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HPLC AND HPTLC - GREEN SIGNAL FOR TARGET MOLECULES IN NATURAL ORIGIN

G. S. Khambe*, D.J.Joshi, J.R.Patil

Vansantidevi Patil Institute of Pharmacy, Kodoli, Tal. Panhala, Dist. Kolhapur, (M S), India.

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HPLC, Herbal medicine, Standardization, analytical Evaluation, Marker Compound

For Correspondence:

G. S. Khambe

Vansantidevi Patil Institute of Pharmacy, Kodoli, Tal. Panhala, Dist. Kolhapur, (M S), India

E-mail:

Sgeetanjali337@gmail.com

ABSTRACT

The growth in popularity in herbal medicine has offered new opportunities to realize significant returns for production on relatively small areas of land. Currently many of the herbal products are going to market without sufficiently assay as to quality. As a result, herbal product quality is known to be variable and damage is done to the credibility of the herbal industry as a whole. HPLC is a popular method for the analysis of herbal medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Thus, over the past decades, HPLC has received the most extensive application in the analysis of herbal medicines. Reversed-phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines.

TRADITIONAL SYSTEM OF MEDICINE

The pharmacological treatment of disease began long ago with the use of herbs. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose.

Name of System	History	
Ayurveda	Ayurveda got organized and enunciated around 1500 BC. Atharvaveda, the last of the four great bodies of knowledge- known as Vedas, which forms the backbone of Indian civilization, contains 114 hymns related to formulations for the treatment of different diseases. It has been conceptualized that the universe is composed of five basic elements named Prithvi (Earth), Jala (Water), Teja (Fire), Vayu (Air) and Akash (Space/Ether). Ayurveda is known as Astanga Ayurveda- means that which is made up of eight parts. The eight major divisions of Ayurveda are as follow as: 1. Kayachikitsa (Internal Medicine) 2. KaumarBhritya (Pediatrics) 3. Bhootavidya (Psychiatry) 4. Shalakya (Otorhinolaryngology and Ophthalmology) 5. Shalya (Surgery) 6. AgadaTantra (toxicology) 7. Rasayana (Geriatrics) and 8. Vajikarana (Aprhodisiacs and Eugenics)	RamachandraRao, 1987
Unani	The Arabs were instrumental in introducing Unani medicine in India around 1350 AD. Some of the renowned physicians who were instrumental in development of the system are- Akbar Mohd Akbar Arzani (around 1721 AD)- the author of the books- QarabadinQadri and Tibbe Akbar; Hakim M. Shareef Khan (1725-1807)- a renowned physician well-known for his book IlajulAmraz.According to the basic principles of Unani the body is made up of four basic	Hippocrates (460-377 BC), Galen (130-201 AD)

	elements i.e. Earth, Air, Water, Fire which have different Temperaments i.e. Cold, Hot, Wet, Dry. They give raise, through mixing and interaction, to new entities. Examination of the pulse occupies a very important place in the disease diagnosis in Unani. In addition examination of the urine and stool is also undertaken. Disease conditions are treated by employing four types of therapies- a- Regimental therapy, b- Dietotherapy, c- Pharmacotherapy and d- Surgery.	
Siddha	Siddha system of medicine is practiced in some parts of South India especially in the state of Tamilnadu. The term 'Siddha' has come from 'Siddhi'- which means achievement. According to the tradition eighteen Siddhars were supposed to have contributed to the development of Siddha medicine, yoga and philosophy.	Agasthiya Guru
Japanese traditional medicine	Many herbal remedies found their way from China into the Japanese systems of traditional healing. Herbs native to Japan were classified in the first pharmacopoeia of Japanese traditional medicine in the ninth century	Saito, 2000
Traditional Chinese medicine	Traditional Chinese medicine has been used by Chinese people from ancient times. Of the more than 12 000 items used by traditional healers, about 500 are in common use	Li Shizhen
Indian traditional medicine	Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment Traditional Systems of medicines always played important role in meeting global health care needs. India has unique distinction of having six recognized systems of medicine in category. They are- Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homoeopathy.	Morgan, 2002

