regulation on food enzymes. Enzymes produced from genetically modified organisms will be subject to the scope of Regulation (EC) 1829/2003 on genetically modified food and feed in relation to the safety assessment of the genetic modification, whereas other aspects of safety and the final authorisation shall be covered under the proposed regulation on food enzymes.

Currently, flavourings are regulated through Council Directive 88/388/EEC relating to flavourings for use in foodstuffs and to source materials for their production. Within this, flavourings can be divided into the following categories:

- Flavouring substances (which describes natural, nature identical and artificial flavourings)
- Flavouring preparations
- Process flavourings
- Smoke flavourings.

Council Directive 88/388/EEC also sets maximum limits for certain undesirable substances obtained from flavourings and other food ingredients with flavouring properties. The proposed regulation on flavourings sets new maximum limits for the presence of these undesirable substances in foods. It also introduces a new annex (Annex IV) which lists source materials to which restrictions apply for their use in the production of flavourings and food ingredients with flavouring properties. EFSA is responsible for the risk assessment of flavourings.

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The proposed regulation on flavourings aims to establish a positive list of flavourings and source materials approved for use in and on foods with their conditions of use in and on foods, as well as setting rules on the labelling of flavourings.

The positive list shall be established by placing the list of flavouring substances referred to in Article 2(2) of Regulation (EC) No 2232/96 laying down a Community procedure for flavouring substances used or intended for use in or on foodstuffs, into Annex I of the proposed regulation on flavourings, at the time of its adoption.

The current register of flavouring substances (as adopted by Decision (EC) 1999/217/EC, as amended, adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96), is valid for the whole of the EU and includes about 2700 flavouring substances. The new proposed regulation defines and contains the following categories of flavourings:

- Flavouring substances (defining natural flavouring substances only)
- Flavouring preparations
- Thermal process flavourings
- Smoke flavourings
- Flavour precursors
- Other flavourings or mixtures thereof
- A new category of flavouring introduced by the proposal.

The definition of a 'natural flavouring substance' has been amended by this proposal as discussed earlier in this chapter. Given that the chemical structure of the molecules is identical, it was sensible to remove the distinction between 'natural' and 'nature identical' flavouring substances because as far as human consumption is concerned, it is the safety of the substance that is important, not its origin.

A 'thermal process flavouring' is defined as a product obtained after heat treatment from a mixture of ingredients not necessarily having flavouring properties themselves, of which at least one contains nitrogen and another is a reducing sugar; the ingredients for the production of thermal process flavourings may be:

(*a*) food; and/or

(*b*) source material other than food.

In Annex V of the proposed regulation on flavourings, conditions for the production of thermal process flavourings and maximum levels for certain substances in thermal process flavourings are specified. A 'flavour precursor' is defined as a product, not necessarily having flavouring properties itself, intentionally added to food for the sole purpose of producing flavour by breaking down or reacting with other components during food processing; it may be obtained from:

(*a*) food; and/or

(*b*) source material other than food.

A flavouring or source material that falls within the scope of Regulation (EC) 1829/2003 on genetically modified food and feed can only be included in the positive list of flavourings under the new proposal if it has been covered by an authorisation in accordance with the Regulation (EC) No 1829/2003.

Appendix A: Exemptions from Food Ingredients Listing

Foods that are exempt from ingredients listing are:

1. Fresh fruit and vegetables, which have not been peeled or cut into pieces.
2. Carbonated water that contains only carbon dioxide, and whose name indicates that it has been carbonated.
3. Vinegar obtained by fermentation from a single product with no additions.

4. Cheese, butter, fermented milk and fermented cream containing only lactic products, enzymes and microorganism cultures essential to manufacture, or cheese (except curd cheese and processed cheese) containing salt for manufacture.
5. Flour to which no substances have been added other than those required to be present in flour by the Bread and Flour Regulations 1998.
6. Drinks with an alcoholic strength by volume of more than 1.2%.
7. Foods consisting of a single ingredient, where the name of the food is identical to the name of the ingredient, or the name of the food enables the nature of the ingredient to be clearly identified.

Appendix B: Exemptions from Durability Indication

1. Fresh fruit and vegetables, which have not been peeled or cut into pieces.
2. Wine, liqueur wine, sparkling wine, aromatised wine and any similar drink obtained from fruit other than grapes.
3. Any drink made from grapes or grape musts and coming within specified codes of the Combined Nomenclature.
4. Any drink with an alcoholic strength by volume of 10% or more.
5. Any soft drink, fruit juice or fruit nectar or alcoholic drink, sold in a container containing more than 5 litres and intended for supply to catering establishments.
6. Any flour confectionery and bread that, given the nature of its content, is normally consumed within 24 hours of its preparation.
7. Vinegar.
8. Cooking and table salt.
9. Solid sugar and products consisting almost solely of flavoured or coloured sugars.
10. Chewing gums and similar products.
11. Edible ices in individual portions.

Appendix C: Permitted Extraction Solvents

1. Propane
2. Butane
3. Ethyl acetate
4. Ethanol
5. Carbondioxide
6. Acetone
7. Nitrous oxide
8. Methanol
9. Propan-2-ol
10. Hexane
11. Methyl acetate
12. Ethylmethylketone
13. Dichloromethane
14. Diethyl ether
15. Butan-1-ol
16. Butan-2-ol
17. Propan-1-ol
18. Cyclohexane.

SUMMARY

- A processing aid is defined as a substance that is not consumed as a food ingredient by itself; is intentionally used in the processing of raw materials, foods or their ingredients, to fulfil technological purposes during treatment or processing; and is capable of resulting in the unintended but technically unavoidable presence of its residues or its derivatives in the finished product, and the residues of which do not present any risk to human health and do not have any technological effect on finished products.

- A flavouring is a material used or intended for use in or on food to impart odour, taste or both.
- A 'thermal process flavouring' is defined as a product obtained after heat treatment from a mixture of ingredients not necessarily having flavouring properties themselves, of which at least one contains nitrogen and another is a reducing sugar.

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REVIEW QUESTIONS

1. Discuss Food Safety Act 1990.
2. Discuss Trade Descriptions Act 1968.
3. Discuss the Weights and Measures Act 1985
4. Discuss the Compound ingredients in food products.
5. Discuss the Additives in the food.
6. Discuss the Flavourings in food.
7. Discuss Quantitative Ingredients Declaration (QUID).

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

UNIT III: STANDARDIZATION OF COSMETICS

NOTES

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- describe the criticism and controversy.
- explain the organic and natural ingredients.
- know about the ingredients.
- discuss about the cosmetic industry.

3.1 INTRODUCTION

Cosmetics are substances used to enhance the appearance or odor of the human body. Cosmetics include skin-care creams, lotions, powders, perfumes, lipsticks, fingernail and toe nail polish, eye and facial makeup, permanent waves, colored contact lenses, hair colors, hair sprays and gels, deodorants, baby products, bath oils, bubble baths, bath salts, butters and many other types of products. A subset of cosmetics is called "make-up," which refers primarily to colored products intended to alter the user's appearance. Many manufacturers distinguish between decorative cosmetics and care cosmetics.

The manufacture of cosmetics is currently dominated by a small number of multinational corporations that originated in the early 20th century, but the distribution and sale of cosmetics is spread among a wide range of different businesses. The U.S. Food and Drug Administration (FDA) which regulates cosmetics in the United States[1] defines cosmetics as: "intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions." This broad definition includes, as well, any material intended for use as a component of a cosmetic product. The FDA specifically excludes soap from this category.

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3.2 HISTORY

The first archaeological evidence of cosmetics usage was found in Egypt around 3500 BC during the Ancient Egypt times with some of the royalty having make up such as Nefertiti, Nefertari, mask of Tutankhamun, etc. The Ancient Greeks and Romans [citation needed] also used cosmetics. The Romans and Ancient Egyptians used cosmetics containing poisonous mercury and often lead. The ancient kingdom of Israel was influenced by cosmetics as recorded in the Old Testament-2 Kings 9:30 where Jezebel painted her eyelids—approximately 840 BC. The Biblical book of Esther describes various beauty treatments as well.

In the Middle Ages, although its use was frowned upon by Church leaders, many women still wore cosmetics. A popular fad for women during the Middle Ages was to have a pale-skinned complexion, which was achieved through either applying pastes of lead, chalk, or flour, or by bloodletting. Women would also put white lead pigment that was known as "ceruse" on their faces to appear to have pale skin.

Cosmetic use was frowned upon at many points in Western history. For example, in the 1800s, make-up was used primarily by prostitutes, and Queen Victoria publicly declared makeup improper, vulgar, and acceptable only for use by actors. Adolf Hitler told women that face painting was for clowns and not for the women of the master race.

Women in the 19th century liked to be thought of as fragile ladies. They compared themselves to delicate flowers and emphasised their delicacy and femininity. They aimed always to look pale and interesting. Paleness could be induced by drinking vinegar and avoiding fresh air. Sometimes ladies discreetly used a little rouge on the cheeks, and used "belladonna" to dilate their eyes to make their eyes stand out more. Make-up was frowned upon in general especially during the 1870s when social etiquette became more rigid.

Actresses however were allowed to use make up and famous beauties such as Sarah Bernhardt and Lillie Langtry could be powdered. Most cosmetic products available were still either chemically dubious, or found in the kitchen amid food colorings, berries and beetroot.

By the middle of the 20th century, cosmetics were in widespread use by women in nearly all industrial societies around the world. Cosmetics have been in use for thousands of years. The absence of regulation of the manufacture and use of cosmetics has led to negative side effects, deformities, blindness, and even death through the ages. Examples of this were the prevalent use of ceruse (white lead), to cover the face during the Renaissance, and blindness caused by the mascara Lash Lure during the early 1900s.

The worldwide annual expenditures for cosmetics today is estimated at $19 billion. Of the major firms, the largest is L'Oréal, which was founded by Eugene Schueller in 1909 as the French Harmless Hair Colouring Company (now owned by Liliane Bettencourt 26% and Nestlé 28%, with the remaining 46% are publicly traded).

The market was developed in the USA during the 1910s by Elizabeth Arden, Helena Rubinstein, and Max Factor. These firms were joined by Revlon just before World War II and Estée Lauder just after.

Beauty products are now widely available from dedicated internet-only retailers, who have more recently been joined online by established outlets, including the major department stores and traditional bricks and mortar beauty retailers.

Like most industries, cosmetic companies resist regulation by government agencies like the FDA, and have lobbied against this throughout the years. The FDA does not have to approve or review the cosmetics, or what goes in them before they are sold to the consumers. The FDA only regulates against the colors that can be used in the cosmetics and hair dyes. The cosmetic companies do not have to report any injuries from the products; they also only have voluntary recalls on products.

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3.3 CRITICISM AND CONTROVERSY

During the 20th century, the popularity of cosmetics increased rapidly. [citation needed] Especially in the United States, cosmetics are used by girls at an increasingly young age. Due to the fast-decreasing age of make-up users, many companies, from high-street brands like Rimmel to higher-end products like Estee Lauder, have catered to this expanding market by introducing more flavored lipsticks and glosses, cosmetics packaged in glittery, sparkly packaging and marketing and advertising using young models. The social consequences of younger and younger beautification has had much attention in the media over the last few years.

Criticism of cosmetics has come from a variety of sources including feminists, animal rights activists, authors and public interest groups. There is a growing awareness and preference for cosmetics that are without any supposedly toxic ingredients, especially those derived from petroleum, Sodium Lauryl Sulfate (SLS), and parabens.

Numerous published reports have raised concern over the safety of a few surfactants. SLS causes a number of skin issues including dermatitis. Parabens can cause skin irritation and contact dermatitis in individuals with paraben allergies, a small percentage of the general population. Animal experiments have shown that parabens have a weak estrogenic activity, acting as xenoestrogens. Prolonged use of makeup has also been linked to thinning eyelashes. Synthetic fragrances are widely used in consumer products. Studies concluded from patch testing show synthetic fragrances are made of many ingredients which cause allergic reactions. Cosmetics companies have been criticised for making pseudoscientific claims about their products which are misleading or not backed by science.

3.4 MAKE UP

Lipstick, lip gloss, lip liner, lip plumper, lip balm, lip conditioner, lip primer, and lip boosters. Foundation, used to smooth out the face and cover spots or uneven skin coloration. Usually a liquid, cream, or powder. Foundation primer is can be applied before to get a smoother finish. Powder, used to set the foundation, giving a matte finish, and also to conceal small flaws or blemishes.

Rouge, blush or blusher, cheek coloring used to bring out the color in the cheeks and make the cheekbones appear more defined. This comes in powder, cream, and liquid forms. Bronzer, used to give skin a bit of color by adding a golden or bronze glow.

Mascara is used to darken, lengthen, and thicken the eyelashes. It is available in natural colors such as brown and black, but also comes in bolder colors such as blue, pink, or purple. There are many different formulas, including waterproof for those prone to allergies or sudden tears. Often used after an eyelash curler and

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mascara primer. The chin mask known as chutti for Kathakali, a performing art in Kerala, India is considered the thickest makeup applied for any artform.Eye liner, eye shadow, eye shimmer, and glitter eye pencils as well as different color pencils used to color and emphasize the eyelids (larger eyes give a more youthful appearance).

Eyebrow pencils, creams, waxes, gels and powders are used to color and define the brows. Nail polish, used to color the fingernails and toenails. Concealer, Makeup used to cover any imperfections of the skin. Also included in the general category of cosmetics are skin care products. These include creams and lotions to moisturize the face and body, sunscreens to protect the skin from damaging UV radiation, and treatment products to repair or hide skin imperfections (acne, wrinkles, dark circles under eyes, etc.). Cosmetics can also be described by the form of the product, as well as the area for application. Cosmetics can be liquid or cream emulsions; powders, both pressed and loose; dispersions; and anhydrous creams or sticks.

Lip stain is a cosmetic product that contains either water or a gel base. To help the product stay on the lips, many stains may contain alcohol. These lip coloring products are available in a variety of formulas, colors, and application types. The idea behind lip stains is to temporarily saturate the lips with color with a dye, rather than applying a colored wax to the lips to color them. A lip stain is usually designed to be waterproof so that the color will be long lasting, and once the stain dries, it should not smear, stain, wear unevenly, or transfer to the teeth. A lip stain may come in a bottle with an applicator which is used to brush the stain onto the lips, and it can also come in a small jar, with users applying the stain with a finger or a cosmetic brush.

Make-up remover is the product used to remove the make-up products applied on the skin. It is used for cleaning the skin for other procedure, like applying any type of lotion at evening before the person go to sleep.

3.5 SPECIAL EFFECTS

In addition to over-the-counter cosmetic products, recent years have seen an increasing market for prescription or surgical cosmetic procedures. These range from temporary enhancements, such as cosmetic colored contact lenses, to major cosmetic surgery. To temporary fashionable enhancement belongs application of false eyelashes or eyelash extensions, in order to enhance the natural eyelashes and make eye appearance more attractive. Many techniques, such as microdermabrasion and physical or chemical peels, remove the oldest, top layers of skin cells. The younger layers of skin left behind appear more plump, youthful, and soft. Permanent application of pigments (tattooing) is also used cosmetically.

3.6 INGREDIENTS

While there is assurance from the largest cosmetic companies that ingredients have passed quality tests and official regulations, and are therefore generally safe to use, there is a growing preference for cosmetics that are without any "synthetic" ingredients, especially those derived from petroleum. Once a niche market, handmade and certified organic products are becoming more mainstream.

Ingredients' listings in cosmetics are highly regulated in many countries. The testing of cosmetic products on animals is a subject of some controversy. It is now illegal in the United Kingdom, the Netherlands, and Belgium, and a ban across the European Union is due to come into effect in 2009.

3.7 ORGANIC AND NATURAL INGREDIENTS

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Even though many cosmetic products are regulated, there are still health concerns regarding the presence of harmful chemicals within these products.[citation needed] Aside from color additives, cosmetic products and their ingredients are not subject to FDA regulation prior to their release into the market. It is only when a product is found to violate Federal Food, Drug, and Cosmetic Act (FD&C Act) and Fair Packaging and Labeling Act (FPLA) after its release that the FDA may start taking action against this violation.[20] With many new products released into the market every season, it is hard to keep track of the safety of every product. Some products carry carcinogenic contaminant 1,4- dioxane. Many cosmetic companies are coming out with "All natural" and "Organic" products. All natural products contain mineral and plant ingredients and organic products are made with organic agricultural products. Products who claim they are organic are not, unless they are certified "USDA Organic."

3.8 COSMETIC INDUSTRY

The cosmetic industry is a profitable business for most manufacturers of cosmetic products. By cosmetic products, we understand anything that is intended for personal care such as skin lotions or sun lotions, makeup and other such products meant to emphasize one's look. Given the technological development and the improvement of the manufacturing process of cosmetics and not least due to the constantly increasing demand of such products, this industry reported an important growth in terms of profit.

The cosmetic industry has not only grown only in the United States, but also in various parts of the world which have become famous for their cosmetic precuts. Some of these include France, Germany, Italy and Japan. It has been estimated that in Germany, the cosmetic industry generated sales of EUR 12.6 billion at retail sales, in 2008 which made of German cosmetic industry the 3rd in the world, after Japan and the United States. Also, it has been shown that in the same country, this industry has grown with nearly 5 percent in one year, from 2007 to 2008. The exports of Germany in this industry reached in 2008 EUR 5.8 billion whereas the imports of cosmetics totaled EUR 3 billion. The main countries that export cosmetics to Germany are France, Switzerland, the United States and Italy and they mainly consist of makeup and fragrances or perfumes for women. After the United States, Japan is the second largest market for cosmetics in the world, a market worth about JPY 1.4 trillion per year.

The worldwide cosmetics and perfume industry currently generates an estimated annual turnover of US$170 billion (according to Eurostaf - May 2007). Europe is the leading market, representing approximately €63 billion, while sales in France

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reached €6.5 billion in 2006, according to FIPAR (Fédération des Industries de la Parfumerie - the French federation for the perfume industry). [26] France is another country in which the cosmetic industry plays an important role, both nationally and internationally. Most products on whose label it is stated "Made in France" are valued on the international market. According to data from 2008, the cosmetic industry has risen constantly in France, for 40 consecutive years. In 2006, this industrial sector reached a record level of EUR 6.5 billion. Famous cosmetic brands produced in France include Vichy, Yves Saint Laurent, Yves Rocher and many others.

The Italian cosmetic industry is also an important player in the European cosmetic market. Although not as large as in other European countries, the cosmetic industry in Italy was estimated to reach EUR 9 billion in 2007. The Italian cosmetic industry is however dominated by hair and body products and not makeup as in many other European countries. In Italy, hair and body products make up approximately 30% of the cosmetic market. Makeup and facial care however are the first cosmetic products to be exported in the United States.

Due to the popularity of cosmetics, especially fragrances and perfumes, many designers who are not necessarily involved in the cosmetic industry came up with different perfumes carrying their names. Moreover, most actors and singers also have their own perfume line (such as Celine Dion). The designer perfumes are, like any other designer products, the most expensive in the industry as the consumer pays not only for the product but also for the brand. Famous Italian fragrances are produced by Giorgio Armani, Dolce and Gabbana and so on.

The European Commission and the FDA are the two bodies making legislation in what concerns cosmetic industry and its various aspects within the European Union, respectively in the United States. In the European Union, the circulation of cosmetic products and their safety are law subjects since 1976. One of the newest amendments of the directive concerning cosmetic industry comes as a result of the attempt to ban animal testing. Therefore, testing cosmetic products on animals is illegal in the European Union from September 2004 and testing separate ingredients of such products on animals is also prohibited by law starting with March 2009.

The FDA joined with thirteen other Federal agencies in forming the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in 1997 which is an attempt to ban animal testing and find other methods to test the cosmetic products.

The cosmetic industry worldwide seems to be continuously developing, now more than ever with the advent of the Internet companies. Many famous companies sell their cosmetic products online also in countries in which they do not have representatives.

SUMMARY

- Inventory control is a subject of study under the broad discipline of materials manage.
- Numerous published reports have raised concern over the safety of a few surfactants.

REVIEW QUESTIONS

1. What is the standardization of cosmetics?
2. What are different ingredients in the cosmetics?

FURTHER READINGS

NOTES

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

UNIT IV: HERBAL PRODUCTS

NOTES

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- define the natural products as therapeutic agents.
- know about the natural product research and development.
- describe the standardization of herbals.
- discuss about the toxicity of excipients.

