

Review Article

Current Quality Control Methods For Standardization Of Herbal Drugs

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Abstract

Herbal formulations are widely accepted as a therapeutic agents for several diseases. The development of authentic analytical methods which can reliably identifies the phytochemical composition, including quantitative analyses of marker/bioactive compounds and other major constituents, is a major challenge to scientists. Standardization is an important parameter for the establishment of a consistent biological activity or simply to maintain the quality of production and manufacturing of herbal drugs. This review covers various standardization parameter according to herbal monograph and elucidated in most comprehensive way to support the researcher in herbal formulation and characterization.

Keywords: Standardization, Phytochemistry, Therapeutic Efficacy, Chemical Constituents.

Introduction**1. INTRODUCTION**

Standardization of herbal formulations is crucial factor in assessing the quality drugs, based on the concentration of their chemical constituents, physical, chemical, phyto-chemical, standardization, and In-vitro, In-vivo parameters. The quality assess-

ment of herbal formulations is very important in order to justify their acceptability in modern system of medicine ^[24]. The major problems faced by the herbal industries are unavailability of strong quality control profiles for herbal formulations. In India, the department of Ayush, Government of India, launched a central scheme to develop a standard operating procedures for the manufacturing process to develop pharmacopeial standards for ayurvedic preparations. India needs to explore the medicinally important plants and this can be achieved only if the herbal products are evaluated and analyzed using sophisticated techniques of standardization. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in natural health care programmes because these drugs having low cost, safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques. ^[28]

Classification of standardization techniques are as follows:

A. Physical evaluation**B. Microscopical evaluation****C. Chemical evaluation (pre phytochemical screening)****D. Spectroscopical analysis****2. PHYSICAL EVALUATION:**

Physical evaluation is very crucial factor in the standardisation of crude drugs. This method helps in evaluation of crude drugs with reference to moisture content, viscosity, melting point, pH etc. ^[16]

Significance - Physical evaluation is important tool for determination of Quality Quantity and purity of crude drugs. ^[16]

2.1 Foreign organic matter determination:

Foreign organic matter means the parts of the organs of the crude drug other than those named in definition and description of drug.

The maximum limit for the foreign organic matter is defined in monograph, if it exceeds the limits then it is the indication of deterioration in quality

of drug take place.

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Method:

Weigh 100 –500 g of the drug sample to be examined. Spread it out in a thin layer. F.M.O. should be detected by inspection with eye or by the use of a lens (6x). Separate and weigh it.

$$\text{Percentage of foreign organic matter} = \frac{n \times W \times 94,100 \times 100}{S \times M \times P}$$

Where,

Table 1: Examples of Drugs with Foreign matter^[13]

Name of Drugs	F.O.M. Limit
Rauwolfia serpentina	Not More Than 2%
Acacia catechu(bark)	Not More Than 2%
Embllica officinalis	Not More Than 3.0%
Curcuma Longa	Not More Than 2.0%
Commiphora wightii	Not More Than 5.0%

Table 2: Crude drugs with moisture content analysis^[13]

Name of Drugs	Moisture content(%w/w)
Rauwolfia serpentine	Not more than 12%
Acacia	Not more than 15%

Method:

Place about 10 g of drug after accurately weighing it in a tared evaporating dish. After placing the above said amount of the drug in the tared evaporating dish dry at 105° for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weightings corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weightings after drying for 30 minutes and cooling for 30 minutes in a desiccators, show not more than 0.01 g difference.^[17]

n= number of chart particles in 25 fields.

S= number of spores in the same 25 field.

W= weight in mg of lycopodium taken.

M= weight in mg of the sample (calculation on the sample dried at 105.c

P= number of characteristics particles per mg of the pure foreign matter.

94,000= number of spores per mg of lycopodium.^[14]

2.2 Loss on drying (LOD):

LOD is the loss of weight expressed as w/w and can be determined by following procedure. The percentage of chemical constituents in crude drugs is mentioned on air dried basis.^[16]

Significance-This test determines water and volatile content in the crude drug.

2.3 Ash value:

The residue remaining after incineration is the ash content of the drug.

Significance- Ash value is an important parameter to prove acceptability and purity in case of drugs that are collected or stored by incorrect way.^[16] High ash value is indicative of contamination, substitution, adulteration in crude drug.^[19]

E.g. Inorganic salts, naturally occurring in drug in the form of adulteration. Ash value is determinant of identity or purity of drug.