Herbal Medicines and its significance

Sr. No	Botanical Name	Family	Local Name	Pare Used	Uses
1	Adhatodavasica Nees	Acanthaceae	Vasaka	Root & leaf	Expectorant used in asthma, bronchitis, cough and dysmenorrhea
2	Bacopa monnieri (Linn.) Penn.	Scrophulariaceae	Brahmi	Leaf	Nervous exhaustion, generalized fatigue, epilepsy, improves memory, anti-ageing and bronchitis, coughs
3	Curcuma longa Linn	Zingiberaceae	Haldi	Rhizome	Aromatic, stimulant, tonic, carminative, blood purifier, anti periodic, alterative, for sprains and wounds, purulent conjunctivitis Antioxidant, anti-viral, anti-inflammatory, anticarcinogenic
4	Emblica officinalis Gaertn.	Euphorbiaceae	Amalaki	Fruit	Increases red blood cell counts and therefore improves anemia, asthma, bronchitis, stomach problems and hemorrhoids
5	Glycyrrhiza glabraLinn.	Leguminosae	Yashti-madhu	Root	For gastrointestinal health. It is a mild laxative, which soothes and tones the mucous membranes and relieves muscle spasms. It is rich in flavonoids and an antioxidant, cancer protecting, botanical boosting and an anti-mutagen, preventing damage to genetic material that can eventually result in cancer.
6	Commiphora mukul Engl.	Burseraceae	Guggul	Gum & resin	Guggul is a resin, the major ingredient in joint care and immuno care, increase white blood cell count and to possess strong immuno-modulating properties.
7	Piper longum Linn.	Piperaceae	Pippali	Fruit	Pippali is a powerful stimulant for both the digestive and the respiratory systems and has showed a rejuvenating effect on lungs. It plays an important role in aiding the thermogenic response, i.e. the release of metabolic heat energy. Pippali a typical Ayurvedic complementary component whose benefit is to increased the bioavailability.
8	Terminalia arjuna W. & A.	Combretaceae	Arjuna	Stem bark	Arjuna is a heart tonic that has been used to support the cardiovascular functions, Revitalizing, circulation.
9	Rauwolfia serpentina Benth.	Apocynaceae	Sarpagandha	Root	High blood pressure, mental agitation, insomnia, sedative, hypnotic. Sarpagandha is the source of reserpine, an anti-hypertensive drug used since 1970
10	Datura metel Linn.	Solanaceae	Datura	Whole plant	Whooping cough, muscle spasm, sciatica, asthma and painful menstruation
11	Evolvulusalsinoides Linn.	Convolvulaceae	Shankhpushpi	Whole plant	General weakness, nervous exhaustion and memory loss
12	Boerhaaviadiffusa Linn.	Nyctaginaceae	Punarnava	Root	Diuretic, laxative, expectorant used in asthma, bronchitis, anemia and anti-inflammatory
13	Asparagus racemosus Willd	Liliaceae	Shatavari	Root	Increases muscle strength, stomach, lungs, and sexual organs, increases breast milk secretion during lactation and male impotence.

