Third Year clinical
Quality control of herbal drugs, 19/6/2017
Time allowed: 2 hours

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Part (II): Quality control testing

30 marks

A-Write short notes on five only of the following, supporting your answer with examples and applications whenever applicable: 10 points

1-Finished herbal products

-Consist of herbal preparations made from one or more herbs.
-If more than one herb is used, the term "mixture herbal product" can also be used.
-Finished herbal products and mixture herbal products may contain excepients in addition to the active ingredients.
-However, finished products or mixture products to which chemically defined active substances (synthetic or isolated from herbal material) have been added, are not considered to be herbal.

2-Types of instant teas

a-Spray dried extracts:

-The solutions containing the drug are sprayed through a nozzle, sink in form of droplets in the current of warm air, losing their moisture and reaching the separator as dry and hollow pellets of extract with small proportion of carbohydrate carrier that have to be taken into account while calculating the amount of dietary requirements.
-Essential oils lost during drying can be added again as active substances.
-The product is low-density powder, soluble in water. Since it is hygroscopic, there is problem of caking.

b-Tea granules :

-The fluid drug extracts are sprayed on to a carbohydrate carrier and dried with heating.
-The dried mass is crushed in a mill to granular aggregates readily soluble in water with slight hygroscopic tendency and caking is seldom observed.
-This type of instant tea is often preferred by patients because of ease of manipulation and sweat taste.
-The number of bread units is to be calculated for diabetics and for children, sucrose, which promotes caries is replaced by other carriers.
-Tea granules are usually inferior to instant teas regarding the content of drug extract by a factor of ten times.

3-Lycopodium spore method

-Wallis showed that lycopodium spores are exceptionally uniform in size (about 25 mm) and that I mg of lycopodium contains an average of 94000 spores. The number of spores mg^{-1} was determined by direct counting and by calculation based on specific gravity and dimensions of the spores.

-Mounts containing a definite proportion of the powder and lycopodium are used and the lycopodium spores are counted in each of the fields in which the number or area of the particles in the powder is determined. The method is laborious and has not been subjected to statistical assessment.

Lycopodium spore methods can be used to evaluate many powdered drugs provided that they contain one of:

1-Well-defined particles which may be counted e.g. pollen grains or starch grains.

2-Single layered tissues or cells the area of which may be traced at a definite magnification and the actual area calculated.

3-Characteristic particles of uniform thickness, the length of which can be measured at a definite magnification and the actual length calculated.

4- Karl-Fischer method for moisture determination

-The assay is particularly applicable for expensive drugs and chemicals containing small quantities of moisture.

-For crude drugs such as digitalis and ipecacuanha , thePowdered material can first be exhausted of water with a suitable anhydrous solvent (dioxan) and an aliquot taken for titration.

-Karl-Fischer reagent consists of a solution of iodine, sulphur dioxide and pyridine in dry methanol. This is titrated against a sample containing Water, which causes a loss of the dark brown color.

-At the end-point, when no water is available, the colour of the reagent persists.

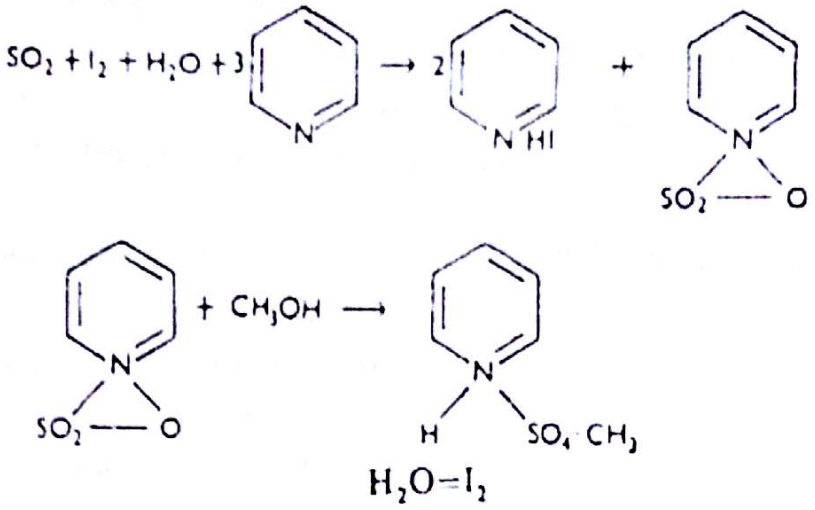
-The basic reaction is a reduction of iodine by sulphur dioxide in the presence of water. Sulphur trioxide is removed as pyridine sulphur trioxide, which reacts with the methanol to form the pyridine salt of methyl sulphate.

-The reagent requires standardization immediately before use.

-The titration is carried out under an atmosphere of dry nitrogen to eliminate interference from atmospheric moisture,

-The principal drawbacks of the Karl Fischer method are the instability of the reagent and the possibility of substances in the sample, other than water, which may react with the reagent.

$$H_2O + I_2 + SO_2 \rightleftharpoons 2HI + SO_3$$



5-Spectroscopic methods for the assay of herbal drugs

a-Comparison of infrared spectra of phytochemicals (pilocarpine and physostigmine) with reference substances as a test of identity.

b-The BP uses ultraviolet absorption characteristics as standards for lanatoside C and a number of alkaloids e.g. morphine and reserpine.

c-fluorescence spectrum is chalacteristic for those substances which exhibit this phenomenon and fluorescence analysis replaced older chemical methods. e.g. quinine-type alkaloids and cinchonine-type alkaloids in cinchona bark.

d- NMR spectroscopy

Although this technique is usually associated with structure determinations of organic compounds the use of ^{1}H-NMR spectroscopy has been described for assay of atropine in extracts of belladonna.

e-Tandem mass spectroscopy (MS-MS)

-Mass spectroscopy is usually associated with the structure elucidation of compounds..
-However, by simultaneous use of two mass spectrometers in series it is possible to determine the amount of a particular targeted compound in complex mixtures.