The ash value is determined by three methods- total ash, acid insoluble ash, water soluble ash.

Method:

Incinerate 2 to 3 g of the ground drug in a tared silica dish at a temperature not exceeding 450°C, cool and weigh. Calculate the % of ash with reference to the air-dried drug. [19]

2.3.1 Acid insoluble ash:

It determines amount of silica present, especially as sand siliceous earth

Procedure:

Boil the ash with 25 ml of dil. HCL. Collect the insoluble matter in a crucible. Wash with hot water

and ignite to constant weight. Calculate the % acid-insoluble ash with reference to the air dried drug. [19]

2.3.2 Water soluble ash:

Method:

Boil the ash for 5 minutes with 25 ml of water. Collect insoluble matter in a crucible wash and ignite for 15 minutes at a temp not exceeding 450°C. Calculate the percentage of water-soluble ash with reference to the air dried drug. (Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash). [19]

Table 3: Examples of Drugs with their Total Ash content [13]

Name of Drugs	Total ash (% w/w)
Acacia Catechu(bark)	Not More Than 15 %
Rauwolfia serpentine	Not More Than 8 %
Centella asiatica	Not More Than 2.0%
Coriandrum sativum	Not More Than 6.0%
Menth x piperita	Not More Than 14%

Table 4: Examples of crude drugs with Acid insoluble ash content [13]

Name of Drugs	Acid insoluble ash (%w/w)
Agar	Not more than 1.0
Amla	Not more than 2.0
Bael	Not more than 1.0
Andrographis paniculata	Not more than 3.0%
Pudina	Not more than 6.0%

Table 5: Examples of crude drugs with Water soluble ash content [13]

Name of Drugs	Water soluble ash (%w/w)
Ginger	Not more than 1.7
Curcuma longa	Not Less Than 12%
Amla	Not More Than 40%

Table 6: Determination of Alcohol Soluble Extractive [13]

Drugs	Alcohol soluble extractive (%w/w)
Amla	Not Less Than 40.0
Ashoka	Not Less Than 15.0
Plantago ovate	Not Less Than 2.0
Curcuma longa	Not Less Than 8.0

2.4.2 Determination of Ether Soluble Extractive (Fixed Oil Content)

Ether soluble extractive value useful for evaluation of crude drugs such as resin, fixed oil etc. There are two types of ether soluble extractives volatile and non volatile ether soluble extractives. [16]

2.5 Refractive index

Refractive index gives idea about purity. When a ray of light passes from through a rarer medium to denser medium it is bent and this bending of light is called as refraction. Thus, the ratio of velocity of light in vacuum to its velocity in a substance is known as refractive index of the second medium. It is constant for a liquid for particular purity value that's why it is considered as important tool for the standardization. It can be affected by wave length of the incident light, temperature and pressure. [4]

Table 7: Examples of drugs showing refractive Index

Drug	Refractive Index
Caraway oil	1.4838-1.4858
Clove oil	1.527-1.535

2.6 Determination of specific optical rotation

It is depend on phenomenon of polarization. Polarization means when plane of polarised light passes through liquid the, light gets rotated its clockwise rotation called dextro and anticlockwise rotation called levo rotator. [22]

It can be calculated by using formula:

$$D_{25} = 100 \times \phi / c$$

Where,

ϕ = Observed rotation in drug at-25°

D = d line of sodium light

l = Length of polarimeter tube.

c = Concentration of substance in percent w/v.

2.7 Determination of pH. [22]

The pH value may be defined as the negative logarithm of hydrogen ion concentration to the base 10. Potentiometrically pH value determine by a glass electrode and a suitable pH meter. [22] The pH for most of the extract ranges from 5 to 7 and can be considered as one of the quality indicator.

E.g. *Andrographis paniculata* - 7.33

2.8 Volatile oil content

Odorous and volatile principal of drug is known as volatile oils such crude drugs are standardized on the basis of their volatile content. [23]

Table 8: Examples of drugs showing Volatile oil content [13]

Drug	Volatile Oil content
Clove	Not Less Than 15
Fennel	Not Less Than 1.4
Pudina	Not Less Than 1.2
Tulsi	Not Less Than 0.4
Dhania	Not Less Than 0.3

2.9 Pesticide residue

World Health Organization and Food and Agricultural Organization set limits of pesticide residue, which are usually present in the herbs. These pesticides are mixed with the herbs during the time of cultivation. DDT, BHC, toxaphene, aldrin these pesticides cause serious side-effects such as poisoning in human if the crude drugs are mixed with these substances. [23]