4.1 INTRODUCTION

By definition, the word *natural* is an adjective referring to something that is present in or produced by nature and not artificial or man-made. When the word *natural* is used in verbiage or written, many times it is assumed that the definition is something good or pure. However, many effective poisons are natural products. The term *natural products* today is quite commonly understood to refer to herbs, herbal concoctions, dietary supplements, traditional Chinese medicine, or alternative medicine. That will not be the case in this chapter. The information presented here will be restricted to the discovery and development of modern drugs that have been isolated or derived from natural sources. While in some cases, such discovery and development may have been based on herbs, folklore, or traditional or alternative medicine, the research and discovery of, along with the development of, herbal remedies or dietary supplements typically present different challenges with different goals. So while the stories of herbs and drugs are very much intertwined, it needs to be fully appreciated that the use of herbs as natural product therapy is different than the use of herbs as a platform for drug discovery and further development.

4.2 HISTORY OF THE USE OF NATURAL PRODUCTS AS THERAPEUTIC AGENTS

Natural products are generally either of prebiotic origin or originate from microbes, plants, or animal sources. As chemicals, natural products include such classes of

compounds as terpenoids, polyketides, amino acids, peptides, proteins, carbohydrates, lipids, nucleic acid bases, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and so forth. Natural products are not just accidents or products of convenience of nature. More than likely they are a natural expression of the increase in complexity of organisms. Interest in natural sources to provide treatments for pain, palliatives, or curatives for a variety of maladies or recreational use reaches back to the earliest points of history.

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Nature has provided many things for humankind over the years, including the tools for the first attempts at therapeutic intervention. Neanderthal remains have been found to contain the remnants of medicinal herbs. The *Nei Ching* is one of the earliest health science anthologies ever produced and dates back to the thirtieth century bc. Some of the first records on the use of natural products in medicine were written in cuneiform in Mesopotamia on clay tablets and date to approximately 2600 bc. Indeed, many of these agents continue to exist in one form or another to this day as treatments for inflammation, influenza, coughing, and parasitic infestation. Chinese herb guides document the use of herbaceous plants as far back in time as 2000 bc.

In fact, *The Chinese Materia Medica* has been repeatedly documented over centuries starting at about 1100 bc. Egyptians have been found to have documented uses of various herbs in 1500 bc. The best known of these documents is the Ebers Papyrus, which documents nearly 1000 different substances and formulations, most of which are plant-based medicines. Asclepius (in 1500 bc) was a physician in ancient Greece who achieved fame in part because of his use of plants in medicine. A collection of Ayurvedic hymns in India from 1000 bc and earlier describes the uses of over 1000 different herbs. This work served as the basis for *Tibetan Medicine* translated from Sanskrit during the eighth century. Theophrastus, a philosopher and natural scientist in approximately 300 bc, wrote a *History of Plants* in which he addressed the medicinal qualities of herbs and the ability to cultivate them. The Greek botanist Pedanious Dioscorides in approximately ad 100 produced a work entitled *De Materia Medica,* which today is still a very well-known European document on the use of herbs in medicine. Galen (ad 130-200), practiced and taught pharmacy and medicine in Rome and published over two dozen books on his areas of interest.

Galen was well-known for his complex formulations containing numerous and multiple ingredients. Monks in monasteries in the Middle Ages (fifth to the twelfth centuries) copied manuscripts about herbs and their uses. However, it should not go unrecognized that it was the Arabs who were responsible for maintaining the documentation of much of the Greek and Roman knowledge of herbs and natural products and expanding that information with their own knowledge of Chinese and Indian herbal medicine. The Persian philosopher and physician Avicenna produced a work entitled *Canon Medicinae,* which is considered to be the definitive summarization of Greek and Roman medicine. Li Shih-Chen produced a Chinese drug encyclopedia during the Ming Dynasty entitled *Pen-ts'as kang mu* in ad 1596, which records 1898 herbal drugs and 8160 prescriptions. John Wesley, the founder of Methodism, had a profoundly negative view on the status of physicians within society and in 1747 wrote a book entitled *Primitive Physic,* which was a popular reference book of the time detailing numerous natural cures.When the colonists originally came to America, they lacked trained physicians and so turned to the Native Americans for advice in healing practices. Such a lack of conventional medicine and physicians in early America spawned the production of various

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types of almanacs and other publications that contained various natural product-based recipes and assorted tidbits of medical information. Indeed, in an effort to curry favor with commoners, physicians themselves turned to the production of self-treatment guides for the general public. Various types of societies and botanical clubs held meetings and published different types of communiqués to educate the public with regard to the availability of natural products and how they could be helpful to an individual's health. Samuel Thompson's *Thompson's New Guide to Health* was one very popular publication. For a variety of different reasons, the interest in natural products continues to this veryday.

The first commercial pure natural product introduced for therapeutic use is generally considered to be the narcotic morphine, marketed by Merck in 1826. The first semisynthetic pure drug based on a natural product, aspirin, was introduced by Bayer in 1899.

4.3 NATURAL PRODUCT RESEARCH AND DEVELOPMENT—AN UPDATE

The World Health Organization estimates that approximately 80 percent of the world's population relies primarily on traditional medicines as sources for their primary health care [44]. Over 100 chemical substances that are considered to be important drugs that are either currently in use or have been widely used in one or more countries in the world have been derived from a little under 100 different plants. Approximately 75 percent of these substances were discovered as a direct result of chemical studies focused on the isolation of active substances from plants used in traditional medicine. The number of medicinal herbs used in China in 1979 has been estimated to be numbered at 5267. More current statistics based on prescription data from 1993 in the United States show that over 50 percent of the most prescribed drugs had a natural product either as the drug or as the starting point in the synthesis or design of the actual end chemical substance. Thirty-nine percent of the 520 new drugs approved during the period 1983 through 1994 were either natural products or derivatives of natural products. Indeed, if one looks at new drugs from an indication perspective over the same period of time, over 60 percent of antibacterials and antineoplastics were again either natural products themselves or based on structures of natural products. Of the 20 top-selling drugs on the market in the year 2000 that are not proteins, 7 of these were either derived from natural products or developed from leads generated from natural products. This select group of drugs generates over 20 billion U.S. dollars of revenue on an annual basis.

Drug development over the years has relied only on a small number of molecular prototypes to produce new medicines. Indeed, only approximately 250 discrete chemical structure prototypes have been used up to 1995, but most of these chemical platforms have been derived from natural sources. While recombinant proteins and peptides are gaining market share, lowmolecular-weight compounds still remain the predominant pharmacologic choice for therapeutic intervention. Just a small sampling of the many available examples of the commercialization of modern drugs from natural products along with their year of introduction, indication, and company are: Orlistat, 1999, obesity, Roche; Miglitol, 1996, antidiabetic (Type II), Bayer; Topotecan, 1996, antineoplastic, SmithKline Beecham; Docetaxel, 1995, antineoplastic, Rhône-Poulenc Rorer; Tacrolimus, 1993, immunosuppressant, Fujisawa;

Paclitaxel, 1993, antineoplastic, Bristol-Myers Squibb. The overwhelming concern today in the pharmaceutical industry is to improve the ability to find new drugs and to accelerate the speed with which new drugs are discovered and developed. This will only be successfully accomplished if the procedures for drug target elucidation and lead compound identification and optimization are themselves optimized. Analysis of the human genome will provide access to a myriad number of potential targets that will need to be evaluated. The process of high-throughput screening enables the testing of increased numbers of targets and samples to the extent that approximately 100,000 assay points per day are able to be generated.

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However, the ability to accelerate the identification of pertinent lead compounds will only be achieved with the implementation of new ideas to generate varieties of structurally diverse test samples. Experience has persistently and repeatedly demonstrated that nature has evolved over thousands of years a diverse chemical library of compounds that are not accessible by commonly recognized and frequently used synthetic approaches.

Natural products have revealed the ways to new therapeutic approaches, contributed to the understanding of numerous biochemical pathways and have established their worth as valuable tools in biological chemistry and molecular and cellular biology. Just a few examples of some natural products that are currently being evaluated as potential drugs are (natural product, source, target, indication, status): manoalide, marine sponge, phospholipage- A2 Ca2+-release, anti-inflammatory, clincial trials; dolastatin 10, sea hare, microtubules, antineoplastic, nonclinical; staurosporine, streptomyces, protein kinase C, antineoplastic, clinical trials; epothilone, myxobacterium, microtubules, antineoplastic, research; calanolide A, B, tree, DNA polymerase action on reverse transcriptase, Acquired Immuno Deficiency Syndrome (AIDS), clinical trials; huperzine A, moss, cholinesterase, alzheimer's disease, clinical trials.

The costs of drug discovery and drug development continue to increase at astronomical rates, yet despite these expenditures, there is a decrease in the number of new medicines introduced into the world market. Despite the successes that have been achieved over the years with natural products, the interest in natural products as a platform for drug discovery has waxed and waned in popularity with various pharmaceutical companies. Natural products today are most likely going to continue to exist and grow to become even more valuable as sources of new drug leads.This is because the degree of chemical diversity found in natural products is broader than that from any other source, and the degree of novelty of molecular structure found in natural products is greater than that determined from any other source.

Where are these opportunities? Well, research into the use of plant-derived natural products alone in just the field of medicine covers a broad spectrum of activities. Examples of such biological activity profiles would include, but are not limited to, nootropics, psychoactive agents, dependence attenuators, anticonvulsants, sedatives, analgesics, anti-inflammatory agents, antipyretics, neurotransmission modulators, autonomic activity modulators, autacoid activity modulators, anticoagulants, hyoplipidemics, antihypertensive agents, cardioprotectants, positive inotropes, antitussives, antiasthmatics, pulmonary function enhancers, antiallergens, hypoglycemic agents, antifertility agents, fertility-enhancing agents, wound healing agents, dermal healing agents, bone healing agents, compounds useful in the prevention of urinary calculi as well as their dissolution, gastrointestinal motility modulators, gastric ulcer protectants, immunomodulators, hepato-protective agents,

myelo-protective agents, pancreato-protective agents, oculo-protective agents, membrane stabilizers, hemato-protective agents, antioxidants, agents protective against oxidative stress, antineoplastics, antimicrobials, antifungal agents, antiprotozoal agents, antihelminthics, and nutraceuticals. Many frontiers remain within the field of natural products that can provide opportunities to improve our quality of life.

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Fungal disease has historically been a difficult clinical entity with which to effectively deal. Fungal diseases can include more than just a mycosis and can also include allergic reactions to fungal proteins and toxic reactions to fungal toxins. Mycoses as a group include diseases that are significantly more serious and life-threatening than nail infestations, athletes foot, or "jock-itch." Indeed, increasing numbers of overtly healthy individuals are becoming victims of the complications of fungal infestation. The reasons for this are that increasing numbers of people are receiving immunomodulatory treatment for an organ transplant or some underlying chronic systemic pathology, antineoplastic chemotherapy for cancer, or have been the recipients of proper or improper use of powerful antibiotics. Additionally there are a number of individuals within society that are infected with the Human Immunodeficiency Virus (HIV). The available drugs to treat mycoses have been limited. Furthermore, in this armamentarium, there are problems with dose-limiting nephrotoxicity, the rapid development of resistance, drug-drug interactions of concern, and a fungistatic mechanism of action. Thus there is an urgent need for the development of more efficacious antifungal agents with fewer limitations and less side effects. Ideally such compounds should possess good distribution characteristics, a novel mechanism of action, and a broad-spectrum cidal antifungal activity. The discovery and isolation of an echinocandin-type lipopeptide (FR901379) and lipopeptidolactone (FR901469) from microbes has been a significant achievement. These compounds are water soluble and inhibit the synthesis of 1, 3-b-glycan, a key component of the fungal cell wall. Furthermore, since the cell wall is a feature particular to fungi and is not present in eukaryotic cells, such inhibitors certainly have the potential to demonstrate selective toxicity against the fungi and not against the animal or human host. The ultimate modifications of the lipopeptide and lipopeptidolactone referenced above led to the discovery of micafungin (FK463), which is currently in phase III clinical trials.This work along with the relatively recent approval of caspofungin (Merck) as a therapeutic agent for the treatment of disseminated aspergillosis are significant achievements in that they demonstrate that a melding of the proper research to identify and develop appropriate targets with the chemical and biological diversity found in natural products can be very rewarding.

Much ado has been made over recent years about endocrine disruptors and their effects on humans. It needs to be recognized that endocrine disruptors are not just synthetic chemicals but can also be natural products. The use of natural product endocrine disruptors may provide significant insight into our understanding of the mechanisms by which the evolution of the genome can protect transactivation of the sex hormone receptors and aid in the development of drugs, which can protect the embryo during its development from hormone disruptive effects.

Diabetes is a multisystemic affliction, having impact on nearly everybody organ. As a disease, it kills more individuals on a per annum basis than AIDS and breast cancer combined. The impact on the quality of life of an individual suffering with diabetes is profound. A number of natural products currently exist that demonstrate hypoglycemic activity. Indeed, depending upon the source that one might use, there are approximately 800 to 1200 plants that exhibit hypoglycemic activity.While

research and development efforts in this particular area thus far are largely restricted to traditional medicine uses, future research may well identify a potent antidiabetic agent.

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The incidences of neuropsychiatric disorders are steadily increasing as our population increases in size and age. Such disorders include, but are not limited to, seizure disorders, schizophrenia, dementia, mania, aggression, memory loss, psychoses, age-related cognitive decline, depression, anxiety states, mood disorders, substance abuse, and substance dependence. There is a large body of data available that suggests the use of many natural products as potential treatments for these conditions and other neuropsychiatric disorders. Indeed, a number of plant extracts have been associated with the treatment of various categories of mental symptoms and various types of receptor selectivity. A very controversial potential psychotherapeutic agent is *Gingko biloba*. A lack of understanding of mechanism of action, misidentification of materials, contamination of materials, intrinsic toxicity, and absence of standardization all contribute to this controversy. Further fractionation, isolation, and characterization of active components of these and other plants will undoubtedly lead to the discovery of novel neuropsychiatric agents as well as the debunking of other alleged therapies.

There are numerous blood-based diseases that afflict humans. These would include, but are not limited to, anemia, blood group incompatibility, blood protein disorders, bone marrow diseases, hemoglobinopathies, hemorrhagic diatheses, leukemia, disorders of leukocyte dysfunction, platelet disorders, and erythrocyte aggregation disorders. A number of natural products have been reported in the literature to be of value in the treatment of Epstein-Barr virus infection, leukemia, thrombosis and coagulopathy, malaria, anemia, and bone marrow diseases. Extracts from the fungus *Trichothecium roseum*, the sea cucumber *Cucumaria japonica*, the legume *Amorpha fruitcosa*, the tree *Magnolia officinalis*, and others may be useful in the therapeutic management of Epstein-Barr virus infection. Extracts from the basidiomycetes *Mycena pura* and *Nidula candida* may be useful in the treatment of leukemia. Compounds isolated from *Streptomyces platensis* may be useful in the treatment of thrombocytopenia. Compounds obtained from the marine sponge *Aplysina archeri* have been reported to inhibit the growth of the feline leukemia virus.

Scalarane-type bishomo-sesterterpenes isolated from the marine sponge *Phyllospongia foliascens* have been reported to exhibit cytotoxic, antithrombocytic, and vasodilation activities. It should be noted that a number of natural products are based on the coumarin nucleus and as such may exhibit antithrombotic and antiplatelet activities. A number of blood-sucking animals have small, low-molecular-weight proteins in their salivas that interfere with the clotting of blood and therefore might be of value as potential anticoagulants.

Streptomyces hygroscopicus ascomyceticus manufactures a macrolide that has been reported to have immunosuppressant activity and may prove to be beneficial in preventing transplant rejection in humans. It is entirely possible that these compounds and others offer sufficient structural diversity, range of biological activities, and differing mechanisms of action that new, safer, and more efficacious drugs to treat blood-based disorders could well burgeon from this library.

A wide variety of natural products are claimed to possess immunosuppressant activity, but it is often difficult to dissect this activity away from associated cytotoxicity. Since the first heart transplant in the late 1960s, medicine has progressed to the point where most organ transplants have become relatively routine procedures.

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The survival of individuals with transplants is owed in large part to the discovery of the fungal metabolite cyclosporine A in 1970 and its widespread use starting in 1978. Indeed, cyclosporine A has achieved such success that it is currently being evaluated for value in the treatment of Crohn's disease, systemic lupus erythematosus, and rheumatoid arthritis. Research efforts abound in the area of natural products and immunosuppression. A methyl analog of oligomycin F isolated from *Streptomyces ostreogriseus* has been reported to quite effectively suppress Bcell activation and T-cell activation in the presence of mitogens at concentrations comparable to that of cyclosporine A. Concanamycin F first isolated from *Streptomyces diastatochromogenes* in 1992 has been found to possess a wide array of biological activities including immunosuppressive and antiviral activities.

The experimental immunosuppressant (+)-discodermolide isolated from the marine sponge *Discodermia dissoluta* exhibits relatively nonspecific immunosuppression, causing the cell cycle to arrest during G2 and M phases. This compound's current primary interest is as a potential antineoplastic agent since it stabilizes microtubules and prevents depolymerization, effectively causing cell cyclic arrest during the metaphase to anaphase transition. This same mode of activity is shared with Taxol (Paclitaxel), the epothilones, eleutherobin, and the sarcodictyins. The didemnins, cyclic peptides, were first isolated from the marine tunicate *Tridemnum solidum* and exhibit immunosuppressive activity through a generalized cytotoxicity mediated by inhibition of progression through the G1 phase of the cell cycle by an unknown mechanism. The trichopolyns I to V from the fungus *Trichoderma polysporum* are lipopeptides that suppress the proliferation of lymphocytes in the murine allogeneic mixed lymphocyte response assay. Triptolide from the plant *Tripterygium winfordii* demonstrates immunosuppressant activity through the inhibition of IL-2 receptor expression and signal transduction. The novel heteroaromatic compound lymphostin, obtained from *Streptomyces* KY11783 has demonstrated immunosuppressant activity through its potent inhibition of the lymphocyte kinase p56lck. Over the last decade, research activities on immunosuppressants of natural product origin have focused on the mechanisms of inhibition of T-cell activation and proliferation. This approach has been fruitful, leading to the generation of significant information about signaling pathways between T cells, greater detail about the roles of T cells in immune function, and the discovery of Tacrolimus (Prograf) from the soil fungus *Streptomyces tsukubaensis*. As immunological research progresses, increasingly more potential targets will be elucidated for immunomodulatory therapeutic intervention. Natural products will undoubtedly provide a sound platform for the delivery of natural-product-based therapeutic agent candidates. Natural-products-based anticancer drug discovery continues to be an active area of research throughout the world.

While cancer incidences and the frequencies of types of cancer may vary from country to country, the most common sites for the development of neoplasia are generally considered to be the breast, colon/rectum, prostate, cervix/uterus, esophagus/ stomach, pancreas, liver, lung, urinary bladder, kidney, ovary, oral cavity, and blood (leukemia and non-Hodgkin lymphoma). Currently, the chemotherapeutic management of these tumors involves a variety of different plant-based chemicals that are either currently in use or in clinical trials and include such drug classes as the vinca alkaloids, lignans, taxanes, stilbenes, flavones, cephalotaxanes, camptothecins, and taxanes. Despite the wide range of organ structure, type, and function, great similarities exist between the organs with regard to the pathogenesis of cancer. As more and more details of the molecular biology of cancer are revealed, more targets will present

themselves for possible therapeutic chemical intervention in the growth and development of neoplasms. A somewhat new approach is that of cancer chemoprevention, where chemoprevention is defined as the prevention, delay, or reversal of carcinogenesis. A few of the more promising cancer chemopreventive agents are (compound, plant source, target): brusatol, *Brucea javanica*, differentiation; zapotin, *Casimiroa edulis*, differentiation and apoptosis; apigenin, *Mezoneuron cacullatum*, antimutagenesis; deguelin, *Mundelea sericea*, inhibitor of ornithine decarboxylase; brassinin, *Brassica* spp., inducer of quinine reductase; and resveratrol, *Cassia quinquangulata*, cyclooxygenase inhibitor. A final note with regard to this approach is that it is important to appreciate that the distinction between chemopreventive agent and chemotherapeutic agent can become quite blurred.

