Review Article

Chromatographic Analysis of Phytopharmaceuticals– A Review

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ABSTRACT

The term "phyto" means plant while "pharmaceuticals" means a compound manufactured for use as a medicinal drug. Hence, phytopharmaceuticals are products derived from plant material. They are produced from fresh or dried, or otherwise preserved plants or parts of plants by expression, extraction, distillation and other operations. As they are complex mixture of many compounds, it is must to analyze each compound. For proper analysis it is very important to understand the chemistry of phytochemicals present in herbal products, based on its characteristic isolation, structural identification and quantification. Hence, chromatographic techniques are used to separate and isolate different compounds. Once the compounds are separated they need to be analyzed by spectroscopic techniques for identification and quantification. Various chromatographic techniques such as Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), High Pressure Liquid Chromatography (HPLC), Gas Chromatography (GC) are used for the analysis of phytochemicals present in the herbal formulations.

Keywords: Phytopharmaceuticals, chromatographic techniques, analysis, HPLC, HPTLC, TLC, GC.

INTRODUCTION

Phytopharmaceuticals are pharmaceutical products comprising of natural components derived from plants as opposed to chemically synthesized components.These products have a better tolerability profile and fewer side effects. They are a complex mixture derived from plant sources that is used as a medicine or a drug. Phytopharmaceuticals contain the active principles together with coexisting materials from the source plant, these additional materials have a greater or lesser beneficial influence upon the activity of the drug. A phytopharmaceutical may frequently not represent the final dosage form administered to the patient. Dry extract for example, are further processed to produce powder mixtures, tablets, suppositories and other dosage forms. They are considered to be intermediate or semi-finished products whose technical qualities are conductive to further processing. For example, de-enzymized gum acacia and gum tragacanth is also considered as phytopharmaceuticals. Pharmaceuticals made from chemical compounds are prone to adverse side effects. The human body will tend to dismiss certain synthetic compounds which do not occur naturally. These dismissals occur in the form of side effects; some as mild as minor headaches, and others as severe as to be potentially lethal while phytopharmaceuticals produce fewer to no side effects.[1]

Phytopharmaceutical analysis is done to check the -[2]

a. Identity: the condition of being specific herb.
b. Purity: the condition of being free from contaminants or adulterants.
c. Content: the amount of active constituents present within definite limit.

As phytopharmaceutical products contain numerous herbs and every herb containing numerous synthetic constituents, selective analytical methods or reference compounds may not be available commercially. The source and nature of the crude materials are variable. The strategies of harvesting, drying, storage, transporting, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) also have a great impact on the quality and purity of the product. Hence analysis of these products is difficult.[3] Pharmaceutical analysis is the branch of practical chemistry which deals with the resolution, separation, identification, determination and purification of a given specimen of a medicine or a pharmaceutical; the detection and estimation of impurities that may be present therein is also included.[4]

Analysis of phytopharmaceutical products is done in a number of steps.

The steps involved are-[5]

a. Sample Preparation
b. Isolation and Purification of analyte
c. Identification of analyte
d. Quantification of analyte

The partition and purification of plant constituents is mainly carried out using one or other, or a combination, of these chromatographic systems: Paper Chromatography (PC), Thin Layer Chromatography (TLC), High Pressure Liquid Chromatography (HPLC), High Performance Thin Liquid Chromatography (HPTLC). The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated.[6]

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Chromatography is an analytical method that is widely used for separation, identification and determination of the chemical components in complex mixtures.

It consists of a mobile phase and a stationary phase. Components of mixture are carried through the stationary phase by the flow of gaseous or liquid mobile phase, separation being based on the differences in migration rates among the sample components.