Table 9: Examples of drugs with Pesticide residue:

Drug	Maximum limit of Aldrin and Dieldrin
Fructose ammi majoris	Not More Than 0.05mg/kg
Folium azadirachti	Not More Than 0.05mg/kg

2.10 Microbial contamination

In medicinal plants main sources of bacterial and molds contamination are soil and atmosphere. Analysis of the limits of *E. coli* and molds is somewhat depends upon harvesting and production practices. The substance known as aflatoxins will produce serious side effects if consumed along with the crude drugs, that's why it should be completely removed or should not be present. [4]

2.11 Radioactive contamination

Radiation sterilization is useful to avoid Microbial

growth in herbals. This process may sterilize the plant material but the hazards of radiation sterilization should be taken into consideration. The radioactivity of the plant samples should be checked according to the guidelines of International Atomic Energy in Vienna and that of World Health Organization. [23]

2.12 Viscosity

Viscosity of liquid is constant for that particular liquid at a given temperature and is an index of its composition, that's why it is an important tool for standardizing liquid drugs. [4] **Significance**-It gives idea about composition of drug and stability.

Table 10: Examples of drugs with their kinematic viscosities [4]

Drug	kinematic viscosity
Liquid paraffin	not less than 64 centistokes
Pyroxylin	1100-2450 centistokes

2.13 Melting point

Melting point for pure chemicals or phytochemicals is constant, but the crude drugs from animal or plant origin contain the mixed chemicals, that's why they are described with certain range of melting point. [4]

Significance-It is one of the parameter to judge the purity and stability of crude drugs. [17]

Table 11: Examples of drugs with their respective Melting Point. [4]

Drug	Melting Point
Colophony	75-85°C
Kokum butter	39-42°C

3. MICROSCOPICAL EVALUATION:

Significance- This method allows detailed examination of drug and it is tool for identification of standard drug .It is considered as crucial factor for qualitative evaluation of organised crude drugs. [17]

3.1 Determination of leaf constant: [15]

3.1.1 Stomatal number

It is average number of stomata per square mm of the epidermis of the leaf.

e.g. Digitalis perpuria: 25-50

3.1.2 Stomatal index [4]

The Stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells each stoma being counted as one cell.

Stomatal index= $S / E+S \times 100$

Where:

S= Total number of stomata in a given area of leaf

E= Number of epidermal cells in the same area of leaf.

e.g. Digitalis perpuria: 1.3-3.5

3.1.2 Vein islet number:

The vein-islet number is average number of vein-islet per square mm of leaf surface midway between midrib and margin. Various species of drugs are distinguished by vein-islet number. E.g. Alexandrian senna and Indian senna are distinguished because of their difference in vein islet numbers which are 27 and 22 respectively.

3.1.3 Vein islet number of various drugs:

Datura metal 19-22

Datura stramonium 12-16

Datura fastuosa 18-24

Cannabis sativa 20-30

Bacopa monniera 6-13

Azadirachta indica 10-18

3.1.4 Vein termination number.

It is defined as the no. of veinlet termination per sq. mm of the leaf surface midway between midrib and margin.

3.1.5 Palisade ratio:

It is defined as average no. of palisade cells beneath each epidermal cell.

E.g. Cassia angustifolia 5.5-10.5

Cassia acutifolia 4.5-9.5

Digitalis perpuria: 3.7-4.2

3.1.6 Trichomes ^[20]

The elongated outgrowth of leaf called as trichomes and they are also known as plant hairs.

Types of trichomes

1. Covering trichomes

a. Unicellular trichomes e.g. Nuxvomica, caanabis

b. Multicellular-unbranched trichomes

(i) Uniseriate- e.g. Datura

(ii) Biseriate –e.g. Calendula officinalis

(iii) Multiseriate- e.g. Male fern

c. Multicellular branched trichomes- e.g. Verbascum Thapsus

2. Glandular trichome

a. Unicellular glandular trichome- e.g. Vasaka

b. Multicellular glandular trichome- e.g. Digitalis purpurea

3. Hydathode trichome – e.g. Piper betal

4. CHEMICAL EVALUATION (PRE PHYTO-CHEMICAL SCREENING) : ^[2,25,26]

Many of the crude drugs have definite chemical constituents and biological or pharmacological activity depends on these chemical constituents. The chemical evaluation of crude drug helpful to identify certain drug or to test their purity. ^[3]