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A recurrent theme in neoplasia is the alteration of cell cycle control. One therapeutic approach to the treatment of neoplasia is the development of a treatment that would return to normal the altered cell cycle. Cyclindependent Kinases (CDKs) control the progression of a cell through its growth cycle. CDKs are regulated through a series of site-specific complex mechanisms, and the components of such mechanisms include activating cyclins and endogenous CDK inhibitors. Processes of such mechanisms involve regulatory phosphorylation. There are natural products such as butyrolactone and staurosporine that are currently known to be able to provide such activity. These compounds and others generated from their platform are adenosine 5'- triphosphate (ATP) site-directed inhibitors and directly antagonize the activity of CDKs. Further research should more fully elucidate the most efficacious endpoint of CDK inhibition and lead to the control of neoplastic growth and possibly even bring about cytostasis or apoptosis.

The introduction of active agents derived from natural sources into the anticancer weaponry has already significantly changed the futures of many individuals afflicted with cancer of many different types. Continued research into natural sources will continue to deliver newer and more promising chemicals and chemical classes of anticancer agents with novel mechanisms of action that will improve survival rates to even higher degrees. Human immunodeficiency virus infection is a devastating, globally widespread disease that consumes significant health-care dollars in the due course of management of patients. Most of the currently useful anti-HIV agents are nucleosides and are limited in use due to severe toxicity and emerging drug resistance. Natural products, with their broad chemical structural diversity, provide an excellent opportunity to deliver significant therapeutic advances in the treatment of HIV. Many natural products with novel structures have been identified as having anti-HIV activities. Betulinic acid, a triterpenoid isolated from *Syzigium claviflorum*, has been found to contain anti HIV activity in lymphocytes. The quassinoside glycoside isolated from *Allanthus altissima* has been found to inhibit HIV replication. Artemisinin, isolated from *Artemisia anuua*, is a sesquiterpene lactone that is of special interest because of its novel structure, potent antimalarial activity, and activity against *Pneumocystis carinii*. A novel phorbol ester isolated from *Excoecaria agallocba* has been reported to be a potent inhibitor of HIV-1 reverse transcriptase. Indeed, most of the natural product chemicals that are attracting interest in this area of research are secondary metabolites such as terpenes, phenolics, peptides, alkaloids, and carbohydrates and are also inhibitors of HIV reverse transcriptase. Other target opportunities in the life cycle of the human immunodeficiency virus available for exploitation are: (1) attachment of virus to cell surface, (2) penetration and fusion of the virus with the cell membrane, (3) reverse transcription via reverse transcriptase, (4) integration into the host genome, (5) synthesis of viral proteins

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including zinc fingers, and (6) processing of viral polypeptide with HIV protease and assembly of viral proteins and DNA into a viral particle, maturation, and extrusion of the mature virus. Infectious viral diseases remain a worldwide problem. Viruses have been resistant to therapy or treatment longer than most other forms of life because their nature is to depend on the cells that they infect for their multiplication and survival. Such a characteristic has made the development of effective antiviral chemotherapeutic agents difficult. Today there are few effective antivirals available for use. In order to confidently wage the war against viruses, research efforts are now turning to the molecular diversity available from natural products. For the period 1983 to 1994, seven out of 10 synthetic agents approved by the Food and Drug Administration (FDA) for use as antivirals were based on a natural product. These drugs are famciclovir, stavudine, zidovudine, zalcitabine, ganciclovir, sorivudine, and didanosine. The viral genome can be composed of either RNA or DNA and HIV, which was discussed earlier is an RNA containing virus. The general potential targets of antiviral chemotherapy are: (1) attachment of virus to host cell, (2) penetration of the host cell by the virus, (3) viral particle uncoating, release, and transport of viral nucleic acid and transport proteins, (4) nucleic acid polymerase release/activation, (5) translation of mRNA (messenger RNA) to polypeptides (early proteins), (6) transcription of mRNA, (7) replication of nucleic acids, (8) protein synthesis (late proteins), (9) viral polypeptide cleavage into polypeptides necessary for maturation, (10) assembly of viral capsids and precursors, (11) encapsidation of nucleic acid, (12) envelopment, and (13) release. Early antiviral research focused on compounds that inhibited viral DNA synthesis, purine, and pyrimidine nucleoside analogs. Today most current antiviral agents target RNA-based viruses and the inhibition of reverse transcriptase in order to block the transcription of the RNA genome to DNA. Such inhibition would prevent the synthesis of viral mRNA and proteins. Protease inhibitors affect the synthesis of late viral proteins and viral packaging activity.

There are no currently available drugs that target early viral protein synthesis. Antiviral compound research has included alkaloids, carbohydrates, chromones, coumarins, flavonoids, lignans, phenolics, quinines, xanthones, phenylpropanoids, tannins, terpenes, steroids, iridoids, thiopenes, polyacetylenes, lactones, butenolides, phospholipids, proteins, peptides, and lectins. While plants have been a common hunting ground, many other sources are now starting to be explored, especially the marine environment. The use of natural products in the field of antiviral research appears to be limited only by the imagination of the researcher.

This review has demonstrated that natural products are indeed viable sources and resources for drug discovery and development. Indeed, without natural products, medicine would be lacking in therapeutic tools in several important clinical areas such as neurodegenerative disease, cardiovascular disease, solid tumors treatment, and immunoinflammatory disease. Furthermore, the continual emergence of new natural product chemical structure skeletons, with interesting biological activities along with the potential for chemical modification and synthesis bode well for the utility of natural products. Finally, the uses of natural products need to be by no means restricted to pharmaceuticals but can also be expanded to agrochemicals. For example, the use of pyrethrins obtained from *Chrysanthemum* spp. as insecticides has been very popular over the years and persists today. Research continues into the use of natural products as pesticides.While the success stories have not been as numerous or spectacular for herbicides as they have been for drugs and pesticides,

there have been victories along the way and the future holds strong potential for this field also.

4.4 STANDARDIZATION OF HERBALS: NEED AND RESPONSIBILITY

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Recent adverse events with Herbal Supplements in countries such as the US, clearly point towards the urgent need for Herbal Standardization and Evaluation of Safety and Efficacy. Plant-based Traditional systems of medicine like TCM and Ayurveda boast of substantial usage data spanning centuries if not millennia indicating safety and efficacy. The opponents of Standardization and state Regulation for herbal products often cite this as the reason to block research based on modern lines. However, most understand why botanicals or Traditional Medicines need to be subjected to modern day scrutiny for product quality, safety and efficacy. This section details the justification, the approaches and the government effort needed for herbal product standardization.

Why Standardization

Substantial original documentation for the more organized systems of medicine still exists. Large expanses of local ethnic medicine, folklore, etc. were passed on to the present generation only by word of mouth. Medical education was personalized to a small group of students or sometimes a single individual.

Although several original codified texts like the Charak Samhita of exist with specific herbal formulas, the physicians down the ages took liberty to modify these formulas according to prevailing local conditions or personalized them for individual patients. In course of time, though the name remained unchanged, the formula of the original preparation went through successive changes. This resulted in the same preparation having different compositions as well as different therapeutic indications.

Thus any Traditional System of Medicine (TSM) is often crowded with duplication, confusing nomenclature of plants, accidental substitution of herbs, etc. due to these "transmission errors". There is no denying that the TSM that has been passed on to us from earlier generations is vastly different from the original works of the earliest authors. Even in the original works, the means of maintaining finished product quality, safety and efficacy mentioned were at best only subjective in the absence of objective means of evaluation available to modern day science. This only contributed to misinterpretations and distortions.

Socio-economic Changes in Herbal Practice

Modern day awareness of the needs for Herbal Standardization and Evaluation have been aptly summarized in the words of Drugs Controller of India, Mr. Ashwini Kumar: "In earlier days, the activity of herb procurement, preparation and dispensing remained mainly the responsibility of practitioners and was on a one to one relationship between physician and his patients. It was a matter of sacred trust. However, the socio-economic changes in modern times, the technological advances, commercial factors, consumer preferences, changing lifestyles, etc., has influenced the way Herbal drugs are being 'manufactured' and distributed in the country. The Practitioner as well as the Consumer now seek assurance from the manufacturer about quality, safety and efficacy of a readymade Herbal Supplement or Medication.

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Changes in Physical Constitution, Social Habits and Environmental Stressors

Though life expectancy may have increased due to advances in medical treatment of life-threatening diseases, the average adult prior to the industrial revolution was much healthier due to the pollution-free environment and simple lifestyle. There has been a remarkable change in the average adult's physical constitution and his natural immunity towards the disease state, the nature and severity of diseases and the dietary and social conditions within which the patient may be consuming the Herbal Supplement.

The disease causing agents/factors then and now are also dramatically different. An average person's diet, lifestyle and other social habits, all which play important roles in disease and treatment, are completely different today. Hence the earlier recommendations for herbs for specific disease states may not hold true today unless validated in today's times.

Phenotypic Changes in Plant Species

Plant constituents are greatly influenced due to climatic factors, intra-species variations and geographical location of their collection and cultivation. The herbs and their properties as described thousands of years ago without doubt must have undergone changes in phytochemical profile in the normal evolutionary process and due to changed environmental and agronomic conditions. Hence the original pharmacological claims of these medicinal plant species need to be revalidated.

Substitution of Drugs Due to Non-availability or Scarcity

Some Traditional literature allows the substitution of herbs that were not found easily or scarce in certain regions. In some cases the substitutes have acquired the status of the original herb and are sometimes wrongly attributed with all the properties of the original herb. With passage of time, the herbs that were meant to substitute the original herb in one application start finding use in other applications not recommended by the authors. Hence constant clinical evaluation and validation is a must.

Evaluation of Herbals as Supplements/Concomitant Therapy

In several countries, regulatory authorities have still limited the use of herbals as supplements and not as the first line of treatment. The high cost of regulatory approvals required in placing a botanical drug on the market as the main line of therapy has meant that herbals are often taken as "Supplements" to other allopathic treatment. The possibility of incompatibilities and complications can be ruled out only after safety trials.

Proliferation of Proprietary Formulas over Traditional Generic Formulas

Traditional literature in Ayurveda for example mentions specific herbal cocktails for all disease conditions. The efficacy and safety of traditional formulas has been confirmed by informal system of trials down the ages but the proprietary branded preparations are not prepared as per Ayurvedic texts hence their final efficacy is open to question. Thus there is a need to confirm their efficacy.

Tough Dietary and Lifestyle restrictions for Effective Therapy

Ayurveda strongly recommends other lifestyle changes in conjunction with herbal medication. Several guidelines in Ayurvedic treatment are either difficult to adhere to in present 21st century lifestyle or are not taken seriously by the patient. The importance of label instructions (e.g., "Take with warm milk" or "mix with honey", etc.), dietary restrictions, dosages, etc. need to be studied using modern techniques. Such research can go a long way in improving patient compliance and achieving better results to herbal therapies.

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Change in the Consumption Pattern of Traditional products

The original recommendations for usage of Herbs and herbal products have undergone mutations in areas of age groups, races, etc. Ginseng is a popular traditional product originating from a certain geographical region where it has been immensely successful on local yellow populations. However, Ginseng has clearly a larger market outside of India and it is consumed by millions people of White/Hispanic/African racial backgrounds. The Traditional Ayurvedic preparation "Chyavanprash" was originally recommended for older men to increase longevity but now even children commonly consume it. Efficacy of traditional products should be established with multicentric trials spanning the complete or partial spectrum of current human usage.

Over-exploitation of Consumer Sentiment on Herbals

In the last half-century, Natural Ingredients have entered several hitherto untouched areas of our lives. There is now a "Herbal variant" of almost every consumable. Several herbal variants are either unjustified or have exaggerated claims. Finished product quality control through regulatory legislation is the answer to the problem of spurious and sub-standard "herbal" products with some times exaggerated claims. Modernization of herb form being administered Several TSM employ the herb in decoction or extract or tincture form but the majority of medication was still in whole herb form. However, today's herbal products, guided by the pharmaceutical practices of over-purification are sometimes presented in the form of near pure phytochemicals or concentrated extracts which bear little resemblance to the original herb in chemistry, efficacy and even safety. Due to the emphasis on higher and higher actives, the other actives in the plant are often excluded to a point where efficacy suffers and toxicity approaches that of synthetic compounds. Such preparations should be not only tested to confirm efficacy but also long-term safety in humans.

In Self-Interest of the Natural Products Industry

Self-regulation or Governmental control is in the selfinterest of the Industry. If the consumer continues to get disillusioned with Herbal products quality due to a few unscrupulous players, then the present rising interest in Natural products will ebb and the entire Industry will be at the losing end.

4.5 PRIVATE SECTOR EFFORT

1. *Pharmacological (Animal) Screening:* The pharmacological screening of plant materials from different ecospheres and traditional formulas from various texts

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has been going on for years in academia and private sector and must now continue at higher speeds available.

2. *Clinical Evaluation:* Taking potentially active herb candidates to the clinic in phase I and II trials to evaluate novel activities can be the concluding effort in herbal standardization that would lead to a healthy and growing market for herbs.
3. *Dose Determination and Safety Studies:* A fresh evaluation of therapeutic and toxic doses should be carried out for even the established uses of common herbs, due to changes over years in patient population, environmental conditions, disease patterns, plant phenotypes, etc.
4. *Development of Analytical Protocols for Finished Products:* Even as Phytochemical Science and Industry has made some progress on analytical methods for the raw materials, the finished product quality control is still a challenge. Industry has now long used the excuse that Multi-Ingredient Product quality control is a costly process and sometimes unattainable.
5. *Privately Funded Clinical Evaluation of Branded Formulations:* Privately funded clinical evaluation of branded formulations has been happening in the past and is only likely to grow as awareness increases amongst physicians and patients alike.

4.6 PUBLIC SECTOR EFFORT

1. *Government Funding and Support:* The Pharmacopoeias often form the basis of quality control in the drug industry of any country. Several countries have already made progress in the compilation of their national Herbal Pharmacopoeias such as the American Herbal Pharmacopoeia, German Monograph E and Indian Herbal Pharmacopoeia. However, this exercise needs to be continued further with added vigor and speed. There is an urgent need for a large collection of cheaply available biologically active compounds.
2. *Drug Testing Labs:* Smaller players in mostly fragmented Herbal markets of the world cannot be expected to have in-house facilities for testing and product development. Third party testing facilities, contract research laboratories and other facilitating agencies should be encouraged with easy finance, etc. to meet the needs of the small-scale sector.
3. *Health Regulatory Policy:* Without a stringently written and implemented regulatory policy on Herbal quality control, there will never be adequate motive for private initiative in this field. Governments should of course first create the public and private infrastructure needed to fulfill the requirements then vigorously implement the existing provisions of health regulations or amend the existing laws to give health authorities more teeth. Private investment in this area will then automatically follow.
4. *Consumer Awareness Program:* Having the infrastructure and Health Administration in place, a consumer awareness program be launched. This will ensure that private companies tighten up and deliver quality at affordable prices. The consumer is smart but she still needs some training in asking the right questions.
5. *International Promotion Program:* After having done the good work, the industry and particularly the government can and should tell the whole world about the modern quality standards that their "traditional" medicine adheres to.

Herbal Standardization is a much-needed public and private effort that will help in elevating Traditional Medicine to the levels it deserves internationally.

4.7 STANDARDIZATION AND QUALITY ASSESSMENT OF HERBALS

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Medicinal plants and herbal medicines are enjoying their acceptance since the origin of mankind. They are time tested. Hindu saints and Vaidyas had their own parameters for testing the formulations. Since last few decades substitution/ adulteration is being done during manufacturing of some formulations. The substitution/ adulteration reveal itself that the "original medicinal plant/part" is replaced by spurious substance of same size and shape through it contains the cheaper variety of drug content. This results in cheating the consumers. In ancient time, every formulation had its own testing procedure.

For example, Ayurveda literature reveals that its own testing procedure for Banslochan (used in formulation called Sitopaladi churan), Banslochan is not easily fragile by hands and after washing with water it convert into transparent material. But in today's manufacturing such test procedure of ancient classics are not totally followed by most of the formularies.

Some Ayurvedic physicians and industry have developed their own procedure, mingle with old procedures to make a hybrid procedure for testing of their products that's why spurious raw material with low therapeutic importance is being mixed in some formulations. Hence, forth WHO and different regulatory bodies came into effect to solve problems associated with herbal formulations so that the consumers should not be cheated by the gray products. WHO have taken some steps to control these kind of adulteration. WHO has have adopted and generated some general methods for standardization and quality control of herbal products. At the same time WHO directed member countries to make their guidelines regarding the standardization of herbals. WHO have also suggested some recent techniques, instruments and parameters for the purpose of standardization and development of any herbal formulations. For the identification of plants and its constituents guidelines suggest the fingerprinting methods.

The herbal medicines are gaining popularity and acceptance in the developed countries due to the failure of the allopathic system of medicine in various chronic ailments. The adverse effects of chemical drugs are the another failure of allopathic system. Herbal formulations are well accepted in the world but they need standardization and quality control profiles to meet global standards. But due to complex nature and inherent variability of the constituents of plant based drugs, it is difficult to establish the quality control parameters of herbal formulations.

Herbal formulations show the number of problems when quality control aspect is considered. This is because of nature of the herbal ingredients and different secondary metabolites present therein. It is also due to variation in the chemical profile of the herbal due to intrinsic and extrinsic factors (growing, harvesting, storage and drying processes).

There are two main important reasons for interest of development of standardization and quality aspect of the herbal products. *Firstly*, the use of medicinal plants, as such as phyto-medicines, dietary supplements, food and beverage ingredients and traditional medicines. *Secondary*, natural product continues remain as important

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source of new drug discovery. Quality is the sum of variable characteristics that significantly impact upon a product. For herbal medicines, such variable characteristics include the origin of the herb, botanical identity, purity, potency, stability and content of the marker compounds. Apart from these, Good Agriculture Practices (GAP) and Good Manufacturing Practices (GMP) are also important and directly assess the quality of the herbal products.

The task force appointed by the planning commission in June 1999, after studying the export market, suggested targets of Rs 3000 crores for 2005 and Rs 10,000 crores by 2050 for export of plant based herbal products. This is a challenging task. To meet this challenge, standardization and quality assessment of herbal products as per international norms will be inevitable. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication.

Authentication of Plant Material

Majority of the crude drugs come from wild sources and it is collected by poor, illiterate tribal without any attention to botanical identification and authentications. At present very few official standards are available for herbal preparations.

Assessment of Quality of Herbal Materials

Monographs exist for some of the commonly used and popular herbal medicines and the Indian Pharmacopoeia contains some monographs to which the respective herbs must comply. However where monographs are not available, quality assessment is usually based on the results of test procedures performed on the plant material itself. Such tests include analysis of the starting material, tests on microbial quality or contaminants such as pesticides and fumigation agents. Quantitative determination of marker compounds with known activity is also assessed as a quality criterion.

Assessment of Quality of Herbal Starting Materials

An assessment of the quality of the starting material and excipients is required. Firstly, information on the site of collection, time of harvesting, stage of growth, drying and storage conditions should be documented and in the case of herbal drugs with constituents with known activity, assays of their content using validated methods are required. This content must be stated as a range in order to ensure reproducibility. Where the constituents are not known, suitable marker compounds may be selected and used. Generally, herbal materials must be tested for microbial contamination, pesticides and fumigation agents, toxic metals and other likely contaminants and adulterants. Acceptance criteria and limits exist but are diverse and appear to be lack of harmony on these (WHO, 1998). For instance, the limits for some pesticides published in the pharmacopoeia 1993 are more restrictive than the WHO limits. In addition, the limits specified for microbial contamination in the European Pharmacopoeia 2002 are more restrictive than the WHO 1998 limits.

Assessment of Quality of Herbal Preparations

Guidelines require that the particulars of the characteristics, identification tests and purity tests for the product be established. These may include details of tests

on the performance of the dosage form such as dissolution or infusion. Chemical fingerprints can be used to trace the stability of the herbal preparation. Since the whole herbal drug or preparation may be considered to be the active, a determination of the stability of a single marker compound may not be adequate; an analysis of the whole herbal material may be more appropriate. **Heigl and Franz**, examined possible changes in the flavonoid pattern of common herbal drugs during long term and stress testing storage conditions used HPLC fingerprint comparisons to demonstrate differences in stability of individual flavonoid components. Such comparisons may allow determinations of substances present in the herbal preparations with respect to their stability and proportions, for quality purposes.