14	Ocimum sanctum Linn.	Lamiaceae	Tulasi	Leaf	Tuberculosis, ringworm, ear infections, common cold, cough, bronchitis, general stress syndrome, skin infections, indigestion, nausea and sinus infection.
15	Terminalia chebula Retz.	Combretaceae	Haritaki	Fruit	In Sanskrit, Haritaki means "carries away" (all diseases). Haritaki is a safe and effective purgative, expectorant and tonic. It is a component of the classic Ayurvedic combination called "Triphala".
16	ZingiberofficinaleRosc	Zinziberaceae	Ginger	Root	To improve digestion and to prevent nausea. Helping bowel movements and relaxing the muscles are controlling the digestive system. Absorption and prevents gastrointestinal side effects.
17	Aloe barbadensis	Liliaceae	Gwarpatha	Leaf	Stomachic, purgative anathematic in piles and rectal fissures, constipation, menstrual, suppression.
18	Abruspreacatorim Linn.	Fabaceae	Ganja	Seed	Purgative, emetic, tonic, aphrodisiac, nervous disorder and cattle poisoning, abortion.
19	Bergenia ciliates Sternb	Saxifragaceae	Bheda	Rhizome	Tonic, used in fever, diarrhea, pulmonary, affections, anti scorbutic, bruised and applied to boils and ophthalmic
20	Syzygiumaromaticum Linn.	Myrtaceae	Lavang	Flower bud	Stimulant, aromatic, carminative, used in flatulence and dyspepsia.
21	Panaxpseudoginseng	Araliaceae	Ginseng	Root	It has acquired a very favorable reputation for treatment of blood disorders, including blood stasis, bleeding, and blood deficiency.
22	Allium sativum	Liliaceae	Lahasun	Bulb	Carminative, aphrodisiac, stimulant in fevers, coughs febrifuge, in intermittent fever, skin diseases, and collie earache
23	Eclipa alba Hassk.	Asteraceae	Bhringaraj	Whole plant	Liver disorders, skin and hair care, improves complexion, viral hepatitis, calms the mind, memory disorders, and strengthens spleen and general tonic.
24	Nardostachysjatamansi DC.	Valerianaceae	Jatamansi	Root	Jatamansi is relaxing plant with established effectiveness for mental health. Ayurvedic practitioners include it in their formulations to address anxiety. It has been shown effective in maintain a restful sleep and with many menopausal symptoms.
25	Syzygiumcumini Linn.	Myrtaceae	Jamun	Seed & stem bark	Astringent, decoctions, gargles and washes, diarrhea, dysentery, stomachic, carminative and diuretic.

Some important Indian pharma Companies relevant to herbal Products

Sr. No	Name of companies	Marketed Product
1	Dabur	DaburChyawanprash, Hajmola Digestive Tablets, DaburAmla hair oil, Vatica (Shampoo), LalDantManjan (Tooth Powder).
2	Baidyanath	Herbal teas, Massage oil, Chyawanprash, Shikakai (soap pod)
3	Zandu	Zandu Bam, Cosmetic products.
4	Himalaya	Liv-52, Abana, Bleminor, Bonnisan, Bresol, Chiropex, Clarina Anti-Acne Cream, Clearvital, Confido, Diabecon, Diarex, Evecare, Gasex Syrup, Geriforte, Hairzone, Herbolax, Himcoline, HiOwna, Koflet, Lucol, Menosan, Mentat, Oxitare, Pilex, Purim, Renalka, Rumalaya, Septilin, Speman, Stylon, Talekt,Himalaya ashvagandha, Himalaya bale, Himalaya Bramhi, Himalaya gokshura, Himalaya guduchi, Haridra Etc.
5	Charak	OstolifeNutra, ObenilNutra, CharakDanil Oil, Vivadona Capsule, Vigorrol Jelly, Moh Nail Care Cream, Richelth Capsule, Vogomex Forte Tablets, Addyzoa Capsule, M2 Tone Tablet, Evanova Capsule, Prosteez Capsule, Neo Tablet, Galakol Tablet, Cytozen Tablet, Cognium Tablet, Femiplex Tablet, Sumenta Tablet, Hyponidd Tablet, Arjunin Capsule, M2 Tone Syrup, Miniscar Cream, Imupsora Ointment etc.
6	Vico	Vico Tooth Power, Vico Turmeric Cream, Vico Tooth Paste
7	Emani	Chyawanprash and other health products

Standardization of crude Drug and Herbal Products

Standardization of herbal formulations is essential in order to assess of quality drugs, based on the concentration of their active principles, physical, chemical, phyto-chemical, and standardization, and In-vitro, In-vivo parameters.