- **Types of Chromatography**

a. Paper chromatography
b. Thin layer chromatography
c. High performance thin layer chromatography
d. High pressure liquid chromatography
e. Supercritical fluid chromatography
f. Gas chromatography
g. Gel chromatography
h. Flash chromatography

Chromatographic methods used in Phytopharmaceutical Analysis[7]–

i. **Paper chromatography (PC)-** it is particularly applicable to water soluble plant constituents, namely the carbohydrates, amino acids, nucleic acid bases, organic acids and phenolic compounds.

ii. **Thin layer chromatography (TLC)-**it is a method of choice for separating all lipid-soluble components, i.e. the lipids, steroids, carotenoids, simple quinones and chlorophylls.

iii. **Gas liquid chromatography (GLC)-** it is mainly used for separation of volatile compounds, fatty acids, mono- and sesquiterpenes, hydrocarbons and sulphur compounds.

iv. **High pressure liquid chromatography (HPLC)**- it combines column efficiency with speed of analysis. Less volatile constituents are separated by this technique.

v. **High performance thin layer chromatography (HPTLC)**- it is the most recent type of TLC plate, coated with the more fine micro particles of silica that are used in the columns for HPLC.

The partition and purification of plant constituents is essentially carried out utilizing one or other, or a combination, of the above chromatographic systems.

Thin Layer Chromatography (TLC)-

The standardized thin-layer chromatographic procedure can be used effectively for the screening, analysis as well as quality evaluation of the plant or its derived herbal products. Newer approaches in thin-layer chromatography enable the analyst to separate and determine the useful natural products in complex mixtures of plant products. Different adsorbents such as cellulose, silica gel, aluminium oxide, celite, calcium hydroxide, ion exchange resin, etc may be spread on to a glass plate or other support and employed for chromatography. It is an advantageous method because of its versatility, speed and sensitivity. [8]

Quantitative determination of sibutramine in adulterated herbal slimming formulations by TLC-image analysis method[9]

Panadda Phattanawasin et al developed a simple TLC-image analysis method for rapid determination and quantitation of sibutramine hydrochloride (SH) adulterated in herbal slimming products. Chromatographic separation of SH was achieved by them on a silica gel 60 F_{254} TLC plate, using toluene-n-hexane-diethylamine (9:1:0.3, v/v/v) as the mobile phase and Dragendorff reagent as spot detection.

They applied TLC-image analysis and TLC-densitometric methods for the detection and quantification of SH in twenty herbal slimming formulations which were guaranteed to contain only natural ingredients obtained from a variety of herbs such as aloe vera, garcinia cambogia, lotus root, konjac and cinnamon. Six formulations from twenty products were shown to be adulterated with sibutramine Hydrochloride. The presence of SH peak was clearly observed in the TLC chromatograms of the adulterated samples (Fig 1).

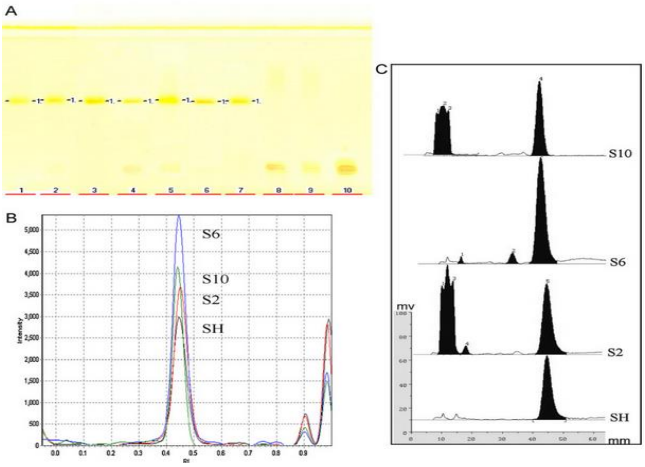

Figure 1: (A) TLC of standard SH (track 1), adulterated slimming formulations (tracks 2–7: S2, S6, S7, S9, S10, S19) and SH-unbound samples (tracks 8–10: S14, S15 and S16), (B) TLC chromatogram of standard SH, S2 (slimming coffee), S6 (slimming gel) and S10 (slimming capsule) obtained from TLC-image analysis and (C) image of TLC-densitometry.