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Parameters for Standardization of Crude Drug/Herbal Medicines (As per WHO)

The following parameters are used for crude drugs/herbal medicines:

1. Authentication (Stage of collection, parts collected, botanical identity like phytomorphology, microscopical and taxonomical identity, etc.)
2. Foreign matter
3. Organoleptic evaluation
4. Tissues of diagnostic importance present in the drug powder.
5. Ash values and extractive values.
6. Volatile matter
7. Moisture content determination
8. Chromatographic and spectroscopic evaluation. TLC, HPTLC, HPLC methods will provide qualitative and semi quantitative information about the main active constituents present in the crude drug as chemical markers in the TLC fingerprint evaluation of herbals (FEH).
9. Determination of heavy metals – *e.g.*, cadmium, lead, arsenic, etc. **(Table 4.1).**
10. Pesticide residue – WHO and FAO set limits of pesticides, which are usually present in the herbs. Mainly pesticides like DDT, BHC, toxaphene, aldrin cause serious side-effects in human beings if the crude drugs are mixed with these agents **(Table 4.2).**
11. Microbial contamination - usually medicinal plants containing bacteria and molds are coming from soil and atmosphere. Analysis of the limits of *E. coli* and molds clearly throws light towards the harvesting and production practices. The substance known as afflatoxins will produce serious side-effects if consumed along with the crude drugs **(Table 4.3).**
12. Radioactive contamination – Microbial growth in herbals are usually avoided by irradiation.

Table 4.1 Limits for heavy/toxic metals

Sr. No.	*Heavy/Toxic Metals*	*As per WHO/ IDA (Permissible limit)*
1	Lead	10.0 ppm
2	Cadmium	0.30 ppm
3	Mercury	1.00 ppm
4	Arsenic	10 0 ppm

This process may sterilize the plant material but the radioactivity hazard should be taken into account. The radioactivity of the plant samples should be checked

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accordingly to the guidelines of International Atomic Energy (IAE) in Vienna and that of WHO.

Table 4.2 Limits for Pesticides residues

Sr. No.	*Name Pesticides/Insecticides*	*Limit as per FDA/EP*
1	Quinolphos	0.01 ppm
2	DDE	1.00 ppm
3	Alderin	0.05 ppm
4	Dieldrin	0.05 ppm
5	DDT	1.00 ppm
6	DDD	1.00 ppm
7	HCH (Hexa chlorocyclohexane)	0.30 ppm
8	Malathion	0.10 ppm
9	Parathion	0.30 ppm

4.8 SEPRATION AND INDENTIFICATION OF ACTIVE PRINCIPLE: PHARMACEUTICAL EXCIPIENTS

Definition of Excipient

The term comes from the Latin word excipiens, present participle of the verb excipere which means to receive, to gather, to take out. This refers to one of the properties of an excipient, which is to ensure that a medicinal product has the weight, consistency and volume necessary for the correct administration of the active principle to the patient. In 1957, excipients were defined as 'the substance used as a medium for giving a medicament', that is to say with simply the functions of an inert support of the active principle or principles.

Again, in 1974 they are described as 'any more or less inert substance added to a prescription in order to confer a suitable consistency or form to the drug: a vehicle'. This historically somewhat limiting definition referred to those substances employed in the preparation of pills, a now obsolete pharmaceutical dosage form later replaced by tablets and capsules. Natural products, such as molasses and honey, were long employed in the preparation of pills up to 1940 and USP 10 also mentioned lactose, glucose, lycopodium, glycerin and gelatin.

To the function of simple vehicle, galenic science then added that of adjuvant in the carrying and release of the active principle of the formulation. Looking at the matter from this angle, the United States' National Formulary of 1994 states that an excipient is any component other than the active principle added intentionally to the medicinal formulation, or 'everything in the formulation except the active drug'. The interpretation of the adverb 'intentionally' brings to mind the multiple roles that an excipient must play today in a modern pharmaceutical dosage form, suitable for administered enterally, parenterally and topically.

Among these roles are to be remembered those of guaranteeing the stability, precision and accuracy of the dose, improving the organoleptic characteristics and the patient's compliance. Modern pharmaceutical technology also requires verification of the physical state of the excipient, which is so important both in the manufacturing phase and to control the release of the active principle, with the object of improving

the bioavailability and consequently the efficacy and tolerability of the medicinal drug.

Origins and Sources of Excipients

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Generally speaking, excipients account for most of the weight or volume of a medicinal product. In a world pharmaceutical market valued at about £215 billion, that of the excipients amounts to about £1.5 billion, 42% of which in North America, 33% in Europe and 25% in the rest of the world. In 1999, all this corresponded to 600 thousand tons of materials most of which for the food, cosmetic and chemical industries and only a small part for the pharmaceutical industry. Excipients are of various origin: animal (*e.g.*, lactose, gelatin, stearic acid), plant (*e.g.* starches, sugars, cellulose, arginates), mineral (*e.g.*, calcium phosphate, silica) and synthesis (*e.g.*, PEGs, polysorbates, povidone, etc.) and they often lack a trade name. Their origin and use do not often guarantee the quality required by the pharmaceutical industry, which must therefore submit them to more thorough-going analytical controls. In order to carry out the numerous functions required, new classes of excipients have now become available, derived from old and new materials, alone or in combination, adapted to the manufacture of high-performance pharmaceutical dosage forms. Looking at the matter from this angle, excipients can no longer be considered mere inert supports for the active principles, but essential functional components of a modern pharmaceutical formulation. It is also to be borne in mind that the ratio of their weight to that of the active principles is usually very high in a formulation, and such as to cause possible action due to their mass.

Like pharmaceutical drugs, they too have their own thermo-dynamic activity which, though generally low, can contribute to reactions leading to degradation or to interactions between the drug and the excipient. In Table 1 the excipients are set out according to their class and the chemical function that can contribute to their reactivity.

Today it is reckoned that over one thousand different materials are used in the pharmaceutical industry to fulfil its various requirements such as diluents, bulking agents, disintegrants, lubricants, colouring agents, sweeteners, etc. They are chemically heterogeneous compounds that go from simple molecules (water) to complex mixtures of natural, semisynthetic or synthetic substances which, from the regulatory point of view, may be subdivided into three categories. In the first category (approved excipients) we find the compounds originating from the food industry (generally recognized as safe: GRAS) or that have been present in pharmaceutical products for a very long time. The intermediate category (essentially new excipients) covers compounds obtained by means of the structural modification of the excipients already approved or those already used in the food or cosmetic industries. The third category covers new compounds, never previously used in the pharmaceutical field and it is growing rapidly due to the present interest in modified-release formulations and the requirements of the modern high-productivity compressing/tabletting machines.

Table 4.3 Chemical classification and roles of pharmaceutical excipients

Chemical classification	*Roles to enhance*
water, alcohols	compliance
esters, ethers, carboxylic acids	dose precision and accuracy
glycerides and waxes	stability

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carbohydrates (mono-, di- and polysaccharides)	manufacturability
hydrocarbons and halogen derivatives	tolerability
polymers (natural and synthetic)	desaggregation
minerals	dissolution
protein	controlled release
various: preservatives, dyes, sweeteners, surfactants, ...	absorption

Principal Requirements of Excipients

Historically, the importance of excipients in pharmaceutical formulations has generally been underestimated, as they were cheap ingredients viewed solely as inert supports for medicaments. Today, this view is outdated and, on the basis of what we have said above, we may say that excipients are rather more than the sugar in the pill.

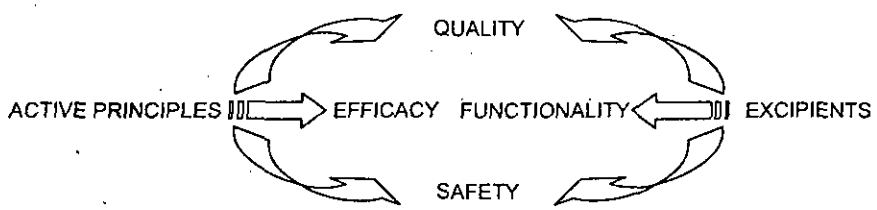

Fig. 4.1 Main requirements for pharmaceutical excipients

At this point we may well ask ourselves what the basic requirements of a modern pharmaceutical excipient are. In Fig. 4.1 the three essential requirements of active principles are compared with those of excipients. Fundamental for both are quality and safety. The requirement of therapeutic efficacy for drugs is replaced by that of functionality for excipients, defined as 'the physical, physicochemical and biopharmaceutical properties' of the same.

Safety has always been the most important requirement and the most studied when dealing with pharmaceutical drugs. Less attention has been devoted to the safety of excipients, because their inertia and innocuity were taken for granted. The considerations made in the preceding paragraphs and the continual evolution of pharmaceutical technology, with the growing use of new 'tailor-made' materials, suggests that a new look at the so-called pharmacological and toxicological inactivity of these components of pharmaceuticals is in order. To this end, we shall examine three issues that may compromise the safety of pharmaceuticals: (*a*) production, distribution and use; (*b*) pharmaceutical-excipient interactions; and (*c*) toxicity, which may be the cause of frequent and sometimes notable 'adverse effects'.

Production, Distribution and use of Excipients

Side effects imputable to excipients were already noted in the 1930s, as in the case of an elixir of sulfanilamide. A number of deaths occurred in Nigeria and India (1990), in Bangladesh (1992) and in Haiti (1996) because of contaminated solvents, *e.g.*, glycerin containing ethylene glycol.

Most excipients are produced in bulk in manufacturing plants that are not devoted to their manufacture. These plants operate with continuous production cycles, and this complicates the traceability of the material, whose quality is often not at pharmaceutical level. Others are isolated from plants or mineral raw materials as opposed to being produced by synthesis. In both cases it is necessary to verify compliance with approved manufacturing guidelines and ISO 9002 standards for quality management systems.

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Particular attention should be paid to the verification of impurities so as to keep them at an acceptable minimum level. Less worrying is their presence when they are functionally necessary and in any case not toxic, such as hemicellulose in microcrystalline cellulose. The definition, classification, sources and verification of tolerated levels of impurities are the subject of a recent publication which gives, among other things, the admissible levels of residual solvents in view of their dangerousness.

The distribution phase of materials and finished products, if not controlled, can also be a source of the poor quality, not to say downright toxicity of excipients. Mention has already been made of contaminated glycerin, which caused the death of eighty children due to acute anuric renal failure. From the manufacturer to the distributor and finally to the patient, this substance travels a long way, during which the characteristics of the excipient can be compromised because of unsuitable warehousing and transport conditions, cross contaminations, errors in labelling and in the traceability of lots.

Finally, some problems arise from improper use, such as parenteral instead of oral administration of a pharmaceutical containing egg albumin. When a generic pharmaceutical is re-formulated, the properties of the new excipients should be carefully evaluated, especially in the case of modified-release formulations. Furthermore, recourse to the use of 'cocktails' of injectables should be allowed only after careful verification of the chemical and physical compatibility of the active principles and their respective solutions.

Pharmaceutical — Excipient Interaction

Excipients constitute the mass or greater volume in the usual enteral or parenteral formulations and, as may be deduced from Table 4.3, they often contain reactive functional groups that may give rise to chemical and physical transformations. Interactions occur more frequently between excipient and active principle than between excipient and excipient and these interactions can be of two types. The physical type of interaction can modify, for example, the speed of dissolution or the uniformity of the dosage of a solid formulation. Indeed, some materials can adsorb drugs to their surfaces, thus increasing the active surface and consequently the wettability and speed of dissolution. The contrary effect may be encountered when the forces of attraction are strong, in which case the drug is released with difficulty and assimilation is compromised. One example is that of lipophilic lubricants (*e.g.*, magnesium stearate) which, when finely dispersed on the particles of the active principle, can slow down dissolution and therefore bioavailability.

The chemical type of interaction, on the other hand, can lead to the degradation of the drug and/or the formation of the so-called degradation impurities. The most frequently encountered reaction is hydrolisis, because water is the preferred and prevalent solvent in liquid formulations. In solid forms which contain hygroscopic components, the presence of humidity must be controlled and reduced. Even the presence of oxygen, when activated by traces of catalysts (heavy metal ions, light, heat...), may give rise to oxidereduction and the formation of free radicals (*e.g.*, lipidic peroxidation). Other, less frequent, reactions are photolysis, isomerisation and polymerisation, which are more likely to occur with certain types of excipients, lowering the title of the active principle and generating dangerous impurities. One example is the presence of polymeric forms in beta-lactam drugs, such as ampicillin, which are thought to be responsible for dangerous allergic reactions. Ionisable pharmaceuticals may react with ionised soluble excipients giving rise to the formation of insoluble products due to charge interactions. In this way, sodium alginate and neomycin cation precipitate in an acqueous solution.

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Another type of interaction may occur between the carbonilic groups of a widely-used excipient like polyvinylpyrrolidone, and pharmaceuticals containing donor groups of hydrogen, like famotidine and atenolol, thus causing problems of incompatibility.

Even silicon dioxide (SiO_2), in anhydrous conditions, behaves like a Lewis acid, giving rise to reactions such as hydrolisis, epimerisation, trans-esterification, etc. One example is the hydrolisis of the imino nitrogen link of nitrazepam with consequent disactivation of the drug.

However, when evaluating potential pharmaceuticalexcipient interactions, it must however be considered that the kinetics of chemical reactions involving solutions are very high, whereas in the case of solid formulations they are low, if not negligible.

Toxicity of Excipients

A discussion of the toxicity of the excipients employed in pharmaceutical formulations is certainly a difficult and extremely diversified task. To simplify matters, the subject may be organised as follows:

- toxic effects encountered in the whole population;
- toxic effects encountered only in specific populations. Into the first category fall all the adverse effects proper to chemical, natural or synthetic substances when a certain dose is exceeded. The second category, however, has to do with phenomena that are often independent of, or only marginally dependent on, the dose. That is to say, they are events linked to specific characteristics of the subjects, such as geneticallytransmitted pathologies (metabolic illnesses, among which phenylketonuria and lactose intolerance) or genetic predisposition (among which diabetes and allergic pathologies).

In principle, excipients ought to be subjected to the same toxicity studies as those requested for active principles, so as to protect the population from undesirable effects. This is assuredly applicable to many compounds, especially those that are used as food additives. However, other substances, that have been used for decades now, can be considered 'safe', given that no adverse effects have been encountered in man. The excipients that have been authorised to be used as food additives have been evaluated as regards toxicology by the JECFA (Joint Expert Committee on Food Additives), which handles the evaluation of the risk from consuming additives or contaminants with food [27a_/d]. In the case of additives, their use is voluntary and has a technological reason, exactly as in the case of pharmaceutical excipients, whereas contaminants are substances that can be vehicled by the food chain, given the ubiquitousness of the distribution of pollutants in the environment. In this article, our preferred source of toxicological data has been the JECFA's conclusions, which take into particular consideration the results of long-term toxicological studies. The JECFA usually terminates its toxicological evaluations with the publication of an Admissible Daily Intake (ADI), which represents the dose that does not carry risks to the population if taken every day for a life-time. This dose is expressed in mg (or microg) per kg of daily weight per day. To establish the total daily dose, we should multiply this number by the bodily weight (usually reckoned as 60 kg). The value of the ADI is extrapolated from studies conducted in laboratory animals, dividing the highest dose without toxic effects in the animal by a safety factor (generally 100).

Toxic Effects Encountered in the Whole Population

In general, it may be said that any compound, and therefore any excipient, can have adverse effects if taken in a sufficiently high dose. This concept was already

well known to Paracelsius in the fifteenth century, when he said 'Omnia venenum sunt, nec sine veneno quicquam existit; dosis sola facit ut venenum non sit.' In practice, every substance is a poison, at the right dose.

Adverse effects due to excipients are, fortunately, infrequent and mild, because excipients are generally selected because of their low toxicity. Their adverse effects may be imputable to direct toxicity, to immunotoxicity, to allergy or intolerance. As we cannot discuss the toxicity of all the compounds on the market, we shall limit ourselves to giving some examples within the principal chemical classes to which they belong. It is, in fact, possible to think that particular structures and/or substituents characterise both the chemical and physical properties, such as the dissociation constant and the distribution coefficient, and the reactivity of an excipient (*e.g.*, oxide-reduction potential).

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A first group of excipients with at least one phenolic function is shown in Table 4.2 where, besides the structural formulae, some of the most frequent side effects are listed. These excipients are added to formulations in very low concentrations (0.01 – 0.3%) as antiseptics and for their antioxidant and anti-free radicals activities. Given their antioxidant activity, this category always includes molecules with strong biological activity.

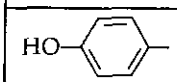
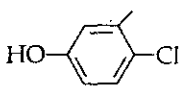
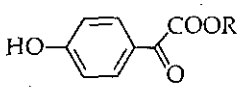
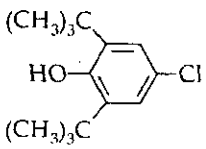
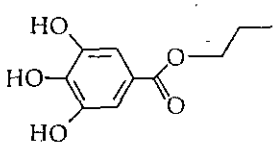
The results of experimental studies conducted on laboratory animals with some of these has given rise to some perplexity, their wide use in foodstuffs notwithstanding. The perplexities surrounding BHA (butyl hydroxyanisol) originated due to the appearance of hyperplasia and carcinoma of the prestomach in rats that underwent chronic treatment with BHA at the dose of 20 g/kg diet/die. Studies conducted in other animal species like man who has no prestomach, such as the pig, the dog and the monkey, however, excluded the possibility of developing hyperplasia of the stomach following chronic administrations of BHA, unless the doses were very high. In any case, deeming that the cancerogenous effect in the rat must not be ignored, the JECFA established an ADI (admissible daily intake) of 0–0.5 mg/kg body weight/die, which means about 30 mg/die 27b. A recent evaluation [27d] indicates that the intake of BHA in the average diet can get close to the admissible daily intake. This means that the additional intake of BHA due to pharmaceutical formulations could in some cases be a determining factor in exceeding the levels recommended by the toxicological committees. Particular attention ought therefore to be given to the use of this antioxidant, at least as regards medicines for oral use. In the case of BHT (butyl hydroxytoluene), the toxic effects most commonly encountered in laboratory animals after chronic administration are lesions to the hepatic cells, which are probably attributable to its inductive action on the hepatic enzymes. On the basis of the various studies taken into consideration, the JECFA defined an ADI of 0–0.3 mg/kg body weight/day, which means 18 mg/die [27c]. What was said about BHA alsoapplies to BHT, namely that it is necessary to verify whether the use of BHT in pharmaceutical formulations can contribute to exceeding the ADI.

A particular group of antioxidants is composed of sulfites: sodium and potassium bisulfite, sodium and potassium metabisulfite, sodium sulfite and sulphurous anhydride. Sulfites are widely used as antioxidants in both the pharmaceutical and the food industries. For this group of substances, the JECFA has established an ADI value of 0–/0.7 mg/kg body weight/die or 42 mg/die [27d]. As in the case of BHA and BHT, evaluations have been conducted on the average intake of this group of substances with food. Once again, based on the most conservative reckoning, we find dietary intake values very close to the ADI; therefore in this case, too, the quantity added by pharmaceutical products should be evaluated with the aim of estimating the total intake more closely.

In the case of sulfites, it must be borne in mind that there is an aspect of hypersensitivity concerning the populations at risk (see below). Hypersensitive reactions are characterised by broncoconstriction, pruritis, urticaria, chest pain, angioedema, and hypotension leading sometimes to loss of consciousness. Normally, these reactions concern patients who have already suffered attacks of asthma and usually appear when they eat food containing sulfites. Much less frequent are adverse phenomena associated with the use of sulfites in pharmaceutical preparations, often due to 'paradoxical broncospasm'. Numerous compounds with alcoholic function are included among pharmaceutical excipients as solvents and preservatives. Table 4.3 lists four with their better- known relative toxic effects. In this case we shall limit ourselves to giving one example of toxicological evaluation, and shall consider the polyethylenic glycols.

Numerous short- and long-term studies have been conducted on these compounds, on several different animal species. It has been observed that toxicity depends in inverse proportion on the molecular weight (the lower the molecular weight, the greater the adverse effects). This phenomenon depends, at least partly, on the lower intestinal absorption of the glycols with higher molecular weight. For this group of compounds, the JECFA has established an ADI of 0 – 10 mg/kg body weight/die, or 0.6 g/die [27a]. In view of the considerations expressed above, the type and quantity of glycols included in pharmaceutical formulations should be carefully evaluated.

Table 4.4 Phenolic excipients

Phenolic excipients		
	carbolated vaseline (0.2%)	-irritant, dermatitis
	bactericidal, fungicidal	-irritancy, delayed
	preservative	-contact dermatitis
	parabens	-contact dermatitis
	antimicrobial (0.01-0.3%)	-hypersensitivity
	BHT (and BHA)	-animal toxicity data
	antioxidant	-one case contact derm
	PG	-methaemoglobinemia
	antioxidant (0.002-0.01%)	-sensitisation, pruritus

The substances belonging to the group of organomercurial compounds are, by definition, non-inert molecules from the biological point of view, as their principal function is that of inhibiting or arresting the growth of micro-organisms. Many of these have been used for a long time (Table 4.6) and some are well known to have caused adverse effects in man. Among the most controversial antibacterials, we cannot fail to mention thimerosal, which is often included as an antibacterial in liquids for the preservation and disinfection of contact lenses. Although significant toxic effects on the cornea or epithelial cells have not been encountered, cases of hypersensitivity are frequent among wearers of contact lenses (about 10% of the population concerned).