Table 12: Detection of Phytoconstituents

Detection of alkaloids				
Extracts were dissolved individually in dilute Hydrochloric acid and filtered.				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Mayer's Test	Filtrates + Mayer's reagent (Potassium Mercuric Iodide)	yellow precipitate	Presence of alkaloids
2	Wagner's Test	Filtrates + Wagner's reagent (Iodine in Potassium Iodide)	brown/reddish precipitate	Presence of alkaloids.
3	Dragendroff's Test	Filtrates + Dragendroff's reagent (Solution of Potassium Bismuth Iodide)	red precipitate	Presence of alkaloids
4	Hager's Test	Filtrates + Hager's reagent (Saturated picric acid solution)	yellow precipitate	Presence of alkaloids

Detection of carbohydrates				
Extracts + 5 ml distilled water and filtered and The filtrates were used for following tests				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Molisch's Test	Filtrates + 2 drops of alcoholic α -naphthol solution	Formation of the violet ring at the junction	Presence of Carbohydrates
2	Benedict's test	Filtrates + Benedict's reagent (Heat)	Orange red precipitate	Reducing sugars
3	Fehling's Test	Filtrates + dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions	Red precipitate	Reducing sugars
Detection of glycosides				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Modified Borntrager's Test	Extracts + Ferric Chloride and immersed in boiling water for about 5 minute cooled and add equal volumes of benzene	Benzene layer + ammoni sol Give pink colour	Presence of anthranol glycosides
2	Legal's Test	Extracts + sodium nitroprusside in pyridine and sodium hydroxide.	Pink colour	cardiac glycosides
3	Froth Test	Extracts + distilled water to 20ml and shaken for 15 minutes	Formation of 1 cm layer of foam	Presence of saponins
4	Foam Test	0.5 gm of extract + shaken with 2 ml of water	If foam produced persists for ten minutes	Presence of saponins
Detection of phytosterols				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Salkowski's Test	Extracts + chloroform and filtered and filtrates + few drops of Conc. Sulphuric acid shaken	Appearance of golden yellow	Presence of triterpenes.

2	Liebermann Burchard test	Extracts + chloroform and filtered filtrates + few drops of acetic anhydride, boiled and cooled + Conc. H ₂ SO ₄	brown ring at the junction	Presence of phytosterols.
Detection of phenols				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Ferric Chloride Test	Extracts + 3-4 drops of ferric chloride solution	Formation of bluish black	colour presence of phenols
Detection of tannins				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Gelatin Test	Extract + 1% gelatin solution containing sodium chloride	white precipitate	presence of tannins
Detection of flavonoids				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Alkaline Reagent Test	Extracts were + few drops of sodium hydroxide solution	yellow colour which becomes colourless on addition of dilute acid	presence of flavonoids
2	Lead acetate Test	Extract + 0.25% w/v Ninhydrin reagent was added and boiled for few minute	blue colour	Presence of amino acid.
Detection of proteins and amino acids				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Xanthoproteic Test	Extracts + few drops of conc. Nitric acid	yellow colour	Presence of proteins.
2	Ninhydrin Test	Extract + 0.25% w/v Ninhydrin reagent was added and boiled for few minutes	blue colour	Presence of amino acid

Detection of flavonoids				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Alkaline Reagent Test	Extracts + few drops of NaOH solution. Intense yellow colour.	which becomes colourless on addition of dilute acid	presence of flavonoids
Detection of diterpenes				
Sr. No	Name of Test	Procedure	Observation	Inference
1	Copper acetate Test	Extracts dissolved in water + 3-4 drops of copper acetate solution	emerald green colour	presence of diterpenes

5. SPECTROSCOPICAL ANALYSIS:

5.1 Ultra Violet Spectroscopy

UV spectroscopy is important technique in the analysis of herbal as well as synthetic drugs. It gives idea about purity of the substance. Its detection capability depends on Beer-Lamberts Law that is absorbance is directly proportional to the concentration and path length. [25]

Table 12: Examples of drugs with their wave-lengths [1]

Drug	UV range (nm)
Morphine	284
Aloe-emodin	225,258,279,287,430
Caffeine	243,326
Scopholine	227,250,288,339

5.2 Infrared spectroscopy: [11]

Significance -It is analytical technique for detection of functional groups.