They then performed the image analysis of the scanned TLC plate to quantify the amount of sibutramine hydrochloride. The polynomial regression data for the calibration plots displayed good linear relationship in the concentration range of 1–6 mg/spot. The limits of detection and quantitation were 190 and 634 ng/spot, respectively. The procedure gave acceptable specificity,

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precision, accuracy, robustness and was applied for determination of SH in herbal formulations.

The proposed TLC-image analysis procedure could therefore be helpful and affordable to local authorities and small laboratories due to its simplicity, low operating cost and uses of inexpensive and general instruments to conduct surveillance programs for quick detection and content determination of undeclared SH to ensure the safety of herbal slimming products.

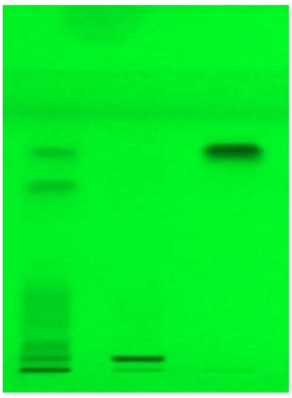
High Performance Thin Layer Chromatography (HPTLC)-

HPTLC is one of the sophisticated instrumental techniques based on the full capabilities of TLC. It is coated with the same micro particles of silica that are used in the columns for HPLC. It is most flexible, reliable and cost efficient separation technique. The advantage of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, and so on enable it to be powerful analytical tool for chromatographic information of complex mixtures of pharmaceutical natural products i.e. phytopharmaceutical products. [10]

Development and validation of HPTLC method for simultaneous estimation of rutin and quercetin in hydroalcoholic extract of triphala churna[11]

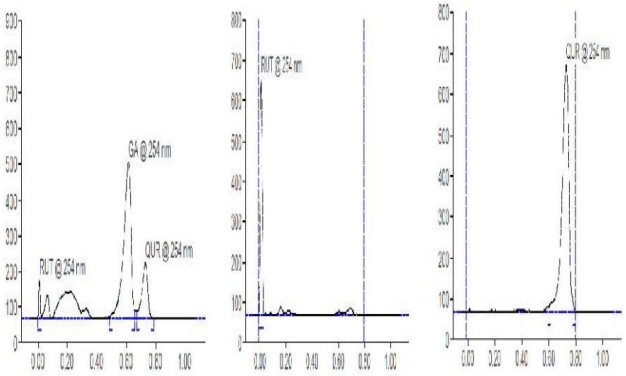
N P Pawar et al, developed a new simple, precise, rapid and selective HPTLC method for the simultaneous determination of rutin and quercetin in ayurvedic formulation triphalachurna. They performed the separation and identification of quercetin, rutin separately on aluminum backed silica gel 60 F_{254} (20cm x10cm of plate size, layer thickness 0.2 mm) which was used as a stationery phase. They applied the samples to the plates as 8 mm bands, with a CamagLinomat V applicator. They developed the plates by ascending technique, in a Camag twin trough glass chamber with a stainless steel lid, utilizing a mobile phase, comprising of ethyl acetate: formic acid: acetic acid: water (10: 1.1: 1.1: 0.6) for both rutin and quercetin. After development, they dried the plates with a hot-hair dryer, viewed in a Camag UV cabinet at 254 nm & 366 nm, and then scanned with a Camag TLC Scanner, using win CATS software (version 1.4.6) , in absorbance mode, with slit dimensions 6.00 x 0.45 mm. The detection wavelength 254 nm was selected. They found that the retention factor (R_f) values were 0.01, 0.76 for rutin and quercetin respectively.

They confirmed the identity of rutin and quercetin by comparing chromatogram of standard rutin and quercetin with that of extract and by comparing retention factor of reference with standard.



D E F

Figure 2: (D) Hydroalcoholic extract of triphalachurna, (E) Standard rutin, (F) Standard quercetin.



L M N

Figure 3: Chromatogram of (L) Triphalachurna, (M) Standard rutin, (N) Standard quercetin.