Another negative aspect of this compound is the fact that it vehicles mercury, a metal that is well known to be toxic. For example, it has been observed that patients undergoing ophthalmic therapy with products containing thimerosal showed

increases in the concentrations of mercury in both blood and tissue. In view of all the negative aspects described above, the use of this antibacterial has been considerably reduced, besides having been banned from numerous pharmaceutical products.

Table 4.5 Excipients with alcoholic functions

C_2H_5OH	
included in hundreds of liquid pharmaceutical preparations	– drug interactions
	– ethanol intoxication (conc. 25 mg/dl)
	– cardiac effects by parenteral infusion in patients with heart desease
$C_6H_5-CH_2OH$	
at low conc, (0.9—2%) as a preservative	– neurological effects
at high conc, (³ 5%) as a solubilizer	– fatal syndrome in premature infants at 99 – 234 mg/kg/day (benzoic acid?)
$CH_3-CH(OH)-CH_2OH$	
as a solvent in i.p., oral, topical	– cardiovascular effects by parenteral infusion
aerosol preparations	– neurological effects
as a preservative	– metabolism to pyruvate and lactate
	– otologic effects
	– thrombophlebitis due to iv diazepam
$HOCH_2-(CH_2-O-CH_2)_n-CH_2OH_2$	
as enhancers for nasal delivery	– only mild local toxicity (no oxalic acid)

The greater part of halogenated hydrocarbons shown in Table 4.7 are derived from methane and ethane, which are very stable chemically (apart from their effect on ozone). Freons are volatile lipophilic excipients used as propellants in sprays based on beta-agonists and cortisones.

When administered in high concentrations to young patients, they may give rise to rare cases of sudden death, which seems due to cardiac effects, particularly arythmias, like those that can be observed with halothane. Chlorbutol is a volatile excipient that has found its use as a bland dental analgesic and as an antimicrobial.

A particular class of excipients is that of the colourants, which are used to improve the aspect of a drug and to avoid confusion during the manufacturing phase or in administration. From the chemical point of view, this class is very heterogeneous for colourants may be of plant, animal or mineral origin, like red and yellow ferrous oxides. Among the synthesised compounds (Table 4.8) the azoics are included for their excellent colouring properties but they are notoriously well known for causing death among the workers in the manufacturing phase, exposed to contact with intermediates and toxic aromatic ammines. Some colourants were banned in some countries (*e.g.*, Italy) because of their cancerogenous activity encountered in laboratory animals, though not confirmed in man. In 1971 some cases of intolerance to Premarin were described: besides oestrogens, this product contained all of 28 excipients. One of these, tartrazine was responsible for the adverse effects.

Toxic Effects Encountered Only in Specific Populations

The case of Premarin and other colourants employed in both the food and the pharmaceutical industries render their inclusion in pharmaceutical products in general inadvisable and more particularly so in antihistaminic preparations, which

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are aimed mostly at allergic patients. As shown below, these toxic effects concern only some consumers but this does not mean that these effects are less important.

The groups at risk to be considered with attention are people with allergies and intolerances. The former involve the immunitary system, whereas the latter are determined by genetically-transmitted metabolic anomalies (phenylketonuria, galactosemia, etc.) or family predisposition (celiachia, diabetes, etc.).

Contrary to what is generally supposed, the greatest risk from colourants is associated with products of natural origin, because the extraction processes may leave traces of proteins, which are mainly responsible for allergic phenomena. In the literature there is the description of the case of an erythromycin syrup which gave some patients bad abdominal pain accompanied by nausea and vomiting, due to the presence of tincture of orange as flavouring. As colouring and flavouring agents do not carry out an important function in a pharmaceutical product, their use should be avoided in products aimed at patients who are already known to suffer from allergic path-ologies.

Table 4.6 Organic mercurial salts

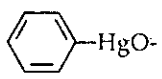
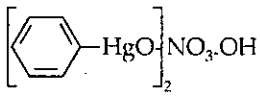
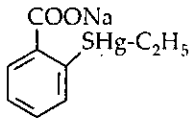
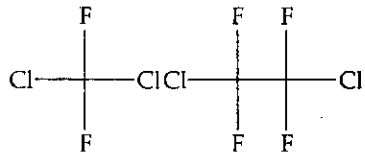
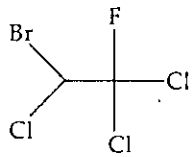
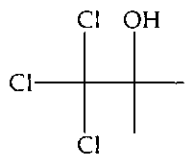
Organic mercurial salts		
	antibacterial, antifungal	teratogenic effects on animals
	(conc. 0.001% ip, 0.004%)	mercurialentis, hypersensit.
	ophthalmics (0.02% vag.)	primary irritants at low concen.
	antiseptic, antimicrobial	hypersensitivity (10% lens weavers)
	(conc. 0.01% eye drops,)	contact dermatitis, mercurial toxicity
Thimerosal	0.02% in biologicals)	due to manufacturing error

Table 4.7 Halogenated hydrocarbons excipients

Halogenated hydrocarbons excipients	
	-cardiac effects at high come.
	-sudden death
Freons as aerosol propellants	
	-cardiac effects
	-bradycardia
	-arrhytmias in dogs
Halotane as anesthetic	
	-drop pressure
	-CNS effects, sonnolence
Chlorbutol as dental analgesic and as antimicrobial	

Many of the four thousand or so products used in cosmetics, both natural extracts and those of synthetic origin, are employed as perfumes in formulations for topical

use. In this sector, too, adverse effects are known to occur in sensitive populations: the most common clinical symptom is eczematous dermatitis. In order to protect sensitive populations, the perfume should be clearly identifiable from the label but, as said before, the use of perfume should be avoided in any case in antihistaminic products, at least in those that are used to reduce the allergic symptom.

NOTES

Gluten is a proteic component of wheat to which bearers of celiac illness are sensitive. The classic manifestation of these patients' intolerance is an enteropathology (malabsorption syndrome), but atypical symptoms such as herpetiform dermatitis, iron deficiency anæmia, alopecia and osteoporosis are also encountered. The pathology is linked mainly to gliadine, composed of different proteic fractions, the most toxic of which is the alpha fraction. The therapy for celiac patients consists solely in the elimination of gluten from the patient's diet for the whole of his life. Naturally, abstention from gluten must include abstention from pharmaceutical products that contain gluten among their excipients.

Lactose, a common pharmaceutical excipient, can also induce intolerance due to the physiological diminution of lactase activity in adults. Many patients experience difficulty in tolerating lactose (in Italy, about 70% of the population), even though symptoms may appear after intake of widely varying amounts. Clinical symptoms: for the more unfortunate even traces of lactose can unleash severe intestinal crises. Consequently, lactose should not be included in antispastic medicaments.

The discovery of BSE (bovine spongiform encephalopathy) and its transmissibility to man have recently brought about restrictive regulations as regards the use not only of lactose but also of bovine gelatin and derivates of tallow.

Medicinal products can be considered a dosed combination of two types of constituents: the active principles and the excipients. The latter are the more important as far as weight is concerned, whether in solid forms, suspensions or solutions. The ideal excipient should be able to fill numerous and important functions, first among which that of guaranteeing the dose, stability and release of the active principle, and the patient's 'compliance'. Furthermore, it should possess particular chemical, physical and mechanical characteristics, so as to optimise the formulation's 'perfor-mance' both during the manufacturing phase (manufacturability) and when used by the patient. This multiplicity of roles fits very ill with the traditional galenic view, that saw these 'non-medicinal ingredients' as chemically and pharmaco-toxi-cologically inert.

For a long time now, much attention has been paid to the required quality, efficacy and safety of active principles but only recently has the necessity emerged of examining not only the quality and performance but also the safety of the excipients. The problem is not simple if one considers that in countries like the United States, Japan and Europe there are now in use over a thousand excipients of varying origin, of more or less complex structure and belonging to different chemical classes. About one fifth of them are present in the respective Pharmacopoeiae, which list the pharmaceutical quality requirements but not physical chemistry requirements, much less do they embark on questions of safety. Some information on this aspect may be gleaned from some texts. This under-estimation of the safety aspect is also a consequence of the fact that the first excipients were taken from the food industry and therefore considered 'as safe', or else they were already used in pharmaceutical products that had been in therapeutic use for a very long time. Today it is required that any chemically new product whose effects on man are not known must pass all the toxicological tests envisaged for an active principle before it can be accepted as an excipient.

In this unit, we have focused our attention on the principal causes that can compromise the safety of excipients, first of all the quality of the raw materials on

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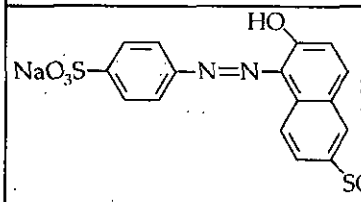
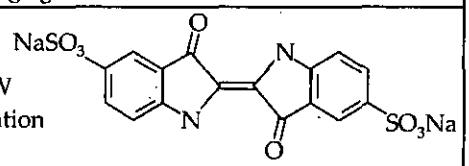
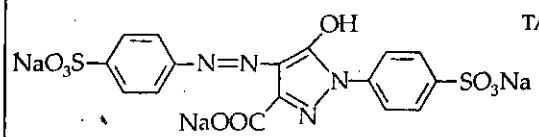
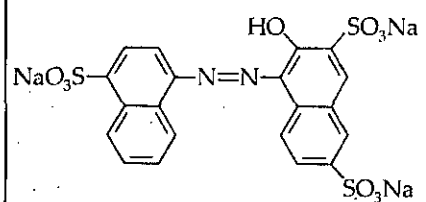
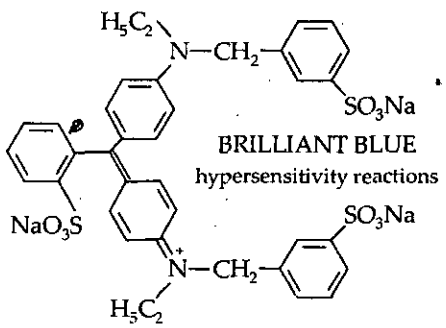
the market, which is often unsuitable for pharmaceutical use. Moreover, these so-called 'inert ingredients' can sometimes interact with the active principles, lowering their titre and generating undesirable impurities, or altering the assimilation processes and the bioavailability of the drug itself. These problems may be overcome by adopting and carefully adhering to good manufacturing practices similar to those for active principles (GMPs).

Very serious risks to the patients can be caused by improper conditions along the distribution chain an-d the transport of the materials. In this sector, too, some guidelines are being drawn up to guarantee the identity, quality and traceability of lots at any moment, from the manufacturer to the end user.

Table 4.8 Dyes and colouring agents

Dyes and colouring agents	
	SUNSET YELLOW Urticaria exacerbation
	INDIGO CARMINE
	TARTRAZINA Headaches, g.i. Disturbance, exacerbation of asthma, dangerous in Aspirin intolerant individuals
	AMARANTH potential carcinogenicity (banned)
	BRILLIANT BLUE hypersensitivity reactions

It has also been mentioned that the improper use of a medicinal product can be dangerous. In order to avoid this, it is necessary to improve the information about the excipients given on the packaging and the patient's information leaflet, so that doctors, pharmacists and patients know all the ingredients included in a preparation.

Furthermore, patients must be instructed to follow prescriptions more closely. The safety of a medicinal product can also be compromised by the intrinsic toxicity of the excipients themselves. Fortunately, this happens rarely because, by definition, excipients are usually chosen from among the materials noted for being very nearly pharmaco-toxicologically inert. In this article we have elected to subdivide them, where possible, into different classes characterised by a particular chemical function to which their activity can be referred (*e.g.*, halogenic function, phenolic function, etc.). Furthermore, examples of adverse reactions imputable to the intrinsic toxicity of the exc-ipient have been listed as distinct from those due to the specific characteristics of patients. The latter involve cases of genetically-transmitted pathologies of metabolic origin and pathologies due to genetic predisposition, such as rather frequent allergies.

The examples given here point towards the advisability of simplifying formulations as much as possible, so as to reduce the number of excipients to those strictly necessary

to fulfil the required functions of the medicinal product. In the same way as for the active principles, the risk/benefit ratio of an excipient should be evaluated on the basis not only of its performance, but also of its safety. It would therefore be useful if the pharmaceutical technologist could avail himself of an inventory of approved excipients, distinguished by a clear international nomenclature (INN system), and listing the relative maximum doses for each administration route. Another important aspect concerns the regulations and guidelines on excipients already published or in an advanced state of revision, along the lines, duly adapted, of those already available for active principles.

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SUMMARY

- Natural products today are most likely going to continue to exist and grow to become even more valuable as sources of new drug leads.This is because the degree of chemical diversity found in natural products is broader than that from any other source, and the degree of novelty of molecular structure found in natural products is greater than that determined from any other source.
- Fungal disease has historically been a difficult clinical entity with which to effectively deal. Fungal diseases can include more than just a mycosis and can also include allergic reactions to fungal proteins and toxic reactions to fungal toxins.
- The incidences of neuropsychiatric disorders are steadily increasing as our population increases in size and age. Such disorders include, but are not limited to, seizure disorders, schizophrenia, dementia, mania, aggression, memory loss, psychoses, age-related cognitive decline, depression, anxiety states, mood disorders, substance abuse, and substance dependence.
- A particular group of antioxidants is composed of sulfites: sodium and potassium bisulfite, sodium and potassium metabisulfite, sodium sulfite and sulphurous anhydride. Sulfites are widely used as antioxidants in both the pharmaceutical and the food industries.
- Medicinal products can be considered a dosed combination of two types of constituents: the active principles and the excipients.

REVIEW QUESTIONS

1. What do you understand by herbal and advantages of herbal products?
2. Discuss the standardization of herbals and its need and responsibility.
3. Discuss the private sector effort in herbals.
4. Discuss the public sector effort in herbals.
5. Discuss active principle: pharmaceutical excipients.

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.

PART 3: STABILITY

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UNIT I: STABILITY OF DRUGS: PHYSICAL STABILITY

★ STRUCTURE ★

LEARNING OBJECTIVES

After going through this unit, you should be able to:

- describe the international conference on harmonization.
- define the physical and chemical stability.
- explain the effects of microbial instability.
- discuss about the mechanism of degradation hydrolysis.

1.1 INTRODUCTION

You all must have noticed in daily life, that all objects suffer from what we may call a *'wear and tear'*. All living beings go through a cycle of birth-growth-reproduction and death. All nonliving beings that are generated or grown or manufactured go through a life span in which they influence and are influenced by their environment. Metals corrode and wood is decomposed by the environment. The environment is spoiled by the presence of some chemicals such as benzene, carbondioxide and methane. So what we may call as a material-environment interaction influences the longevity and purity of all items.

Food as we all know has to be well preserved if we want it to retain its quality. If raw materials such as rice and wheat grains are not stored at the proper temperature

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and humidity they are spoiled by microbial contamination. Food is spoiled by three varieties of decomposition; physical, chemical and microbiological. Have you noticed that rice grains left alone stay fine for years together; rice made into flour is less stable (with time it develops a musty odor and off color) and rice processed into food is stable only for a few hours. My point is "more processing- less stable"; "more contact with water – less stable".

All this is true for drugs also. Pure drugs, solids, liquids, or gases are usually more stable than their formulations. When they are formulated into medicines decomposition happens faster because of the presence of excipients, and moisture and because of processing. So when we say stability, we actually are talking about two varieties of stability; that of the drug and that of the formulation. For example a capsule may become soft, an emulsion may break, a suspension may cake and an ointment may bleed.

Even if solid pure powders of drugs are stored under ideal temperature and humidity conditions – even then there is some degradation. For example consider aspirin. If you take a bottle of an old sample of pure aspirin and smell it you can clearly feel the unmistakable odor of acetic acid. If you granulate aspirin and make it into tablets the rate of decomposition will be faster and if you formulate it into a suspension it will decompose totally into acetic acid and salicylic acid in less than 25 days. This is because the breakdown is a chemical reaction involving the collisions of the molecules, collisions having sufficient energy and the molecules having the proper orientation. This breakdown is affected by various factors such as presence of oxygen, acidity, alkalinity, moisture and light. This breakdown is what we are calling degradation. This is the reason why the manufacturers of medicines are bound by rules to put an expiry date on the medicine. In fact if they cannot put an expiry date then they have to explain reasons for that.

So the researchers in charge of the formulation development do extensive studies to understand the mechanism of degradation and the rate of degradation. Degradation happens because the molecules are hitting one another (like agitated football players in a big field running madly without looking and hitting one another).But there is a method in this madness! And it is a pharmaceutical technologist's job to find the factors that enhance or reduce this madness (Light? Heat? Air? Acidity? Alkalinity?). And it is a pharmaceutical chemistry man's job to find the mechanism of this madness- how is it triggered and how is it happening? Oxidation? Hydrolysis? Racemisation? Photolysis?

So then dear students, I would like to bring your attention on to three different angles of stability, physical, chemical and microbiological. It is imperative that the medicine that we are giving, is stable in its entirety from every angle throughout its shelf life period. Often for a new formulation many batches go into the market and a lot of time elapses before all angles of the stability of the product are understood.

The study of the changes that happen in a dosage form as it stands on the shelf of a drug store constitute the contents of the chapter titled as "Drug Stability". Physical stability implies that the formulation is totally unchanged throughout it shelf life and has not suffered any changes by way of appearance, organoleptic properties, hardness, brittleness, particle size etc. The drug release nature (rate and mechanism) should not be altered. Different formulations suffer from different physical challenges and are tested for different stability criteria. Drug release is important from safety and efficacy angle and the physical stability of the formulation is significant because

NOTES

of its effect on pharmaceutical elegance and on drug content uniformity and drug release rate. Chemical stability implies the lack of any decomposition in the chemical moiety that is incorporated in the formulation as the drug. Chemicals present in the formulation as preservatives or as other excipients may also decompose and their decomposition may influence the physical and chemical stability of the drug. But to test for the chemical stability of a formulation we test for the drug content of the formulation. When we say microbiological stability, we imply that the formulation has not suffered from any microbiological attack and is meeting the standards with respect to lack of contamination/sterility which we have claimed when we kept the product on the shelf.

1.2 INTERNATIONAL CONFERENCE ON HARMONISATION (ICH)

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a unique project that brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration.

The ICH guidelines are submitted to the Committee for Human Medicinal Products (CHMP) for endorsement once they have reached Step 2 or Step 4 of the ICH Process. The CHMP, in consultation with the European Commission decides on the duration for consultation with interested parties (up to 6 months).

The European Agency for the Evaluation of Medicinal Products publishes and distrubutes the Step 2 guidelines for comments. At Step 4 the guidelines are endorsed by the CHMP and a timeframe for implementation is established (usually 6 months).

The guidelines are subsequently published by the European Commission in the Rules Governing Medicinal Products in the European Union (http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/index.htm). Step 2 and Step 4 guidelines are available from the European Medicines Agency site on the Internet:

<http://www.emea.europa.eu/htms/human/ich/background.htm>

MHLW

When Step 2 or Step 4 has been reached, the ICH texts are translated into Japanese. Subsequently Pharmaceutical and Medical Safety Bureau (PMSB) Notification for the promulgation or consultation of guidelines written in Japanese is issued with a deadline for comments in the case of consultation drafts, or an implementation date for finalised guidelines. The notifications on guidelines in Japanese and also English attachments (ICH Texts) are available from PMSB or on the Internet by the Pharmaceutical and Medical Devices Agency (PMDA). http://www.pmda.go.jp/ich/ich_index.html

FDA

When Step 2 or Step 4 has been reached, FDA publishes a notice with the full text of the guidance in the Federal Register. Notices for Step 2 guidances include a date for receipt of written comment; Step 4 guidances are available for use on the date they are published in the Federal Register. FDA guidances and guidelines are available on the Internet:

CDER: http://www.fda.gov/cder/guidance/index.htm

CBER: http://www.fda.gov/cber/guidelines.htm

1.3 PHYSICAL STABILITY

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The job of the medicine is to give the therapeutic effect and so the most important thing is that the required drug content is available till the expiry date is over. But the appearance and lack of any change in a physical sense are also important as the patient only observes the exterior and any change from the original position may frighten him or bother him. Some physical changes can have deleterious effects too. An emulsion may crack into two phases and thus different doses drawn from it may have different drug contents. A suspension may have a hard cake as a sediment; as a result of which the supernatant may be bereft of all drug and a dose drawn from the bottom of the bottle may have toxic amounts of drug. A tablet may become soft and ugly or it may become very hard and show very slow dissolution time as a result of which bio-availability may not be good. *Stability is the capacity of a drug product to remain within specifications established to ensure its identity, strength, quality and purity.*

So it is absolutely essential that for all formulations all areas in which instability is likely to occur are understood and stability is tested for. With each formulation the problems are different and their effects and solutions possible are also different. Table 1.1 tries to summarise the instability possibilities in different formulations.