IR Spectra may be measured on plant substances in an automatic recording IR spectrophotometer.

The sampling of the solid sample is done by using mulling technique.

Some important IR Frequencies

Amines -3300-3500 cm^{-1}

Alkanes-2940-2860 cm^{-1}

Carboxylic acid-3520 cm^{-1}

Cynide-2225 cm^{-1}

Hydroxyl-3400-3500 cm^{-1} .

5.3 Mass Spectrometry:

It gives idea about molecular weight and molecular formula. The idea of molecular weight is generated from molecular ion peak. The index of hydrogen deficiency is useful for prediction of validated molecular formula and no of unsaturation. [11]

5.4 NMR Spectroscopy –

It is spectroscopic technique which gives idea of no and types of protons present in particular structure of compound. [11]

5.5 Chromatographic evaluation

5.5.1 Thin Layer Chromatography

TLC is one of the most important tool for separation of compound. It is widely used technique of chromatography. It is based on principle of adsorption [18]. In this method stationary phase is a finely

divided solid and it is applied as a thin layer on supporting plate and the mobile phase is a liquid which is allowed to flow on the surface of the plate

by capillary action.

Common adsorbent material used- Silica gel, Alumina, Kieselguhr.

Table 13: Examples of drugs with their solvent system ^[1]

Drug	Adsorbent	Solvent system
Rauwolfia alkaloids	Silica gel 60 F ₂₅₄	Ethyl acetate: Methanol: Water (100:13.5:10)
Colchicum alkaloids	Silica gel 60 F ₂₅₄	Ethyl acetate: Methanol: Water (100:13.5:10)
Funiculum vulgare	Silica gel 60	Toluene:Ethyl acetate(93:7)
Tribulus terrestris	Silica gel G	Toluene:Ethyl acetate(8:2)

5.5.2 HPTLC:

In HPTLC a layer thickness of 100-150micron is used to achieve separation. HPTLC uses open layers of adsorbents on plates or foils to separate component of samples. ^[1]

Significance of HPTLC: Identification and detection of adulterants in herbal product and it is also important in identification of pesticide content, mycotoxins and in quality control of herbs and health foods ^[27]

Examples:

i. 18 beta-glycyrrhetic acid

Stationary Phase: Silica gel

Mobile Phase: Ethyl acetate:Menthol:Ammonia(10:3:1)

Quantification: UV absorbance (260nm) . ^[17]

ii. Panaxadiol and Panaxatriol

Stationary Phase: Silica

Mobile Phase: Chloroform:Ether(1:1)

Detection: Spraying with 10% Sulphuric acid in methanol, heating at 105°C for 10 min.

Quantification: UV absorbance (544nm and 52nm) . ^[17]

iii. Flavonol Glycosides:

Stationary Phase: Silica gel

Mobile Phase: Chloroform:Benzene:Ethanol:Acetic acid:Water (11:4:2:1:2)

Detection: Spraying with 8% AlCl₃ in ethanol. .

Quantification: UV absorbance 370nm. . ^[17]

iv. Carvone:

Stationary Phase: Silica gel

Mobile Phase: Chloroform : acetone (100:2)

Detection: By dipping in anisaldehyde sulphuric acid reagent, heating at 80° for about 10 min.

Quantification: UV absorbance 410nm. . ^[17]

v. Aloin:

Stationary Phase: Silica gel

Mobile Phase: Ethyl acetate : formic acid: Water (17: 2: 3)

Quantification: UV absorbance 350 nm. ^[17]

vi. Andrographis Paniculata

Pre-coated plates: TLC Silica gel 60 F, 0.25mm, 108 x 20cm.

Prewashing: methanol

Mobile phase: Chloroform : methanol(8:1)

Preconditioning: Chloroform : methanol (8:1)

Development and drying: Automatic development chamber.^[1]

Stationary Phase: Silica gel 60F₂₅₄

Mobile Phase: Ethyl acetate : Diethyl amine (88:12)

Quantification: UV absorbance 236 nm.