High-performance liquid chromatographic (HPLC)-

HPLC is the latest chromatographic technique to be added to the phytochemist's armoury. This technique is mainly used for those classes of compounds which are non-volatile, e.g. higher terpenoids, phenolics of all type, alkaloids, lipids and sugars. It works best for compounds which can be detected in the ultra-violet or visible regions of the spectrum. It works best for compounds which can be detected in the ultra-violet or visible regions of the spectrum. Thus, over the past decades, HPLC has received the most ample application in the analysis of herbal medicines. Reversed-phase (RP) columns are considered to be the most popular columns used in the analytical separation of herbal medicines. [12]

High pressure liquid chromatographic method for the quantification of phenolics in chyavanprash[13]

Chyavanprash is used widely as an immune modulator and rejuvenator. The rejuvenating and tonic properties of 'Chyavanprash' are considered majorly due to their antioxidant principles, which in turn is due to the presence of phenolic compounds.

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Govindarajan et al, developed a simple HPLC method for the partition and quantitative estimation of the major antioxidant compounds from Chyavanprash. This method enabled the efficient separation of phenolic compounds catechin, quercetin-3-O-rutinoside, syringic acid and gallic acid. They used Waters Symmetry® (150mm×3.6mm, i.d., 5m pore size), guard column as stationary phase and solvent system as water: phosphoric acid (99.7:0.3, v/v) labeled as solvent A and acetonitrile :water :phosphoric acid (79.7:20:0.3,v/v)labeled as solvent Busing a gradient elution in 0–5min with 88–85% A, 5–10min with 85–75% of A, 10–20min with 75-70% of A, 20–25min with 70–50% of A, 25–30min with 50–30% A and 30–35min with 30–88% of A.

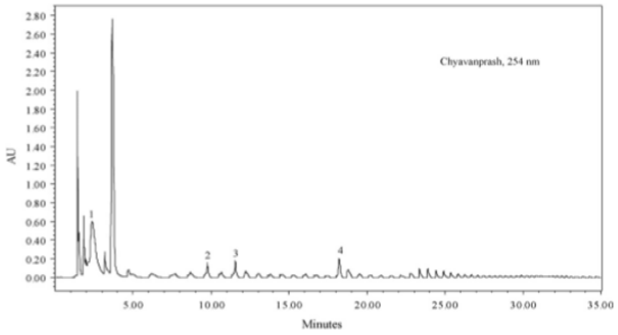

Figure 4: Chromatograms registered for Chyavanprash at 254 and 280 nm, showing the phenolics: (1) gallic acid; (2) catechin; (3) syringic acid; (4) rutin.

They identified four phenolics in Chyavanprash, i.e. gallic acid, catechin, syringic acid and rutin (Fig 4). The chromatograms also showed many other peaks apart from the 10 standards studied (Fig.5) work is still in progress to identify them also.

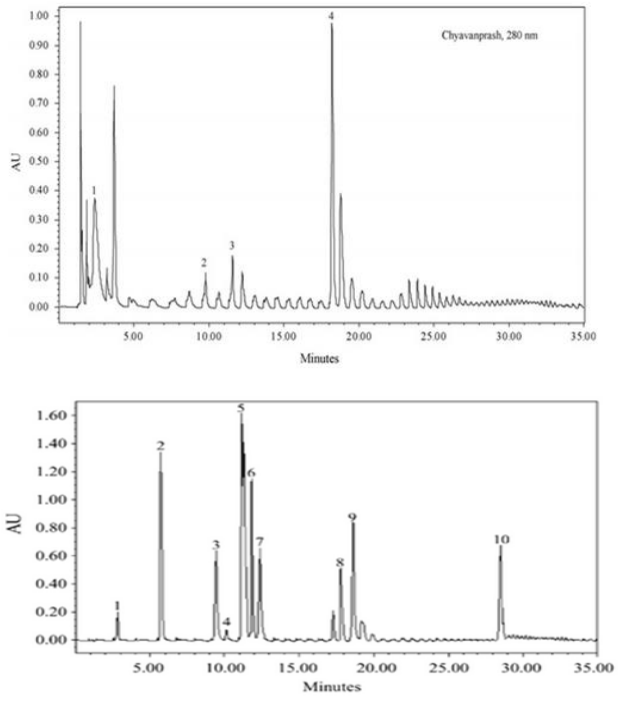

Figure 5: Chromatograms registered for standards at 280 nm, showing the phenolics: (1) gallic acid (2) protocatechuic acid (3) catechin (4) caffeic acid (5)vanillic acid (6) chlorogenic acid (7) syringic acid (8) rutin (9) ferulic acid (10) quercitrin.