Standardization of raw materials includes the following steps:-

Authentication- Each and every step has to be authenticated, area of the collection, parts of the plant collection, the regional situation, as phytomorphology botanical identity, microscopic and histological analysis(characteristic features of cell walls, cell contents, starch grains, calcium oxalate crystals, hairs, fibers, vessels etc.)

Pharmacognostic evaluation It includes color, odor, taste, texture, size, shape, microscopical characters, and histological parameters.

Physico-chemical parameters It includes foreign matter, total ash, acid-insoluble ash, swelling and foaming index, assay, successive extractive values, moisture content, viscosity, PH, Disintegration time, friability, hardness, flow capacity, flocculation, sedimentation, alcohol content.

Chemical parameters It includes limit tests, chemical tests etc.

Chromatographic and spectroscopic analysis It includes TLC, HPLC, HPTLC, GC, UV, IR, FT-IR, AAS, LC-MS, GC-MS, fluorimetry etc.

Microbiological parameters It includes the full content of viable, total mould count, total coliforms count.

WHO GUIDELINES FOR QUALITY STANDARDIZED HERBAL FORMULATIONS

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

2) Stability assessment and shelf life.

3) Safety assessment; documentation of safety based on experience or toxicological studies.

4) Assessment of efficacy by ethnomedicalinformations 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).

ANALYTICAL EVALUATION:

In general, quality control is based on three important pharmacopoeias definitions:

Identity: Identity can be achieved by macro- and microscopical examinations. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification.

Purity is closely linked with the safe use of drugs and deals with factors such ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation includes microbial contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods

such as photometric analysis (UV, IR, MS, and NMR), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations.

Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are not known. Sometimes markers can be used. In all other cases, where no active constituent or marker can be defined for the herbal drug, the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeias.

HPLC and HPTLC- Method Developments of Marker Compound:

High performance liquid chromatography (HPLC), also known as high pressure liquid chromatography, is essentially a form of column chromatography in which the stationary phase consists of small particle (3-50µm) packing contained in a column with a small bore (2-5mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). The three forms of high performance liquid chromatography most often used are ion exchange, partition and adsorption.

High-performance liquid chromatography (HPLC) has been employed to analyze several components in a medicinal preparation composed of several crude drugs. Among the analytical methods for standardization of Indian herbal medicines HPLC is the most popular one, due to its versatility, precision and relatively low cost. This represents a progress in comparison of the one or two marker quantitative approach. One of the main advantages of HPLC is that many detectors can be coupled with it, such as UV, DAD, ELSD, FLD, RID, MS, and NMR, etc., which supplies much more possibilities for detecting different constituent types. In recent years, coulometric electrode array detection (HPLC- CEAD) and charged aerosol detection (CAD) [13, 14] have been also introduced to the analysis of herbal formulations. Most frequently, the method is used on reversed phase (RP) C18 columns, a binary solvent system containing acidified water, a polar organic solvent (acetonitrile or methanol), and UV–vis diode array detection (DAD). HPLC method with various detectors has been developed for qualitative and quantitative analysis of various phytoconstituents such as chiconic acid in Posidoniaoceanica (HPLC-UV detector) [15], simultaneous determination of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin in kampo medicines (HPLC-photodiode array detector) [16], phenolic compounds in Achilleamillefolium L. (reverse phase HPLC- dual λ absorbance detector such as UV/Vis and Photodiode arrey) [17], Chlorogenic acid in Tobacco residuals (HPLC system with UV

detector and C18 reversed phase column)[18] and phenolics in chyavanpras (RP column with HPLC UV–vis diode array detection) [19].

Preparative and analytical HPLC are widely used in pharmaceutical industry for isolating and purification of herbal compounds. There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high pressure HPLC (pressure >20 bar) (Chimezie et al., 2008). The important parameters to be considered are resolution, sensitivity and fast analysis time in analytical HPLC whereas both the degree of solute purity as well as the amount of compound that can be produced per unit time i.e. throughput or recovery in preparative HPLC.