-Plant extracts or even in dried plant material can be used. Sensitivity to picograms of targeted compounds can be achieved with high specificity and nearly instantaneous response. Regarding sensitivity it compares with RIA but is much more rapidly performed.

-The method has been used for the analysis of cocaine in plant materials, pyrrolizidine in Senecio and other genera, taxanes from single needles of *Taxus* species and aflatoxin B_1 in peanut butter.

6- 5S-rRNA spacer domains and Multiplex Amplification Refractory Mutation System (MARMS) techniques for quality control of Panax species

-5S-rRNA spacer domains (5S-rRNA gene) were also isolated from *P. notoginseng (Sanki)* and other *Panax* species. The spacer domains is highly conserved and showed 75% DNA homology among all *Panax* species, but not the adulterants.

-Recently, Multiplex Amplification Refractory Mutation System (MARMS) allowed the detection of many sites of nucleotide difference and provided reliable authentication of five *Panax* species.

-MARMS is amplification strategy in which a polymerase chain reaction (PCR) primer is designed to be able to discriminate among templates that differ by a single nucleotide residue ARMS-PCR

-Identifying a herb within a herbal mixture of numerous herbs is challenging and a Ginseng marker primer (SIM2) that specifically amplified fragment from the DNA of *Panax* species, was also identified.

-A gradient PCR method using the SIM2 primer was used to uniquely identify *Panax* species in herbal medicines and herbal preparations containing diverse components. This study suggests the possibility of developing a *Panax* species identification kit for herbal medicines and their preparations

-This technique (gradient PCR method using the SIM2 primer) may be applied to many other herbs in the future and a new simultaneous identification method for many herbal medicines may be possible, thus providing a new level of quality control for herbal preparations.

B-Match the following statements with the answers in table1 by writing the number of each statement in front of the correct answer: 20 points

1-A problems encountered with the use of herbal medicines and products.

2-Herbal teas preferred by people sensitive to coffee.

3-A method of extraction, suitable for hard to very hard drugs.

4-A drug used in dry cough, acute, chronic irritation of mouth & throat.

5-A problem, which complicates the quality control of herbal drugs.

6-It is the average number of palisade cells beneath each upper epidermal cell.

7-A GC column used in moisture determination of herbal drugs.

8-Commodities for which moisture determination was performed by NMR.

9-The composition of total ash.

10-A reagent used in the assay of tannin content.

11-The use of a tea made of equal parts of valerian, caraway, matricaria and peppermint.

12-The use of Restharrow root.

13-It is the volume in milliliters occupied by I g of a drug, after it has swollen in an aqueous liquid 4 hr.

14-One way for determination of essential oils in herbal drugs.

15-A physical constant determined for quality control of liquid natural products.

16-Quantitaive chemical tests useful in the evaluation of balsams.

17-An example of phytopharmaceuticals.

18-An example for the use of HPLC in identity preceded by sample derivatization.

19-A chemical marker used in standardization of coneflower *Echinacea purpurea* extract.

20-Mixture natural products assayed as a whole after alkaline hydrolysis.

21-It is a type of PCR but the segments of DNA to be amplified are haphazard.

22-It is a difference in homologous DNA sequences detected by presence of fragments of different lengths after digestion of DNA samples with specific restriction endonucleases

23-Differentiation of *Panax* species using chemical finger printing.

24-It is a universal HPLC monitoring with high sensitivity for analytes lacking chromophore.

25-A relatively old HPLC/MS hyphenation system.

26-A method for analysis of underivatised dencichine.

27-A method used to identify major semi-volatile compounds in Gensing.

28-A method used in analysis of fangchinoline and tetrandrine in *Stephania tetrandra.*

29-Chemical nature of Z-ligustilide in dong quai (*Angelica sinensis*).

30- HPLC-MS interface in which ion generation takes place under atmospheric pressure.

Table (1):

Answer	Number
Stomach and bowel	11
Escin from *Aesculus hippocastanum*	20
Palisade ratio	6
High-speed countercurrent chromatography	
Hydrophilic interaction chromatography with MS-MS detection.	26
Evaporative Light Scattering Detector	24
Refrative index detector	
Acid value, ester value and saponification value	16
Mild diuretic and to treat gout and rheumatism	12
RAPD	21
Chemical Ionization (CI)	30
Methoxyl determination & volatile acidity	
House hold teas	2
Use of Clevenger apparatus	14
Saponins, essential oil and mucilage,	
Moving belt interface	25
Nonaqueous capillary electrophoresis	28
Starch & cotton	8
RFLP	22
Sedative	
Refractive index	15

Only silicate	
Colts foot leaves	4
Infusion	
Teflon-6 coated with polyethylene glycol	7
Butylidene phthalide	29
Optical rotation	
Ginkgo biloba extract	17
Triterpenoid saponins of *Phytolacca dodecandra*	18
Mixing herbal products with synthetic drugs	1
Phenylpropanoid ester echinacoside	19
Swelling index	13
Preparation of bath for alleviating rheumatic pain	
HPLC profile of ginsenosides Rgl to Re and notoginsenoside Rl,	23
Sodium phosphomolybdo tungstate	10
Two-dimensional gas chromatography-quadrupole mass spectrometry	27
The active principle(s), in most cases unknown	5
Decoction	3
Carbonates, phosphates, silicates and silica.	9