R_f Value: 0.24

vii. HPTLC chromatogram of Quinine^[12]

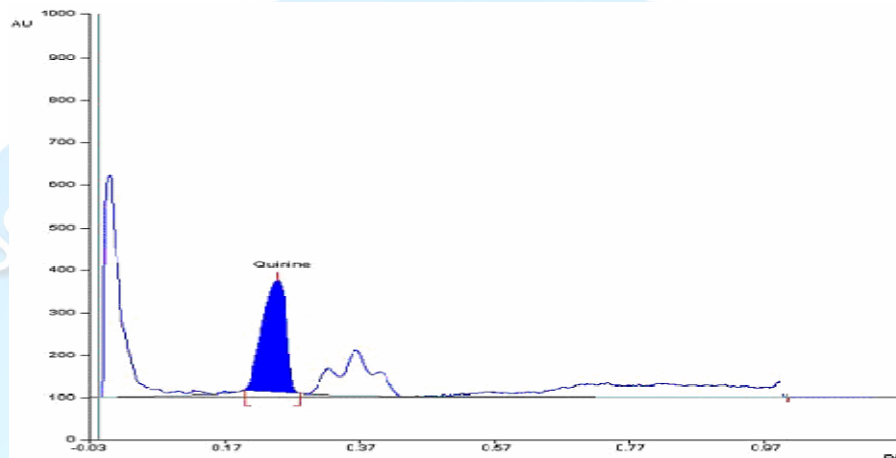


Fig.1 HPTLC chromatogram of Quinine

viii. HPTLC chromatogram of Vasicine^[21]

Pre-coated plates: Silica gel 60 F₂₅₄

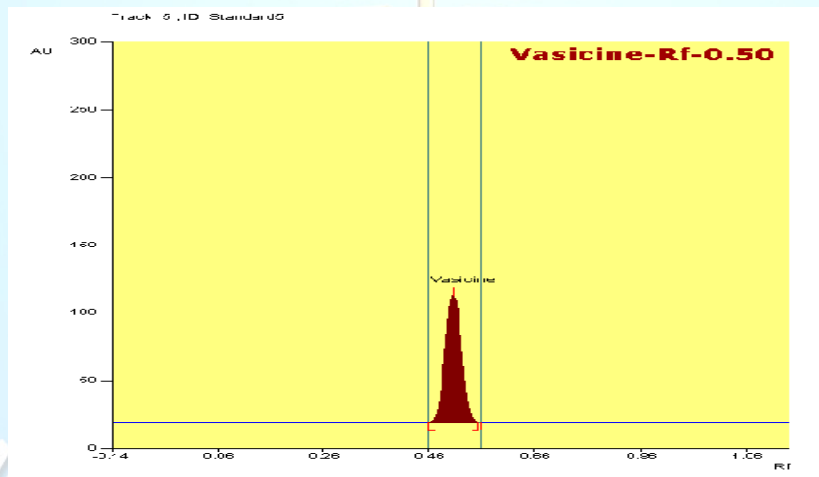


Fig.2 HPTLC chromatogram of Vasicine

5.5.3 HPLC

HPLC is useful for isolation and purification of herbal compounds. There are two types of preparative HPLC: low pressure HPLC (near about 5 bar) and high pressure HPLC (>20 bar).^[7] This is very

important in pharmaceutical industry because it is efficient purification technique and it spend less time on the synthesis conditions.^[6]

Examples:

i. HPLC study of Azadirectin^[29]

Mobile Phase: Aceto nitrile: methanol: tri ethyl amine (60:40:1)

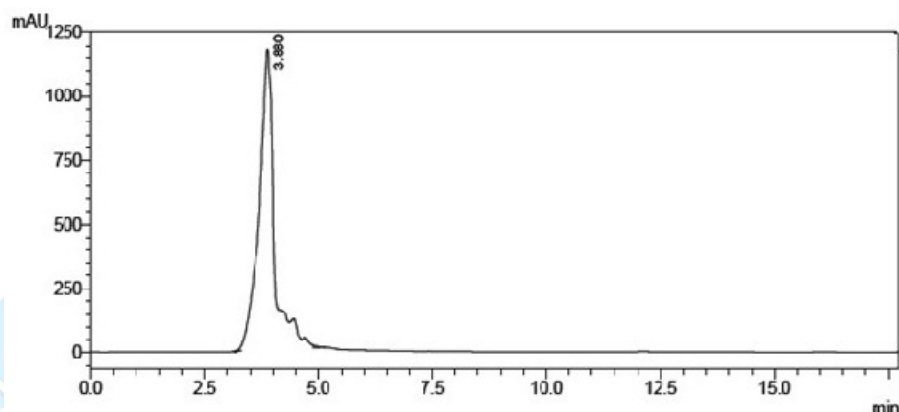


Fig.3 HPLC Spectra of Azadirectin

ii. HPLC study of clove ^[9]