1.4 CHEMICAL STABILITY

Let us try to understand what is known as chemical stability. I said earlier that the rate of decomposition depends on the frequency and intensity of the collisions of the molecules. Let us take off in this class from that point. When you cut an apple and expose it to the air very quickly it gets deep brown colour-it is getting oxidized. Milk is being turned into curd by a reaction known as fermentation. Every one knows the smell of a poorly stored sample of oil- which is called a rancid oil. What has happened to the oil? It has been hydrolyzed! Lipolytic enzymes in the presence of moisture hydrolyze oils and fats producing glycerol and free fatty acids and thus develop rancidity in fats and oils. So to store oil properly we have to exclude air, light, microorganisms and moisture. In all these reactions; hydrolysis, or oxidation or fermentation a common villain is moisture. All reactions are speeded up in the presence of moisture. Why? **Moisture gives a fertile ground for the growth of microorganisms.** Moisture takes part as a reactant in many chemical reactions and plays the role of a **solvent vector** in many reactions. It has better thermal conductivity than solids and allows better heat transfer-hence molecules have more kinetic energy and you observe more decomposition. So, we can put a **ditto** below foods and write about drugs. All that is true for foods is also true for drugs.

But I want to take you a little deeper first and make you see how a chemical reaction is happening-whether it is oxidation or hydrolysis. Let us try to remember the second law of thermodynamics-a spontaneous reaction always proceeds towards greater entropy (more disorder) and towards loss of free energy. A chemical reaction also tends to occur if it increases the disorder of the system.

Table 3.4

Formulation	*Likely physical instability problems*	*Effects*	*Stability testing*	*Steps to prevent instability*
Oral solutions	1. Loss of flavour 2. Change in taste 3. Presence of off flavours due to interaction with plastic bottle 4. Loss of dye 5. Precipitation 6. Discoloration	Change in smell or feel or taste	A "tester" should smell taste or feel the product and judge it qualitatively and quantitatively. The depth of taste may be judged for example as degree of saltiness or sourness on a scale of 1-5. The depth of flavour may be judged as type of flavour or level of flavour on a scale of 1-5. A colour standard may be used to describe the "intensity" of the discoloration. Clarity should be studied.	Use of proper excipients and suitable packing materials.
Parenteral solutions physical instability occurs due to: 1. Interaction of the contents with the container. 2. Changes in Chemical composition.	1. Discoloration due to photo chemical reaction or oxidation. Ex: thiamine hydrochloride 2. Presence of precipitate due to interaction with container or stopper. 3. Presence of "whiskers". If some mall pinholes are present in the ampule due to improper sealing the solution wicks out, the liquid evaporates and the solid settles on the outside. It further helps in wicking out more solution and long lines of crystals form on the outside of the vial which are called whiskers. This may happen due to small hole (<0.5 μm) going undetected or the crack developing during storage. 4. Clouds: A cloud will appear in the product due to: (*i*) Chemical changes	Change in appearance and in bio-availability. Change in appearance and in bioavailability.	Use of a colour standard to describe the "intensity" of the discoloration. Extent of precipitate may be counted using a Coulter Counter or the number of vials having a precipitate may be counted. Since this precipitate formation is due to a reaction with container or stopper we have to store vials for testing by placing vials in different positions such as (1) upright, (2) on the side and (3) upside down. Periodic observation Study of clarity, pH, sterility, pyrogenicity, volume (for plasticcontainers) and extractables (for plastic containers) Clouding which may be a prelude to precipitation may occur due to loss of viscosity and this change in viscosity may be followed with a Cup and Bob	Use of antioxidants (0.5%) Acetylcystane or 0.02 — 1% Ascorbic acid) or Chelating agents (0.01 — 0.075 sodium edetale) to prevent discoloration.Change in stopper or material of the container will eliminate the problem. Checking of the manufacturing process Increasing solubility by the use of cosolvents (eg: polyethylene glycol) or by other methods such as micellar approach or complexation will reduce clouding.

	(an ester *e.g.,*: polysorbate may hydrolyse producing an acid which is poorly soluble). (*ii*) Solubility product may be exceeded. (*iii*) The original preparation of a supersaturated solution or the use of a metastable form (ex: calcium gluceptate).		Viscometer. Drastic changes in viscosity may happen due to bacterial contamination.	
Suspensions	Settling, caking, crystal growth	Loss of drug content uniformity in different doses from the bottle and loss of elegance.	1. Subjective tests involving shaking the bottle. 2. Rotating the bottle under reproducible conditions and analyzing the supernatant for drug content. 3. Freeze-thaw testing. 4. X-ray diffraction study 5. Study of sedimentation rate and sedimentation volume 6. Chemical testing for the amount of the preservative 7. Study of ph.	Design of product based on proper pre-formulation studies.
Emulsions	Creaming or cracking	Loss of drug content uniformity in different doses from the bottle and loss of elegance.	1. Study of globule size 2. Study of rheological behaviour with a Cup and Bob Viscometer. 3. Study of pH.	Design of product based on proper pre-formulation studies.
Semisolids (Ointments and suppositories)	1. Changes in: (*a*) Particle size, (*b*) Polymorphic state, or hydration or solvation state (*c*) Consistency (*d*) drug release rate	Loss of drug content uniformity, loss of elegance and change in drug release rate.	1. Consistency by penetrometer 2. Feel to the touch 3. X-ray diffraction studies. 4. In-vitro drug release study with a diffusion cell.	Design of product based on proper pre-formulation studies.

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	2. Caking or coalescence. 3. Bleeding			
Tablets	1. Change in 2. (*a*) Disintegration time (*b*) Dissolution profile (*c*) Hardness. (*d*) Appearance	Change in drug release	Testing for hardness, disintegration time, dissolution, appearance, friability, moisture content.	Design of product based on proper pre-formulation studies.
Capsules	Change in (*a*) Appearance (*b*) Dissolution, and (*c*) Strength	Change in drug release	Testing for strength, moisture content, appearance, brittleness and dissolution.	Design of product based on proper pre-formulation studies.

Unimolecular Reaction

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Remember that we are talking about several types of reactions, such as, (1) the splitting up of a molecule into two units, (2) the transformation of an isomer from one form to another, (3) the reaction between two different molecules, and so on. For all these reactions some basic steps are necessary. I will explain a unimolecular reaction first, with the example of the transformation of a Cis-dichloroethylene to trans-dichloroethylene.

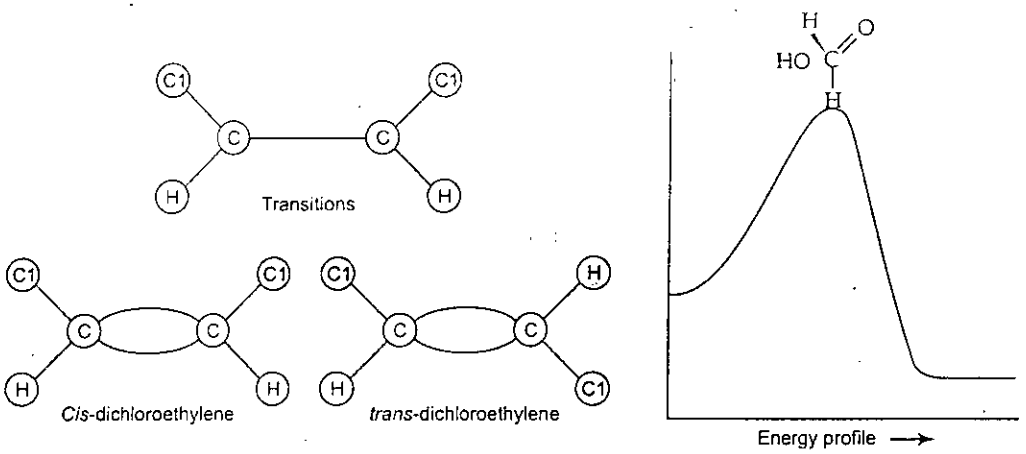

Fig. 1.1 Energy profile for the conversion of Cis-to trans-dichloroethylene

The energy of a molecule going through this conversion process is represented in Fig. 1.1. A molecule of the cis compound which is stable and is having low energy, picks up energy as it changes to the transition state configuration and then releases energy as it rolls downhill to the trans form. The abscissa of the graph, called the reaction coordinate is a measure of the progress of the reaction. For this reaction to take place, a molecule of cis-dichlorothylene must gain an amount of energy shown in Fig. 1.1 as Ea, which is called the activation energy for the process. However, all the molecules which have sufficient activation energy do not transform into the product; some of them shed their excess energy and return to the starting state rather than proceeding to form the reaction product. We can write a rate equation as:

$$\text{Rate} = \begin{pmatrix}\text{rate for}\\ \text{enegized}\\ \text{molecules}\end{pmatrix} \times \begin{pmatrix}\text{fraction of}\\ \text{molecules}\\ \text{energized}\end{pmatrix} \times \begin{pmatrix}\text{total}\\ \text{number of}\\ \text{molecules}\end{pmatrix}$$

In concentration units

$$\text{Rate} = \begin{pmatrix}\text{rate for}\\ \text{energized}\\ \text{molecules}\end{pmatrix} \times \begin{pmatrix}\text{fraction of}\\ \text{molecules}\\ \text{energized}\end{pmatrix} \times \begin{pmatrix}\text{concentration}\\ \text{molecules}\end{pmatrix}$$

It was established by experimentation that

$$\begin{pmatrix}\text{Frtion of molecules having energy}\\ \text{equal to the activation energy}\end{pmatrix} = e^{-(E_a/RT)}$$

Wher R is the gas constant and T is the absolute temperature

$$\text{So rate} = \begin{pmatrix}\text{Rate for energized}\\ \text{molecules}\end{pmatrix} \times e^{-(E_a/RT)} \times A$$

Where [A] = concentration of reactant A.

NOTES

When experiments were done on several chemical reactions, the exponential dependence of rates on temperature was established. The rate for energized molecules is a temperature independent factor and varies over wide limits (109 – 1016 sec-1) but many results cluster around the value of 1013 sec-1. So for a unimolecular reaction:

$$\text{Rate} = k\ [A]$$

$$K = p\, e^{-(E_a/RT)} \qquad \ldots(1)$$

Where ρ = frequency factor

= rate for energized molecules

$$2.303 \log k = 2.303 \log \rho - Ea/RT.$$

Bimolecular Reaction

In a bimolelcular reaction two molecules react with each other to form products. Molecules must collide to react. These collision rates are much faster and only a fraction of the molecules colliding result in reaction. The rates of these reactions also increase with temperature just as unimolecular reactions do and they also have to reach or attain an activation energy to result in a reaction.

Taking an example of the reaction of a hydroxide ion with methyl iodide,

$$HO^- + CH_3\,I \rightarrow CH_3OH + I^-$$

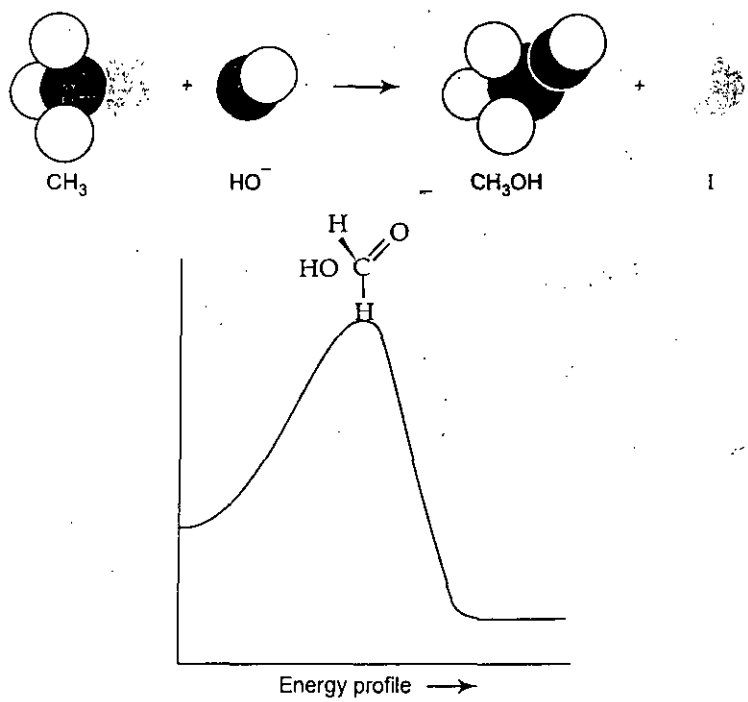

Fig. 1.2 Energy profile for the reaction of hydroxide ion with methyl iodide

Just as we have written for the unimolecular reaction let us write a rate expression in terms of molecular collisions for the bimolecular reaction also.

$$A + B \rightarrow \text{products}$$

the rate is $$\text{rate} = \begin{pmatrix}\text{Rate of collision between A and B}\end{pmatrix} \times \begin{pmatrix}\text{fraction of collisions with enough energy}\end{pmatrix} \times \begin{pmatrix}\text{fraction of energetic collisions resulting in reaction}\end{pmatrix}$$

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The last factor in the rate expression is called the "orientation" factor. It tells us that all the energetic collisions do not result in a reaction - this is because they are not properly oriented to cause a reaction. This term corresponds to the ρ factor in the unimolecular rate expression (Eq.1). In this example, collision of a hydroxide ion with the iodine atom of methyl iodide would not result in reaction. Only collisions involving direct contact of the oxygen atom with the carbon atom would be effective in causing reaction to occur. The collision rate is a number that depends on the concentrations of the molecules colliding.

$$\text{rate} = \begin{pmatrix}\text{Collision rate}\\ \text{when [A]=}\\ \text{[B]=IM}\end{pmatrix} \times \begin{pmatrix}\text{fraction of}\\ \text{collisions}\\ \text{with enough}\\ \text{energy}\end{pmatrix} \times \begin{pmatrix}\text{fraction of}\\ \text{energetic}\\ \text{collisions resulting}\\ \text{in reaction}\end{pmatrix} \times [A] \times [B]$$

or rate = $\rho e^{-(Ea/RT)}$ [A] [B]

The bimolecular rate constatn for the reaction

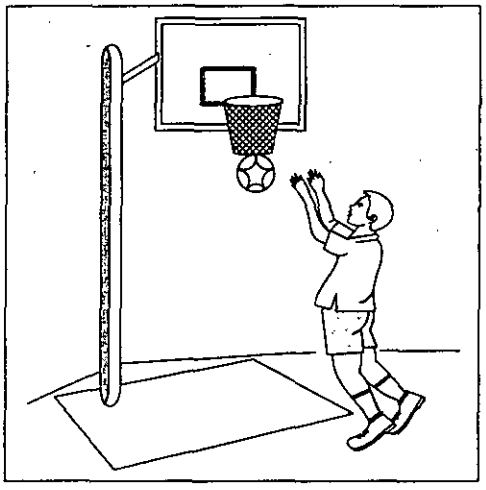
$A + B \xrightarrow{k}$ Products

In this model is $k = \pi e^{-(EA/RT)}$

You can see that the expression for k is the same as that for a unimolecular reaction. But the difference lies in the interpretation and units of ρ. For bimolecular rate processes, ρ has units of liter mole-1 sec-1 whereas for unimolecular reactions, ρ is given in sec-1. On the basis of their experience scientists give us these guidelines:

1. A typical reaction will proceed at an appreciable rate at room temperature if Ea is 10 kcal mole-1 or less.
2. For such a reaction, the fraction of high energy molecules increases with temperature in such a way that a 10°C rise in temperature will roughly double the reaction rate.

Now, I want to compare a unimolecular reaction to a person trying to hit a goal in a game of basketball. The man gathers strength, raises and throws the ball. His attempts are many, everytime he gathers sufficient energy but the hits are few and the 'hit' comes only whenevery move is properly coordinated, *i.e.*, the right orientation is there.



To understand a bimolecular reaction visualize two young girls playing a skipping game with a long rope. One girl swings the rope, starting from below her feet, coming from behind – above her head and in its forward sweep it includes the second girl

and comes from beneath her feet by then one swing is over. The second girl jumps into the air as the rope is going below her feet. The girls try a number of times, but perfect smooth circle of the rope is completed only some times, *i.e.* when both the girls are perfectly oriented to each other and are in perfect coordination.

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Degradation in solid dosage forms and solid drugs is usually not much because of one strong reason *i.e.,* the usual range for activation energies for tablet formulation decomposition is about 10 to 20kcal/mole-1 except if diffusion or photolysis is rate determining. Then the rate is about 2 to 3 kcal/mole-1 which rarely occurs in tablet degradation. For reactions in which the heat activation energies range is more than 50 k cal/mole-1, the rate of degradation is not of any practical significance at the temperature of shelf life storage of solid formulations.

I explained all these points before going into degradation mechanisms because I want you to have an idea of how actually a chemical reaction is taking place. So a formulation suffers chemical instability when the drug degrades and the drug content as claimed on the label is not available in it. Other chemicals which are used in the formulation as excipients may also decompose and though their content is not theraupeutically necessary the decomposition is of high pharmaceutical significance. It may alter the appearance as well as the release rate of the drug. Decomposition of the drugs is happening when the drug molecules are energized and are taking a particular orientation, but after taking the orientation or positioning, what exactly is happening? Are the molecules splitting into two? Are they loosing some electrons? Or they gaining some electrons? Are they racemizing?. This later step is what we are calling as the pathway or mechanism of degradation. Some well known mechanisms are hydrolysis, oxidation-reduction, racemization, decarboxylation, ring cleavage and photolysis. But the most frequently seen mechanisms are hydrolysis and oxidation reduction. Let us try to understand how these reactions are happening a little later.

1.5 MICROBIOLOGICAL STABILITY

Contamination from microorganisms is a big problem for all formulations containing moisture but it can be a bother in solid dosage forms also if some natural polymers

are used because many natural polymers are fertile sources of microorganisms. In the type of hygienic manufacture carried out today where "Quality Assurance" is a prerequisite as per the cGMP procedures, there are definite procedures to prevent microbial contamination in all formulations. But way back in the sixties microbial contamination of pharmaceutical formulations was a big problem. One case of outbreak of Salmonellosis in Sweden attributed to contaminated tablets is discussed in "Pharmaceutics, The Science of Dosage Form Design" by Michael E. Aulton. In this case the infection was traced to the original defatted thyroid powder imported from Hungary which was used to make the tablets. The Pharmaceutical Society of Great Britain set up a working party in 1968 to investigate microbial contamination of pharmaceutical preparations in manufacturing establishments and in hospital and retail pharmacies. This investigation shed light on a number of issues including microbial content of some drugs and medicines and suggested many measures to reduce contamination.

Sources of Microbial Contamination

Water	Low demand gram-negative groups: Pseudomonas, Xanthamonas, Flavobacterium, Achromobacter
Air	Mould spores: Penicillium, Mucor, Aspergillus Bacterial spores: Bacillus spp. Yeasts
Raw Materials	Micrococci
Earths	Anaerobic spore formers: Clostridium spp
Pigments	Salmonella
Gums	Actinomyces
Animal products	Salmonella, Coliforms
Personnel	Coliforms, Staphylococci, Sterptococci, Coryembacteria

Table 1.2 taken from "Pharmaceutics, The Science of Dosage Form Design" by Michael E. Aulton gives the types of organisms present in different sources. Extremely hygienic manufacture ensures a product that is free of contamination in the case of all non-sterile preparations and a sterile preparation in the case of all parenterals. There are two strategies followed in the manufacture of microbiologically stable, acceptable pharmaceutical preparations. The first step is to prevent contamination of the product. The second is to formulate the final product so that it is hostile to microorganisms and it is usually done by the addition of preservatives.