This is very important in pharmaceutical industry of today because new products (Natural, Synthetic) have to be introduced to the market as quickly as possible. Having available such a powerful purification technique makes it possible to spend less time on the synthesis conditions.

The herbal medicines which are evaluated by hplc are as follows;

Sr No	Example of herbal medicine	HPLC Conditions	Chemical Constituents to be Separated
1	Extraction of goldenseal root powder	Column: Zorbax Eclipse XDB-C18 (3.5 µm, 4.6 × 150 mm) Guard column: Zorbax Eclipse XDB-C18 (5 µm, 4.6 × 12.5 mm) Solvent: 68% 30 mM ammonium acetate, 14 mM TEA, pH ~ 4.85 32% acetonitrile Wavelength: 230 nm Flow rate: 1 mL/min Column temperature: 30 ˚C Injection volumn: 10 µL Run time: 17 min[23].	An accurate method for the determination of key alkaloids in goldenseal, including berberine and hydrastine. HPLC analysis can be applied toseveral other alkaloids, including canadine, hydrastinine, and palmatine, and may be applicable to other berberine-con- taining plant roots.
2	Ginkgo	Method for Ginkgo analysis • Mobile phase: A = water, B = tetrahydro-furan/methanol with 3a volume ratio of 10/25 • Flow rate: 0.7 mL/min • Gradient: 0 min, 12 %B; 10 min, 16 %B; 15 min, 22 %B; 20 min, 30 %B • ELSD: temperature = 40 °C, pressure = 50 psi, gain = 7, filter = 3 seconds[25].	Ginkgodinoides
3	Astragali	Method for Astragali analysis • Mobile phase: A = water, B = acetonitrile • Flow rate: 0.7 mL/min • Gradient: 0 min, 20 %B; 1 min, 30 %B; 5 min, 35 %B, 30 min, 100 %B.	For analysis of astragaloside in Astragali
4	Kankasava	The separation was achieved with a column RP C-18 (250mm×4.6mm×5 micron) using mobile phase mixture of methanol &10 moldihydrogen phosphate buffer in a ratio of 50:50 v/v at a flow rate	For the quantitative estimation of atropine in kankasavapolyherbal branded formulations [27, 28].

		of 1 ml/min, and analysis was screened with UV detector at 254 nm.	
5	licorice	Instrument: Agilent 1200 Series Rapid Resolution System, Detector: Multiple wavelength detector (MWD), 254 nm/100 BW, 450 nm reference, Mobile phase: A = 1% Acetic acid in water B = 1% Acetic acid in acetonitrile.	20 % Glycyrrhizinic acid, 5 % Lutein
6	Da-Chai-Hu-Tang (Chinies Medicine)	The chromatographic separation was performed on an Agilent ZORBAX C18 column (250 mm × 4.6 mm i.d., 5.0 μm), and the mobile phase composed of methanol and water containing 1% (v/v) acetic acid was used to elute the targets in a gradient elution mode. The flow rate and detection wavelength were set at 0.8 ml/min and 280 nm, respectively.	For the simultaneous separation and determination of naringin, hesperidin, neohesperidin, baicalin, wogonoside, baicalein, wogonin, emodin and chrysophanol in Da-Chai-Hu-Tang.
7	Ostericumkoreanum	An analytical method has been developed by HPLC-UV detection at a wavelength 254 nm. HPLC chromatographic separation was successfully achieved with a Develosil RPAQUEOUS C 30 (4 . 6 × 2 5 0 mm , 5 μ m) column and a mobile phase of acetonitrile-water (60:40, v/v).	For the simultaneous determination of four biological marker compounds including bisabolangelone, oxypeucedanin, imperatorin, and isoimperatorin extracted from Ostericumkoreanum.
8	Vernoniacinerea Linn.	Chromatographic separation of the two compounds was performed on a waters symmetry shield C18 column (150 x 4.6, 5 μm) as stationary phase with a mobile phase comprising of methanol : acetonitrile (30:70) v/v at a flow rate of 1.0 mL min -1 and UV detection at 210nm with a run time of 12.0 min.	For simultaneous determination of β-sitosterol and Lupeol in whole plant powder of Vernoniacinerea Linn.
9	Nymphaeastellata, Willd.	Chromatographic separation was analyzed with a HiQSil C-18 column by isocratic elution using 0.01 % (v/v) orthophosphoricacid:acetonitrile (95:5 v/v) as the mobile phase. The flow rate was 1.2 mL min-1 and detection was set at 265 nm.	For the quantitative estimation of flavanoids, gallic acid, astragallin, quercetin and kaempferol.
10	Madhujeevanchurna (MJC)	HPLC assay method was developed and validated for Quercetin using an isocratic RP-HPLC method which employed an SS Wakosil II- C 18 column (250 mm × 4.6 mm i.d., 5 μm) with a mobile phase consisting of phosphate buffer (pH 6.8) and methanol (5:2 v/v), and UV detection at 254 nm at a flow rate of 1 ml/min.	For the quantitative determination of Quercetin in Madhujeevanchurna (MJC)