Mobile Phase: methanol: acetonitrile : water (10:50:40)

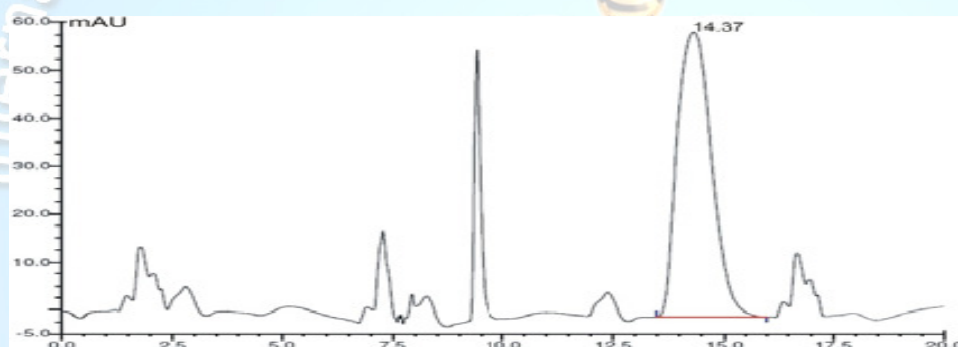


Fig.4 Chromatogram of Methanolic extract of Clove

Table 14: Examples of drugs with columns and mobile phase

Drug	Column	Temperature	Mobile phase
Glycyrrhizin	Permaphase AAX	50°C	Isopropanol : Phosphate buffer (30:70)
Atropine	Merckosorb SI 60	20°C	Diethyl ether : Methanol (60:40)
Cocaine	Merckosorb SI 60	20°C	Cloroform : Methanol (80:20)

5.5.4 Liquid Chromatography-Mass Spectroscopy (LC-MS)

This technique gives high detection, sensitivity and

specificity. LC-MS gives accurate determination of high molecular weight proteins, peptides. ^[5]

5.5.5 Gas liquid chromatography & gc-ms:

Significance: GC is an important tool for detection of volatile substances.

GLC separate the volatile substances by percolating a gas stream over a stationary phase. The basic of separation in GLC is the partitioning of sample in and out of the film of liquid spread over an inert solid. The nitrogen and helium are most common gases used in GC.^[16] Advantages of these methods are their high sensitivity, stability and high efficiency. Especially, the hyphenation with MS provides reliable information for the qualitative analysis of the complex constituents ^[10]

Examples:

i. Analysis of anethole in Fennel oil

Test sample: Fennel oil

Stationary phase: FFAP

Carrier gas: Helium

Sample size: 0.1micro litre

ii. Analysis of eugenol in clove oil

Test sample: Clove oil

Stationary phase: FFAP

Carrier gas: Helium

Sample size: 0.20 micro litre

6. CONCLUSION:

The pharmacopoeial standards in Ayurvedic Pharmacopoeia of India are not Sufficient to ensure the quality of plant materials because the materials received in the manufacturing premises are not manufactured in a condition that effective microscopic examination can be done. That's why Physical chemical, Microscopic, Analytical, and chromatographic analysis are preferred to determine the proper quality of plant material. Non standardized procedures may lead to the improper examination of the phytochemical present in the plants and may lead to the variations thus leading to the lack of reproducibility. This review contains in brief all the information related to standardization procedures, that will helpful researchers to ensure the quality and to standardize the phytoconstituents.