Gas Chromatography (GC)-

Gas chromatography (GC), also called as gas liquid chromatography (GLC), is a technique for separation of mixtures into components by a process which depends on the redistribution of the components across a stationary phase or support material in the form of a liquid, solid or combination of both and a gaseous mobile phase. It is well-known that many pharmacologically active constituents in herbal medicines are volatile chemical compounds. Thus, the analysis of volatile compounds by gas chromatography is very important in the analysis of herbal medicines. GC provides both qualitative and quantitative data on plant substances, since measurements of the area under the peaks shown on the GC trace are directly related to the concentrations of the different components of the original mixture. The GC apparatus can be set up in such a way that the separated components are further subjected to spectral or other analysis. Most frequently, GC is automatically linked to mass spectroscopy (MS) and the combined GC-MS apparatus has emerged in recent years as one of the most important of all techniques for phytochemical analysis. [14]

Analytical method development and validation of pain relief herbal formulations[15]

Rucha J. Shah et al, developed a rapid, sensitive, precise, and robust gas chromatography method for the simultaneous estimation of menthol, methyl salicylate and linseed oil in laboratory prepared pain relief herbal formulation and marketed pain relief formulation. They carried out the determination on capillary gas chromatography using flame ionization detector.

They performed gas chromatography on Shimadzu GC2010 apparatus, equipped with split-splitless injector attached to DB01 column (30 m × 0.53 mm, film thickness 3 µm) and to flame-ionization detector (FID). They used nitrogen as the carrier gas and temperature set as follows: injector and detector both at 250˚C while column oven temperature program was set from 100-155˚C (2min) at a rate of 7˚C/min than upto 250˚C (15mins) at a rate of 50˚C/min. They performed data equisition and integration using GC solution software.

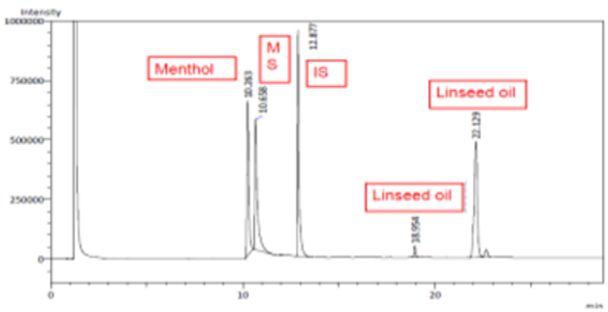

Figure 6: Chromatogram of standard mixture

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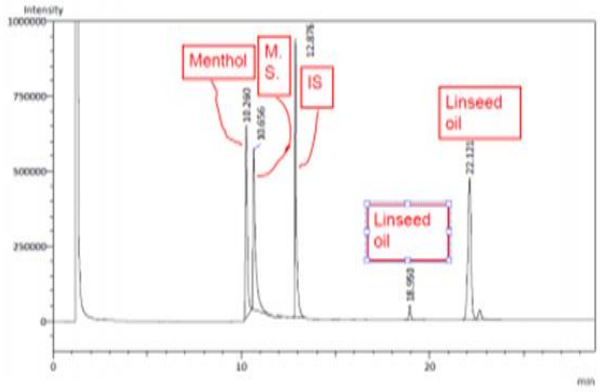