For sterile preparations there is either a terminal sterilization process or a closely controlled aseptic manufacturing procedure. In every case the final product is so made to protect the product during storage and minimize contamination while the product is in use. When discussing microbiological stability we have to discuss parenterals as one class and the rest of the formulations as one class. Parenterals are either terminally sterilized or manufactured by an aseptic manufacturing procedure. To prevent contamination to the formulation during storage and use many steps are taken such as:

1. suitably designing the containers,
2. usually using single dose containers,
3. sticking to proper storage conditions and
4. adding an antimicrobial substance as preservative.

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Preservatives used in Pharmaceutical Preparations

The following Table 1.2 given in "Pharmaceutics The Science of Dosage Form Design" by Michael E. Aulton gives a list of usual preservatives used in pharmaceutical preparations.

Table 1.2

Preparation	*Preservative*	*Concentration % w.v*	*Special factors*
Injections	Phenol Cresol Chlorocresol Phenylmrcuric nitrate Benzyl alcohol	0.5 0.3 0.1 0.001 1.0	Not for intraocular, intracardiac or intacisternal or over 15 mil single dose. Closures pretreated.
Eye drops	Phenylmercuric nitrate or Acetate Chlorhexidine Acetate Benzalkonium chloride	0.002 0.01 0.01	Dropper teat pretreated Silicone rubber teats
Mixtures	Chloroform Benzoic acid Methyl paraben Alcohol Sulphur dioxide	0.25 0.1 0.1 12-20 400 parts/10^6	Adsorptin. Volatile P^H (p^Ka, 4.2) Adsorption Volatile Volatile
Creams	Parabens Chlorocresol Dichlorobenzyl alcohol Cetyltrimethyl ammonium bromide Phenylmercuric nitrate	0.1-0.2 0.1 0.05-0.2 0.01-0.1 0.001	*K^o_w R high K^o_w R high K^o_w R high
Tablets	Methylparaben	0.1	
K^o_w values	*Mineral oil*	*Vegetable oil*	
Chlorocresol	1.5	117	
Methylparaben	0.02	7.5	
Propylparaben	0.5	80.0	

R = ratio of total to free preservative in non-ionic surfactant - water system.

Parenterals and ophthalmic preparations have to be totally free from microorganisms *i.e.*, they have to be sterile. This requirement is met by (1) placing a suitable preservative or combination of preservatives wherever required in the products, (2) storing the products properly, (3) stoppering them properly and by following proper aseptic procedures during administration and during any admixture procedures followed prior to administration. In spite of all these precautions if any microbial growth takes place and is observed the product is condemned and the entire batch from which the product has come is recalled. The storage of these products is done under conditions recommended by WHO which prescribe temperature, humidity, cleanliness as well as colour of the walls of the room.

Non-sterile preparations have less stringent requirements regarding exclusion of microbes. They need not be sterile but it has to be shown that some specifically named organisms are not present in them. Table 1.3 taken from Michael E. Aulton gives the microbial standards for pharmaceutical preparations.

Table. 1.3 Microbial standards for pharmaceutical preparations

Requirement	*Authority*
Exclusion	
1. Complete exclusion - sterility Injections, Ophthalmic preparations	BP, EP,
2. Exclusion of named organisms	USP
Raw materials:	
e.g. : Aluminium Hydroxide -	BP
Ps. Aeruginosa/1 g, E. coli/1g	
Cochineal. Gelatin - E. coli/1g	
Pancreatin, Thyroid	
- Salmonella/10 g	BP
Maize starch	BP
Tragacanth E. Coi/1g	BP
Oral dosage forms:	
Free from E. Coli	USP
Free from E. Coli and Salmonella/1 ml.	EP
Topical preparations:	
Free from Ps aeruginosa and S. Aureus	USP
Fee from Enterobacteria, S.aureus and Ps. Aeruginosa	EP
Limit upon number of viable organisms	
Oral dosage froms:	USP
Limit upon total aerobic count of non-specified viable organisms, e.g. Milk of Magnesia 100 cells/ml	
Raw materials:	
• 10^4 aerobic bacteria/ml	EP
• 10^2 *yeasts or moulds/ml*	
• 10^2 enterobacteria/ml Topical preparations:	
• 100 organisms/g or ml	EP

BP, British Pharmacopeia 1980; BP Addendum 1986; USP, United States Pharmacopeia XXI; EP, European Pharmacopeia (draft proposals).

Vulnerability to Microbial Attacks

So dear students, we now understand that different formulations have differing levels of stringency regarding microbial presence. You may be thinking "Drug products are made under such extremely hygienic conditions and are stored under very good conditions. There is no chance for any contamination to enter into them". But however clever we are microorganisms seem to be cleverer still! The risk of contamination is more in non sterile than in sterile products and more in multiple use formulations than in unit dose systems. Most of the raw materials used in the manufacture of medicines support the growth of microbes and so medicines including dry powders and tablets and capsules are susceptible to microbial spoilage. Microbes can survive in a vast range of habitats including volcanic regions and icy regions! They can use glass and concrete as nutritional sources! So a majority of medicines are a good source of nutrition to the microbes and if the microbe gets access to the medicine it can definitely survive and proliferate. This growth sometimes

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gives off offensive odours and colours. Thus, we can spot the breakdown of the system due to microbes. Sometimes the microbes grow without any visible sign and this can be really troublesome because then we will administer the medicine without realizing its harmful potential.

Understanding the vulnerability of drug systems to microbes is important. When scientists studied the interaction of microorganisms within foods such as milk and meat products they discovered some curious things. One variety of microbes first attack the medicines and degrade complex nutrients and alter pH levels. Then another variety attacks and thrives on the simple nutrients. For example, syrups may be first contaminated by osmophilic yeasts which can thrive at high sugar concentrations. They then utilize the sugars and create conditions suitable for other microbes. When such syrups are examined there may not be any evidence of the yeasts which started the entire spoilage process.

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Effects of Microbial Instability

Contamination of a product may sometimes cause a lot of damage and sometimes may not be anything at all. For example, spores of the mould Mucor may be present in a dormant form and never produce spoilage and will not harm the patient who takes the medicine. But if Salmonella enters a medicine, it may not cause any visible damage but would cause a serious health hazard to the patient who consumes it.

If contamination happens in parenterals or in ophthalmic preparations it can be very serious in its effects. But contamination in other nonsterile products is usually not so damaging. It results in general spoilage such as discoloration, breakdown of emulsions and the production of gas and other odours. This is good in one way, if prevents the patient form consuming the medicine. In some cases active drugs may be destroyed without any outward signs. Thus, salicylates, phenacetin, paracetamol, atropine, chloramphenicol and hydrocortisone can be degraded to a variety of therapeutically inactive products. Preservatives, especially those that are aromatic in structure can themselves act as a ready source of nutrition to microbes.

Suppose some microorganisms have entered the raw materials used in some parenteral preparation and then are killed in the process of sterilization - still there is some harm. Some toxic substances produced by the bacteria may cause harm. Pyrogens which are the metabolic products of bacterial growth are usually lipo-polysaccharides and they represent a particularly hazardous product released by gramnegative bacteria. If administered inadvertently to a patient they may cause chills and fever.

What to do to Prevent Microbial Spoilage

The proper preservative has to be used. By 'proper' I mean that the preservative must have the required oil/water partition coefficient, it must be non-toxic, odourless, stable and compatible with other formulation components while exerting its effects.

Containers

Traditional glass containers do not interact with the preservatives. If the closure is airtight there is no problem of contamination. But plastic containers pose problems

such as permeation through the container or interaction with it. Rubber also reacts with preservatives but it is still used for teats and closures. These teats and closures are treated with the preservatives they are to be in contact with, in order to minimize subsequent uptake during storage. For sterile preparations there is either a terminal sterilization process or a closely controlled aseptic manufacturing procedure. In every case the final product is so made to protect the product during storage and minimize contamination while the product is in use.

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1.6 MECHANISMS OF DEGRADATION: HYDROLYSIS, OXIDATION AND PHOTOLYSIS

Let us now see why and how the drugs placed in the products are decomposing. Are they splitting into two? Are they changing into other inactive or toxic forms? Why is this happening and can we prevent it? Degradation happens by a few pathways but the most often seen mechanism is Hydrolysis. Oxidation and photolysis are the next two mechanisms most often seen.

Hydrolysis

The word "hydrolysis" literally means "splitting by water". Before the mechanism of hydrolysis was properly understood scientists thought when some compounds are added to water, the water splits them. For example, a solution of sodium acetate produces acetic acid and hydroxide ions. At one time, the equation for this process would have been written (assuming that there is a chemical reaction between water and sodium acetate).

$$AcONa + H_2O \Leftrightarrow AcOH + NaOH \quad ...(1)$$

But today we know that what is involved is not a reaction with water, in the presence of water sodium acetate is dissociating into acetate ion and sodium ion and the resultant acetate ion is reacting with water to form acetic acid, but this reaction is not fast at all; it happens only to a small extent. So this process is better represented as:

$$AcONa \Leftrightarrow AcO + Na^+ \quad ...(2)$$

$$AcO^- + H_2O \Leftrightarrow AcOH + OH^- \quad ...(3)$$

Equation (2) is essentially complete and the acid base reaction of equation (3) happens only to a small extent. Equation (3) represents "hydrolysis" and we say that the acetate ion is slightly "hydrolyzed" in water to form acetic acid and hydroxide ion. Similarly chemists say that ammonium ion (NH_4^+) is hydrolyzed by water to form ammonia and hydronium ion.

$$H_4N + Cl^- \rightarrow H_4N^+ + Cl^-$$

$$H_4N^+ + H_2O \Leftrightarrow NH_3 + H_3O^+$$

The term "hydrolyze" is used to include almost any reaction with water. So we must see that though water may be involved in some reactions as the reacting species, in many cases it plays a passive supporting role, that is, it is something like a facilitator. It many times acts as a solvent vector between two reacting species in solution. In solid dosage forms the reactions will be taking place in saturated solutions. Now I want you to observe a few facts regarding hydrolysis:

1. Hydrolysis reactions involve, nucleophilic attack of labile bonds by water on the drug in solution.

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2. The reactions involving lactam groups are fastest and are followed by those involving esters, amides and imides in that order.
3. These reactions usually follow first order.
4. If this type of reaction happens due to any other solvent it is called solvolysis.
5. These reactions are catalyzed by H_3O^+ (*c*) presence of divalent metal ions (*d*) ionic hydrolysis (Protolysis) (*e*) heat (*f*) light (*g*) solution and (*h*) high drug concentrations.

The molecules having ester or amide functional groups are most susceptible to hydrolysis. Anesthetics, antibiotics, vitamins and barbiturates are examples for drugs that decompose due to hydrolysis. The ester and amide groups have so many similarities that they are called "bioisosteres". Bioisosteres are structures that have similar sizes, shapes and electronic structures.

Let me tell you a few things about local anesthetics so that you will know that we are talking about real drugs. Most of the local anesthetic drugs are either benzoic acid or aniline derivatives. The benzoic acid derivatives are esters developed from cocaine, whereas the aniline derivatives are amides developed from isogramine. These drugs have chemical structures that usually have the following formula:

Lipophilic center → Ester or amide group → Bridge → Hydrophilic center

The lipophilic center helps in the penetration of the cell membrane and the hydrophilic center helps in transportation and in binding to the receptor. When the two centers are in good balance the best local anesthetic action is obtained. So esters or amides are really providing links between two other moieties and as the saying goes "any chain is only as strong as its weakest link". So it is at the point of an ester or an amide that decomposition or splitting usually happens.

Ester Hydrolysis

Esters are compounds having the structure $R - C - O - R_1$, where R and R_1 are carbon groups. An ester can be thought of as being derived by reaction of an alcohol with a carboxylic acid, with the elimination of a molecule of water. The hydrolysis of an ester into a mixture of an acid and alcohol involves the rupture of a covalent bond between a carbon atom and oxygen atom. These reactions usually happen in the presence of water but happen much faster when either an acid or an alkali is present. Acids, alkalies and certain enzymes, which are capable of supplying the hydrogen or hydroxyl ions to the reaction mixture catalyse this hydrolysis. The alkaline hydrolysis of an ester is irreversible and an acid hydrolysis is reversible.

Let us take a brief look at the scheme of ester hydrolysis given by Walters and explained very clearly by Leon Lachman and Patrick De Luca in "Theory and practice of Industrial Pharmacy".

The ester usually is cleaved at the acyl-oxygen linkages, that is between the carbonyl carbon and the oxygen of C_2H_5 ($O^- C_2H_5$). The scheme of ester hydrolysis is this:

$$\underset{\text{ester}}{R_1-\overset{\overset{O}{\|}}{C}-OR} + H^+ + OH^- \longrightarrow \underset{\text{acid}}{R_1-\overset{\overset{O}{\|}}{C}-OH} + \underset{\text{alcohol}}{ROH}$$

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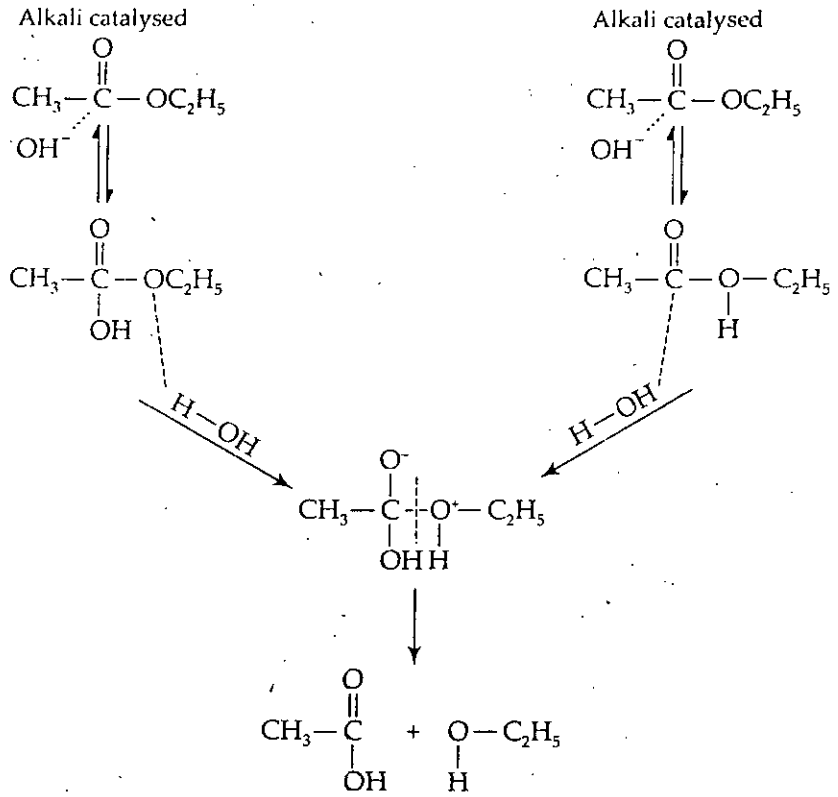


So the general form of the kinetic equation to express acid or base-catalyzed hydrolysis are as follows. So the general form of the kinetic equation to express acid or base-catalyzed hydrolysis are as follows:

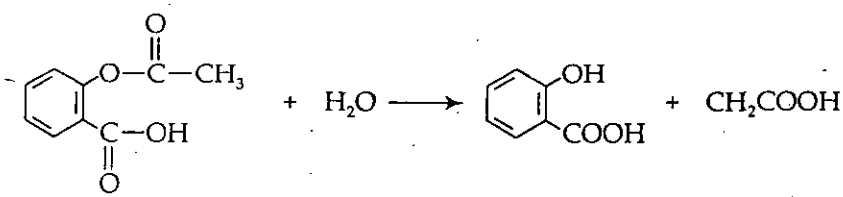
$$d(ester)/dt = K\ (ester)\ (H^+)$$

$$d(ester)/dt = K\ (ester)\ (OH^-)$$

So, since the rate seems to depend on the concentrations of two ingredients, it looks as through they are second order equations. But in reality we keep the acid or the alkali in huge excess, so that the small change in the concentration is negligible. Hence the concentration of (H^+) or (OH) is constant throughout the reaction. So we treat it as a pseudo-first order equation and the above two equations reduce to the form.

$$\mathbf{d(ester)/dt = - K\ (ester)}$$

This is an expression for a first order equation. Many drugs have been studied with respect to their decomposition by hydrolysis, but I will take the example of aspirin. Aspirin was most widely studied and a thorough study was performed by Edwards and is discussed by Lachman. The degradation of aspirin in various buffer solutions was studied and the reaction rate was treated as pseudo first - order.



Aspirin Salicylic acid Acetic acid

Aspirin hydrolysis takes place even when the drug is in the solid powder form and if we take an old sample of aspirin and open the lid the unmistakable smell of acetic acid is sure to hit us. The presence of free salicylic acid is recognized and a test for fee salicylic acid is a required test on all samples of aspirin powder. Another

advantage in studying the reaction rate of aspirin hydrolysis is that we can follow the concentration of salicylic acid, *i.e.*, the product of the reaction and from that calculate the remaining amount of aspirin. We must remember to calculate in molar quantities.

Amide Hydrolysis

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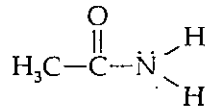
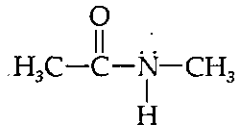
Amides contain the $-\overset{O}{\overset{\|}{C}}-\overset{|}{N}-$ group. We can say that they are derived from carboxylic acids and ammonia or amines.

Two examples are:

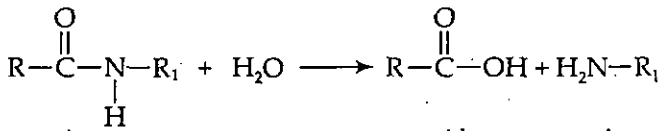


Acetamide



N-methl acetamide

Nylon and a number of other synthetic fibers and films are amides. Proteins are also amides. In all these substances, the constituent units are very large molecules, in which amide groups are the principal building blocks. Hydrolytic cleavage of an amide results in the formation of an acid and an amine.



amine acid amine

Amides are more stable than the esters. Drugs such as niacinamide, phenethicillin, barbiturates, and chloramphenicol have been reported to degrade by amide hydrolysis. Kosky studied the stability of salicylamide and some N-substituted derivatives, and postulated both basic and acid hydrolysis for degradation. Kosky found that in the acid medium, salicylanilide was more stable than salicylamide, which in turn was more stable than benzamide. Aminoalkyl substituents on the nitrogen increased the stability of benzamide. Salicylamide was more stable in basic than in acidic medium, probably due to the protection given by the negative charges on the phenolate ion. The N-alkyl and N-amino alkyl salicylamides were highly resistant to acid and base hydrolysis. This was probably because of the combined steric hindrance by the hydroxyl group in the orthoposition and the alkyl and aminoalkyl group on the nitrogen.

Drugs having ester groups and amide groups in their molecular structure degrade via hydrolysis in the presence of water. Common ester labile bonds are formed between an alcohol and a carboxylic acid. The ester bond is hydrolyzed by hydrogen and hydroxyl ions as shown in the following reactions:

The acyl oxygen in the ester group is protonated and the carboxyl group is further polarized. Nucleophilic attack at the acyl carbon is increased by water. A base, which is a powerful nucleophile, attacks on the acyl carbon and the carbon oxygen bond is broken. The rate of degradation of an ester labile group is dependent on the characteristics of R_1 and R_2. For a given R_1, the rate of degradation decreases with the higher ackyl group of R_2OH because the higher the alkyl, the fewer electrons

are withdrawn whereas for a given R_2 the degrdation rate increases with the increase in electron – withdrawing group (eg. Cl, NO_2) of R, COOH. The rate of degradation by hydrolysis increases by replacing methyl to ethyl and propyl. The higher alkyl groups possessing the greater electron–donating characteristics increase the electron density at the acyl carbon and thus the attack of OH^- is inhibited. On the contrary electron attracting groups such as chlorine and NO_2 increase the rate of degradation.

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Substituents can have a dramatic effect on reaction rates. For example, the test - butyl ester of acetic acid is about 120 times more stable than the methyl ester which in turn is approximately 60 times more stable than the vinyl analog.

Amides

An amide is a compound of the type $R-\overset{\overset{O}{\|}}{C}-NH_2$ and is formed by reaction between a carbonylic acid and an amine and is less susceptible than ester groups to hydrolysis. This is because of the lesser electrophilicity of the carbon-nitrogen bond. The amide group is hydrolyzed

$$R_1-\overset{\overset{O}{\|}}{C}-\underset{\underset{R_3}{|}}{N}-R_2 + H_2O \longrightarrow R_1-\overset{\overset{O}{\|}}{C}-OH + \underset{\underset{R_3}{|}}{HN}-R_2$$

The rate of degradation of the amide group by hydrolysis is dependent on the characteristics of the substituents R_1, R_2 and R_3.