7. REFERENCES

1. Agarwal SS, Paridhavi M. Herbal Drug Technology Published by University Press India Ltd., Hyderabad; 2007. Page no. 245-290.
1. 2.Amita P. Shalini Tripathi. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry 2014; 2 (5): 115-119.
2. Anjoo Kamboj (2012). Analytical Evaluation of Herbal Drugs, Drug Discovery Research in Pharmacognosy, Prof. Omboon Vallisuta (Ed.), ISBN: 978-953-51-0213-7.
3. Archana G, Shiv J.K, Pramod K.Sharma, Vipin K.Garg, Sharad V, Nitin K. Identification, evaluation and standardization of herbal drugs: A review Scholars Research Library;2 (6):302-315.
4. Bhutani KK, Finger-Printing of Ayurvedic Drugs, The Eastern Pharmacist, 2000; 507: 21-26.
5. Bhutani KK. Herbal medicines enigma and a challenge for science and guidelines for new initiatives. J Nat Prod 2003; 19(1): 3-8.
6. Chimezie A, Ibukun A, Teddy E, Francis O. HPLC analysis of nicotinamide, pyridoxine, riboflavin and thiamin in some selected food products in Nigeria. Afr J Pharm Pharmacol 2008; 2(2):29-36.
7. Douglas A Skoog, D West, F Holler, S Crouch 2004, Fundamentals of Analytical chemistry, Eighth Edition, page no. 707-1013.
8. Farhin I, Sujata D, Neha Narkhede. HPLC-UV method Development and Quantification of Eugenol from methanolic Extract of some spices. International Journal of chemical and physical sciences; Vol. 3, No. 6, Nov-Dec 2014.
9. Guo F.Q., Huang L.F., Zhou S.Y., Zhang T.M., Liang Y.Z., Comparison of the volatile compounds of Atractylodes medicinal plants by headspace solid-phase microextraction-gas chromatography-mass spectrometry. Anal.Chim. Acta 570: (2006) 73-78.
10. Herbone JB. Phytochemical methods Chapman and Hall, London, New York, 3rd edition Page no.21-29.
11. Himanshu Mishra ,Bhupendra K. Mehta and Dharam C. Jain. Optimization of extraction condition and HPTLC –UV Method for De-

- termination of Quinine Different Extracts of Cinchona species; Rec. Nat. Prod (2008) 2:4 107-115.
12. Indian Herbal Pharmacopoeia (Revised New Edition 2002), Published by Indian drug Manufacturers association Mumbai, Page no. 1-345
 13. K.Kirtikar and B.D.Basu, Indian medicinal plants, V-1, p-503-507.
 14. Khandelwal KR. Practical Pharmacognosy Techniques & Experiments. 24th edition, Nirali Prakashan (2002) Page no. 24.1-25.6.
 15. Kokate CK, Purohit AP, Gokhale SB. 2005. *Pharmacognosy*, 46th edition Nirali Prakashan, Volume 1. Page no. 6.19 – 6.28
 16. Kokate CK, Purohit AP, Gokhale SB. 2005. *Pharmacognosy*, 50th edition Nirali Prakashan, Volume 1. Page no. 7.4- 7.28
 17. Liang YZ, Xie P, Chan K, J., Quality control of herbal medicines, Chromatography B, 2004; 812: 53–70.
 18. Mukherjee PK. Quality control of Herbal drugs. First edition, Business Horizons (2002) Page no. 183-189.
 19. Nitin V, Kiran A. Wadkar, Manish S. Kondawar. Review on Standardization of Herbal churna. Int.J. Res Ayurveda Pharm. 2014; 5(3): 397-401.
 20. P. Ravi Kumar Development of HPTLC Method for Estimation of vasicine Herbal Formulations in Asfrez 12, World Journal of Pharmacy and Pharmaceutical Sciences, Volume 4, Issue 03, 1075-1083.
 21. R D Chaudhari, Herbal Drug Industry, A practical approach to industrial pharmacognocny, First edition, 1996 page no. 506.
 22. S Shrikumer; U Maheshwari; A Sughanti; TK Ravi. WHO guidelines for herbal drugs standardization. 2006.
 23. Satheesh Madhavi NN, Kumud Upadhya, Asha bishti. Phytochemical screening and standardization of poly herbal formulation for Dyslipidemia. Indian journal of physiology and pharmacology, 3(3), 2011. 2.
 24. Sharadha R. Kale, Rajendra R. Kale Biochemistry and Clinical Pathology Nirali Prakashan; As per E.R 1991 Page no. 1-19.
 25. Sharma US, Sharma UK, Singh A, Sutar N, Singh PJ. In vitro anthelmintic activity of *Muraya koenigii* linn. Leaves extracts. International journal of pharma and bio sciences 2010; 1(3): 1-4.
 26. Soni K, Naved T. HPTLC-Its applications in herbal drug industry. The Pharma Review 2010, 112-117
 27. Sunita Panchawat, Kamal Singh Rathore, Sssodia Nema RK. Standardization and evaluation of herbal drug formulations. 2010.
 28. Supriya D, V. Pranay, M Singaiah, J Satwik, Dr. V.V.L.N Prasad, Dr. Prakash V.D. Studies on extraction and HPLC Analysis of Azadirachtin from kernels of Neem Seeds J. Adv. Pharm. Edu & Res.