Figure 7: Chromatogram of laboratory formulation

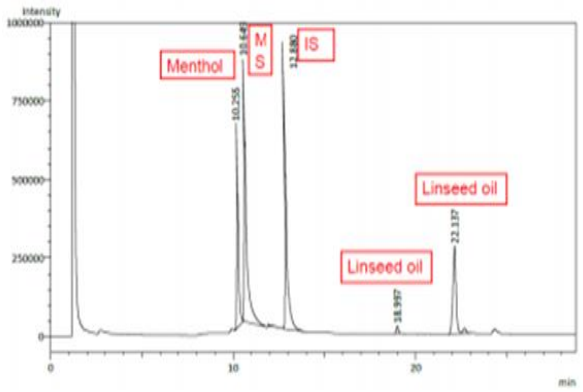

Figure 8: Chromatogram of marketed formulation

Good separation of menthol (tr= 10.368), methyl salicylate (tr= 10.795) and linseed oil (tr= 21.912 and 25.180) and terpene hydrate (tr= 13.914) was obtained. The recovery of Menthol, Methyl Salicylate and Linseed oil was observed to be 98.34%, 99.1% and 102.74% respectively. The correlation coefficients of linear regression analysis (r^2) were found to be 0.999, 0.999 and 0.996 respectively. They found that the proposed GC method provided a good resolution of menthol, methyl salicylate and linseed oil.

CONCLUSION

- Nowadays, phytopharmaceuticals have extensive acceptability as therapeutic as well as nutritional agents.
- Since most of the phytopharmaceutical products are polyherbal and as each herb contains many phytoconstituents, their analysis becomes very difficult and tedious.
- Hence, it is of utmost importance to develop an authentic analytical method which can reliably profile the phytochemical composition and quantitative analysis of phytochemicals and testing of marker/bioactive compounds and other major constituents present in the formulation.
- The above discussed chromatographic techniques were not only found to be simple and selective but it also gave rapid and precise results in phytopharmaceutical analysis.

REFERENCES

1. List PH, Schmidt PC, Phytopharmaceutical Technology, Wiley India Pvt Ltd, New Delhi, India, 2010, 1-2.
2. Vallisuta O, Olimat SM, Drug Discovery Research in Pharmacognosy, CBS publishers and distributers Pvt Ltd, India, 2012, 29.
3. Vallisuta O, Olimat SM, Drug Discovery Research in Pharmacognosy,CBS publishers and distributers Pvt Ltd, India, 2012, 30.
4. Shaikh A, Development of stability indicating method for validation of losartan potassium and hydrochlorothiazide drugs in solid dosage form by RP-HPLC, International Journal of Pharma and Bio Sciences, 4(1), 2013, 621 – 638.
5. Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd edition, Thomson Science, New York, London, 1998, 17, 32.
6. Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd edition, Thomson Science, New York, London, 1998, 7.
7. Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd edition, Thomson Science, New York, London, 1998, 3-7,8
8. Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd edition, Thomson Science, New York, London, 1998, 11.
9. Phattanawasin P, Sotanaphun U,Sukwattanasinit T, Akkarawaranthorn J, Kitchaiya S, Quantitative Determination Of Sibutramine In Adulterated Herbal Slimming Formulations by TLC-Image Analysis Method, Forensic Science International, 219, 2012, 96–100.
10. Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd edition, Thomson Science, New York, London, 1998, 12.
11. Pawar NP, Salunkhe VR, Development And Validation Of HPTLC Method for Simultaneous Estimation of Rutin And Quercetin In Hydroalcoholic Extract of TriphalaChurna, International Journal of PharmTech Research, 4(4),2012,1457-1463.
12. Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd edition, Thomson Science, New York, London, 1998, 15.
13. Govindarajan R, Singh DP, Rawat AK, High pressure liquid chromatographic method for the quantification of phenolics in chyavanprash, Journal of Pharmaceutical and Biomedical Analysis, 43, 2007, 527–532.
14. Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd edition, Thomson Science, New York, London, 1998, 14.
15. Shah RJ, Patel AI, Vikani KV, Patel NL, Analytical Method Development and Validation of Pain Relief Herbal Formulations, Indo American Journal of Pharmaceutical Research, 6(4), 2016, 5112-5117.

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