Lactam

Antibiotics possessing the β-lactam structure, which is a cyclic amide are hydrolyzed rapidly by ring opening of the β-lactam group. The ring opening of the β-lactam is much faster than that of other amide groups because a four membered ring is joined to a five or six membered ring and a weaker bond exists between carbon and nitrogen of β-lactam.

The following Table 1.4 the modern Pharmaceutics (Third Edition) by Gilbert S. Banker and Christopher T. Rhodes gives all the types of drug compounds that are susceptible to hydrolysis and also gives good examples.

Table 1.4 Some Functional Groups Subject to Hydrolysis

Drug type	*Examples*
Esters	Aspirin, alkaloids, Dexmethasne sodium phosphate, Estrone sulfate, Nitroglycerin
Lactones	Pilocarpine, Spironolactone
Amides	Thiacinamide, Chloramphenicol
Lactams	Penicillins, Cephalosporins
Oximes	Steroid oximes
Imides	Glutethimide, Ethosuximide
Malonic ureas	Barbiturates
Nitrogen mustards	Melphalan

Oxidation

Oxidation is the mot important path way of drug decomposition. Oxygen is present everywhere in the atmosphere and exposure to oxygen will decompose drug

substances that are not in their most oxidized state through auto oxidation. Oxygen is a diradical and most auto oxidations are free radical reactions. A free radical is a molecule or atom with one or more unpaired electrons. Oxidation/ reduction reactions involve the transfer of electrons or the transfer of oxygen or hydrogen from a substance. Oxidation of inorganic and organic compounds is explained by a loss of electrons and the loss of a molecule of hydrogen, respectively as, Inorganic compounds:

$$(SO_3)^{-2} + 2\ OH \Leftrightarrow (SO_4)^{-2} + H_2O + 2e^-$$

Organic Compounds: Loss of Hydrogen

When an oxidation reaction involves molecular oxygen, the reaction occurs spontaneously under mild conditions. It is known as auto oxidation. In an auto oxidation process, free radicals formed by thermal or photolytic cleavage of chemical bonds. (For example, peroxide (ROOH) or redox processes with metal ions presenting raw material impurities are involved.

$$Fe^{++} + ROOH \rightarrow Fe^{+++} + OH^- + RO.$$

The free radical formed RO reacts with oxygen to produces a peroxide radical, and the reaction propagates as:

$$RO + O_2 \rightarrow ROOO.$$

$$ROOO + RH \rightarrow ROOH + RO.$$

The free radical reaction continues until all the free radicals are consumed or destroyed. Banker we learn very interesting things. As little as 0.0002 M copper ion will increase the rate of Vitamin C oxidation by a factor of 105. Hydroperoxides contained in polyethylene glycol suppository bases have been implicated in the oxidation of codeine to codeine – N – oxide. Many oxidation reactions are catalyzed by acids and bases. The following Table 1.5. Banker gives a list of functional groups susceptible to Auto oxidation.

Table 1.5 Some Functional Groups Subject to Autoxidation

Functional group	*Examples*
Phenols	Phenolsin steroids
Catechols	Catecholamines (dopamine, isoproterenol)
Ethers	Diethylether
Thiols	Dimercaprol (BAL)
Thioethers	Phenothiazines (Chlorpromazine)
Carboxylic acids	Fatty acids
Nitrites	Amyl nitrite
Aldehydes	Paraldehyde

The products of oxidation are usually electronically more conjugated; thus the appearance of, or a change in, color in a dosage form is suggestive of the occurrence of oxidative degradation.

1.7 PHOTOLYSIS

We have seen in the introductory note on chemical stability that when molecules absorb energy and are energized or activated they go to a high energy state and

then release that energy in a chemical reaction and come back to their original position. When this energy of activation is supplied by light and is absorbed by the compound the decomposition reaction is called photolytic. The activated species then returns to ground state by either (*a*) emitting light of a different frequency (this is fluorescene or phosphorescene) or (*b*) decomposition (Photolysis). An excellent example for photolysis is the photolysis is the photo degradation of sodium nitroprusside in aqueous solution. Sodium nitroprusside is administered by intravenous infusion for the management of acute hypertension. If the solution is protected from light, it is stable for at least 1 year; if exposed to normal room light, it has a shelf life of only 4 hours.

The energy associated with the radiation increases as its wavelength decreases, so that the energy of U.V. is greater than that of visible which is greater than that of J. R. Look at following Table 1.6 which I have reproduced from Aulton's book on Pharmaceutics. Relationship between wavelength and associated energy of various forms of light.

Table 1.6

Type of radiation	*Wavelength*	*Energy*
U.V.	50–400	Kcal mol^{-1}
Visible	400–750	287–72
		72–36
i.r.	750–10,000	1–36

The higher energy range of visible light and U.V. light cause photolysis. Conventional tungsten filament light bulbs are safe and do not contribute to photolysis. Oxidation to a great extent and hydrolysis to some extent are catalysed by light. Photo-induced reactions are common in steroids; an example is the formation of 2-benzoyl cholestan – 3 – one following is radiation of:

Cholest – 2 en – 3 – ol benzoate.

Photolysis is prevented by suitable packing in amber coloured bottles, cardboard outers and in aluminium foil over wraps and blisters. Jen, T. Cartensen in his book on Drug Stability, Principles and Practices gives extensive examples of photolysis. In pharmaceutical systems most reported photolysis reactions have been first order. A good example is the photolysis of cefatoxime. The wavelength of the irradiating light plays an important part in photolysis.

1.8 RATE KINETICS

The rate of decomposition then, depends on the frequency and intensity of the collisions of the molecules. This in turn is dependent on the nature and concentration of the chemicals involved or more specifically on their activation energy, collision frequency and what is called as a orientation factor. They can be predicted because they are happening due to molecular collisions which in turn are dependant on temperature, pH and light. The science that deals with these rates is called "kinetics" and as it describes decomposition it is called the kinetics of decomposition.

When we talk about kinetics, several 'orders' of kinetics can be there, such as zero order, first order, second order and so on, but fortunately for all of us drug

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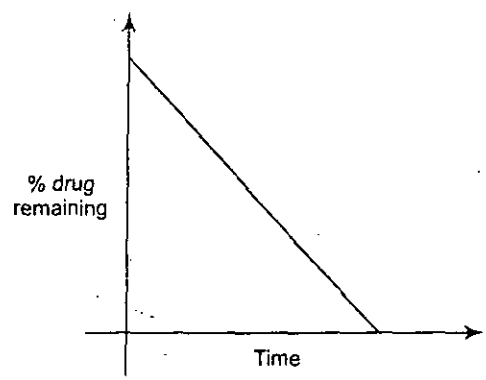
decomposition rates do not follow complex orders; they are mostly of zero order or first order.

Rate kinetics is discussed in four different contexts; 1. drug stability 2. drug release from dosage forms in dissolution studies 3. absorption, distribution, and elimination studies on the drugs in the body and 4. explaining the drug action at the molecular level when the response is a rate process. In all the cases the concepts are the same and the equations are also same.

At this stage it is imperative for us to get the concepts about zero order and first order because they are very important. These are explained most beautifully by Alfred Martin in his Physical Pharmacy book.

A man called A decides to go by car from a town called Anakapalli to a city called Visakhapatnam. Let us assume they are separated by a distance of 40 Km. The man A declares that he will proceed from Anakapalli to Visakhapatmam at a steady speed of 10 Km/hr. So if he starts at 10 a.m. in Anakapalli he will reach Visakhapatnam at 2 p.m. In his journey he will neither accelerate nor decelerate. We can say that A is making a journey at a zero order rate.

A man called B also decides to make the same journey by his car; but he declares that his speed at any given time is one fourth of the distance to be covered in Km/hr. For the sake of easy calculation let us assume B breaks down his time periods into half hours. Then in the first half hour he travels a distance of 40/8 *i.e.*, 5 km; in the second half hour 35/8 *i.e.*, 4.375; in the third half hour 30.625/8 *i.e.*, 3.828; in the fourth half hour 26.797/8 *i.e.*, 3.349 and so on. So as the destination nears his speed will go on decreasing because his speed is one fourth of the distance to be covered. So theoretically speaking B will never reach his destination. B is traveling by first order kinetics! You must all have seen a nurse arrange an infusion into a patient's vein at a fixed number of drops per minute; the infusion is flowing by zero order. Suppose you throw a big rock into a stagnant pool of water, suddenly there are waves, and the extent of waves go down with each passing moment, but it will take a lot of time for the pool to become still again. This phenomenon is following a first order kinetics. The rate of decomposition is proportional to some power of the concentration of the reactants and it can be shown like this – $dc/dt = KC^n$ different values to n we will get equations for different orders. If $n = 0$ then $-dc/dt = KC$. If $n = 1$ then $-dCa/dt = KCa$ where Ca is the concentration of the reactant. Let us understand these two orders and let us also learn how to differentiate between these two. For zero order reaction a plot of time vs % drug remaining will be like this:



If the drug decomposition is following first order kinetics then plot of time vs % drug remaining will be like this:

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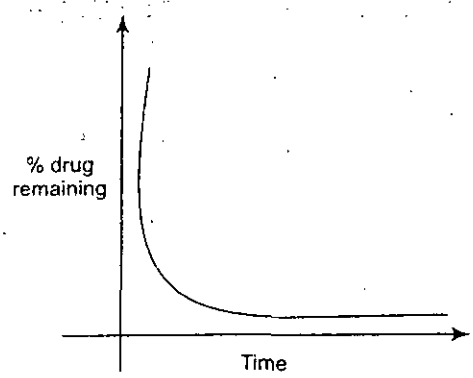
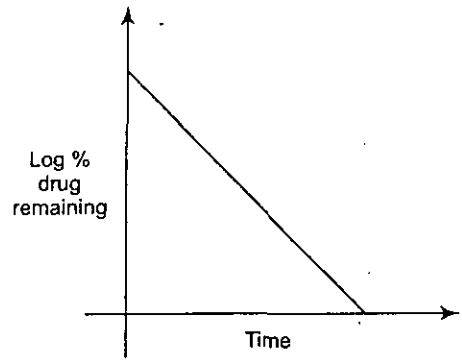
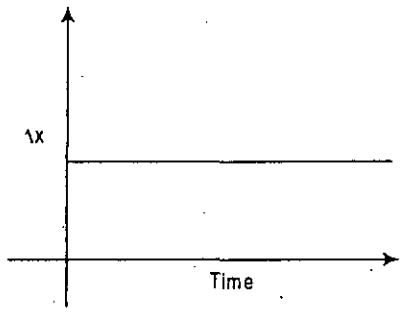
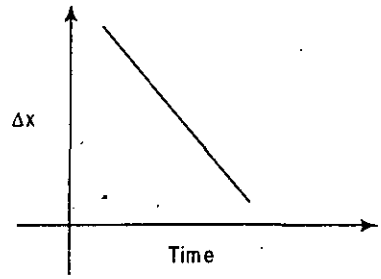


If we plot log % remaining Vs time, the plot will be like this,



Zero order	*First order*
1. When the reaction rate is independent of the concentration of the reacting substance, it is dependent on the zero power of the reactant and therefore is considered to be of the zero order reaction. The limiting factor is usually solubility of the drug or absorption of light.	1. When the reaction rate is dependent on the first power of concentration of a single reactant (rate = KC_a). It is considered to be first order. A reactant decomposes directly into one or more products.
2. Mathematically, $-dCa/dt = K$, $X = Kt$ + Constant or $Ct = C_0 - K_0 t$ where, C_a = Concentration of reacting material A. K= proportionality factor = reaction rate t = time X = amount reacting C_t = concentration at time 't' C_0 = initial concentration.	2. Mathematically, $-dCa/dt = KCa$ and $-\log Ca = Kt / 2.303$ + Constant or $K = 2.303 / t_2 - t_1 \log C_1/C_2$ or $K = 2.303 / t \log a/a-x$ where $a = C_0$, X = amount reacting in time 't' and $a - X$ = the amount remaining after time 't'
3. $t_{1/2} = 0.5\ C_0 / K_0$	3. $t_{1/2} = 0.693/K$
4. Slope of the line = K_0	4. slope × 2.303 = K_1
5. Units are : wt/time like mg/hr	5. Units are: hr^{-1}
6. If Δx = differences in amount remaining per unit time then, 	6. If Δx = differences in amount remaining per unit time then, 

7. The reaction comes to a conclusion in a finite time.	7. Theoretically it never comes to a conclusion. But practically it comes so close to completion that it may be considered as complete.
Examples:	Examples:
1. Intravenous infusion.	1. Absorption, distribution, elimination rates.
2. Drug released from transdermal drug delivery systems.	2. Microbial death kinetics.

By looking at all these differences you may feel it is very easy to find the order of the reaction by looking at the data, it is not so easy practically though theoretically it may look like that. The reason is when we actually follow the decomposition of some drugs the data appear as though they are satisfying criteria of both the orders. In such a situation to find the order of the reaction we have to follow the least square method of linear regression.

Second order, third order and more complex reactions are there but drug decomposition usually follows zero order or first order. When we know the order and the reaction rate constant we can predict the expiry date. In the case of vitamins, this data helps in determining the amount of overages we want to add to prolong the shelf life by a particular period of time. By knowing kinetic data and using some statistical methods we can also draw a stability line and 95% and 99% confidence limits to that line. Thus understanding rate kinetics is a key element in understanding the stability of drugs.

1.9 TEMPERATURE

Temperature has a high degree of influence on all varieties of chemical reactions and usually they are accelerated by a raise in temperature. This is understandable as we know that with increased temperature the molecules move faster with increased kinetic energy and the rate of collision of molecules increases. Also, a greater available energy causes more molecules to have enough activation energy and the fraction of collisions with enough energy increases. It is said that typically a 10°C increase in temperature produces a 2 – 5 fold increase in decomposition.

The effect of temperature on the rate constant k is indicated by the Arrhenius equation which

$$K = Ae^{-(Ea/RT)}$$

$$\text{Log } k = \text{Log } A - Ea/2.303\ RT$$

Where A is a constant that is termed the frequency factor, a is the energy of activation, R is the gas constant and T is the thermodynamic temperature. For drug compounds whose decomposition is log K is temperature, if log K is plotted against 1/T, a straight line is obtained. This is kown as Arrhenius plot. The constants Ea and A may be determined from the slope and intercept of this line, which are equal to – Ea/2.303R and log A respectively. We usually take on the X axis $1/T \times 10^{-3}$ and log K on the Y axis. The activation energy Ea is the energy needed to cause reaction and is usually in the range of 15–60 kcal mol^{-1} with a mean of 19.8 (Aulton). Values for a wide range of reactions are 10–100 kcal mol^{-1}. If we are using Ea in the calculation of shelf life and if we are not sure of this value, we should make a conservative estimate and assume a low value (*e.g.,*: 10 kcal mol^{-1}) for it. This assumption gives a shorter shelf life but it is better to avoid any risk to the patient.

Non-Arrhenius behavior, or decreasing decomposition with temperature has been observed in Pharmaceutical systems as is well explained by Banker. This may be attributed to the possible evaporation of solvent, multiple reaction pathways, change in physical form of the formulation when the temperature of the reaction is changed.

A good example for this is the increased rate of decomposition of ampicillin on freezing. Savello and Shangraw showed that for a 1% sodium ampicillin solution in 5% dextrose, the percentage of degradation at 4 hour is approximately 14% at – 20°C, compared with 6% at 0°C and 10% at 5°C. This decrease in stability in frozen solutions is most frequently observed when the reaction obeys second or higher order kinetics. For example, the formation of nitrosomorpholine from morpholine and nitrite obeys third – order kinetics and the rate of nitrosation is drastically enhanced in frozen solutions.

This behaviour which is contradictory to our basic understanding of reaction mechanisms was reviewed by Pincock and is discussed in Banker. In reactions following second or higher order kinetics, an increase in rate may be brought about by concentration of the reactants in the liquid phase, the solute molecules being excluded from their lattice when the solution freezes. This may also happen due to a change in pH on freezing.

pH

Acidic and alkaline pH influence the rate of decomposition of most drugs. Many drugs are stable between pH 4 and 8. Weekly acidic and basic drugs show good solubility when they are ionized and they also decompose faster when they are ionized. So if the pH of a drug solution has to be adjusted to improve solubility and the resultant pH leads to instability then a way out of this tricky problem is to introduce a water-miscible solvent into the product. It will increase stability by (*a*) suppressing ionization, (*b*) reducing the extreme pH required to achieve solubility, (*c*) enhancing solubility and (*d*) reducing the water activity by reducing the polarity of the solvent. For example, 20% propylene glycol is placed in chlordiazepoxide injection for this purpose.

Reactions catalysed by pH are monitored by measuring degradation rates against pH, keeping temperature, ionic strength and solvent concentration constant. Some buffers such as acetate, citrate, lactate, phosphate and ascorbate buffers are utilized to prevent drastic change in pH.

Sometimes pH can have a very serious effect on decomposition. As little as 1 pH unit change in pH can cause a change of ten fold in rate constant. So when we are formulating a drug into a solution we should carefully prepare a pH – decomposition profile and then formulate the solution at a pH which is acceptable physiologically and stability-wise also.

1.10 PACKAGING AND STABILITY

Packaging of the drug product is very important when its stability is being considered. The immediate container and closure are particularly important in affecting product stability. Glass, plastic, rubber (natural and synthetic) and metal are the four types of containers commonly utilized for packing drug products.

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Glass

Glass is resistant to chemical and physical change and is the most commonly used material, but it has the limitations of:

1. Its alkaline surface may raise the pH of the product.
2. Ionic radicals present in the drug may precipitate insoluble crystals from the glass
3. The clarity of the glass permits the transmission of high energy wavelength of light which may accelerate decomposition.

All these limitations are overcome by the technologists in the following way: (1) the first problem is overcome by the use of Borosilicate glass which contains fewer reactive alkali ions, (2) Treatment of glass with chemicals or the use of buffers helps in overcoming the second problem, (3) Amber coloured glass which transmits light only at wavelengths above 470 nm is used for photolytic drug products.

Plastics

Plastics include a wide range of polymers of varying density and molecular weight, each possessing different physicochemical characteristics. The problems with plastic are:

1. Migration of the drug through the plastic into the environment.
2. Transfer of environmental moisture, oxygen, and other elements into the pharmaceutical product.
3. Leaching of container ingredients into the drug.
4. Adorption or absorption of the active drug or excipients by the plastic.

For all these problems the solution is to suitably pretreat the plastic chemically. The drug product packed in the final container must be tested for stability.

Metals

Various alloys and aluminium tubes may be utilized as containers for emulsions, ointments, creams and pastes. They may cause corrosion and precipitation in the drug product. Coating the tubes with polymers or epoxy may reduce these tendencies.

Rubber

Rubber also has the problems of extraction of drug ingredients and leaching of container ingredients described for plastics. The use of neoprene, butyl or natural rubber, in combination with certain epoxy, Teflon or varnish coatings reduces drug-container interactions. The pretreatment of rubber vial stoppers and closures with water and steam removes surface blooms and also reduces potential leaching.

SUMMARY

- The job of the medicine is to give the therapeutic effect and so the most important thing is that the required drug content is available till the expiry date is over.
- Stability is the capacity of a drug product to remain within specifications established to ensure its identity, strength, quality and purity.
- Rate kinetics is discussed in four different contexts; 1. drug stability 2. drug release from dosage forms in dissolution studies 3. absorption, distribution, and

elimination studies on the drugs in the body and 4. explaining the drug action at the molecular level when the response is a rate process.

- Temperature has a high degree of influence on all varieties of chemical reactions and usually they are accelerated by a raise in temperature.

REVIEW QUESTIONS

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1. Discuss the stability of herbal medicines.
2. Discuss the international conference on harmonisation (ICH).
3. Discuss the physical stability of medicine.
4. Discuss the chemical stability of drugs.
5. Discuss the microbiological stability.
6. What is photolysis?

FURTHER READINGS

1. **DNA Based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation:** Edited by Peter Walker and Rohana Subasinghe, Daya, 2005
2. **Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Vols. I and II:** V. Rajpal, Eastern Pub, 2008
3. **Stability of Drugs and Dosage Forms:** Yoshioka, Springer.
4. **Formulation, Characterization, and Stability of Protein Drugs: Case Histories:** Pearlman, Springer, Hardbound.