HPTLC:

The analysis and quality control of herbal medicines are moving a step ahead towards an integrative and comprehensive direction, in order to tackle the complex nature of herbal medicines. High-performance thin layer chromatography (HPTLC) is one of the sophisticated instrumental techniques for qualitative and quantitative analysis of the herbs and herbal drugs. This article emphasize on HPTLC based analytical method development and evaluation of validation characteristics.

High Performance Thin layer Chromatography (HPTLC) technique is a sophisticated and automated form of the thin-layer chromatography (TLC) with better and advanced separation efficiency and detection limits, and is often an excellent alternative to GC and HPLC. Applications of HPTLC include phytochemical and biomedical analysis, herbal drug quantification, active ingredient quantification, fingerprinting of formulations, and check for adulterants in the formulations. HPTLC is useful in detecting chemicals of forensic concern. Various advance techniques in reference to HPTLC like hyphenations in HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser have made HPTLC a power analytical tool in the field of analysis. Experts are of the opinion that HPTLC future to combinatorial approach and the utilization of instrumental HPTLC toward the analysis of drug formulations, bulk drugs, and natural products will increase in the future.

Advantages of HPTLC:

1) Ability to analyze crude samples containing multi-components.
2) The separation process is easy to follow especially with colored compounds.
3) Several samples can be separated parallel to each other on the same plate resulting in a high output, time saving, and a rapid low-cost analysis.
4) Choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step.
5) Two-dimensional separations are easy to perform. Stability during chromatography should be tested using two-dimensional development.
6) Better accuracy and sensitivity.
7) Specific and sensitive colour reagents can be used to detect separated spots (Dragendroff reagent/Kedde reagent).Contact detection allows radiolabelled compounds to be monitored and microbial activity in spots to be assessed.

Disadvantages of HPTLC :

1) bulky instrumentation, large space requirement, many folds expensive.

2) Requires stringent condition of operation like dust free environment and temperature controlled conditions.
3) Technically skilled person with the knowledge to run the system.

Steps Involved In HPTLC:

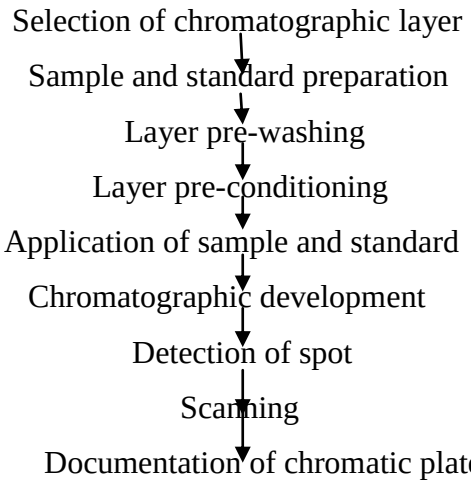

Figure 1: Steps involved in HPTLC

Table 2: Generally used Mobile phase in detection of some chemical compounds

SN	Chemical Compound	Mobile Phase
1	Polar Compounds Anthraglycosides, Arbutin,Alkaloids, Cardiac Glycosides, Bitter Principles, Flavonoids, Saponin	Ethyl Acetate: Methanol: Water [100:13.5:10]
2	Lipophilic Compounds,Essential oils, Terpenes, Coumarin, Napthoquinons, Velpotriate	Toluene: Ethyl Acetate [93:7]
3	Alkaloids	Toluene: Ethyl Acetate: Diethyl Amine [70:20:10]
4	Flavonoids	Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water [100:11:11:26]
5	Saponin	Chloroform: Glacial Acetic Acid: Methanol: Water [64:32:12:8]
6	Coumarin	Diethyl Ether: Toluene [1:1] Saturated with 10% Acetic Acid
7	Bitter Drug	Ethyl Acetate: Methanol: Water [77:15:8]
8	Cardiac Glycosides	Ethyl Acetate: Methanol: Water [100:13.5:10] OR [81:11:8]
9	Lignans	Chloroform: Methanol: Water [70:30:4] Chloroform: Methanol [90:10] Toluene: Ethyl Acetate [70:30]
10	Triterpenes	Ethyl Acetate: Toluene: Formic Acid [50:50:15] Toluene: Chloroform: Ethanol [40:40:10]

Table 3: HPTLC determination of pharmaceutical products in various formulations

Drug	Dosage form	Technique used
Bisdemethoxycurcumin, demethoxycurcumin and curcumin	Mouth ulcer poly herbal formulation.	The separation was performed on TLC aluminium plates Precoated with silica gel G60 F254. Good separation was achieved in the mobile phase of Chloroform: methanol: Glacial acetic acid (7.5: 2.0: 0.5 v/v/v) at Rf = 0.18, 0.31, 0.56 for bisdemethoxycurcumin, demethoxycurcumin and curcumin respectively.
Moxonidine	Tablet	Stationary phase:Silica gel, 60F 254 Mobile phase: methanol:toluene:triethylamine(4:6:0.1(v/v/v). Densitometric quantification at 266 nm.
CefiximeTrihydrate and AmbroxolHydrochlorid	Pharmaceutical dosage form	Stationary phase: silica gel 60 F254. Mobile phase: acetonitrile:methanol:triethylamine (8.2:1:0.8, v/v/v). Densitometric measurements of spots at 254 nm
Ofloxacin and Ornidazole	Solid dosage form	Stationary phase: silica gel60F254. Mobile phase: dichloromethane: methanol: 25% ammonia solution (9.5:1:3 drops v/v) Detection was carried out at 318 nm. The mean R f value of ofloxacin and ornidazole was found to be 0.16 and 0.56, 0.78
Olanzapine	Formulations	Stationary phase: silica gel 60F254. Mobile phase: methanol:ethyl acetate (8:2, v/v). Olanzapine was quantified by densitometric analysis at 285 nm.
Lamivudine and Zidovudine	Fixed-dose combination tablets	Stationary phase: pre-coated plate of silica gel GF aluminum TLC plate. Mobile phase: toluene:ethylacetate:methanol (4:4:2, v/v/v). Densitometric absorbance mode at 276 nm. The Rf values were 0.41±0.03 and 0.60±0.04 for Lamivudine and Zidovudine
Voriconazole	Bulk and in cream formulation	Stationary phase: silica gel 60RP-18F-254S. Mobile phase: Acetonitrile: Water (6:4 v/v). Densitometric analysis at 257 nm.
Paracetamol and Lornoxicam	Bulk drug and pharmaceutical formulation	Stationary phase: silica gel 60 F 254. Mobile phase: acetone:methanol:toluene:formic acid (2:2:6:0.1 v/v/v/v). Densitometric evaluation at 252 nm The Rf values and drug content of paracetamol and lornoxicam were 0.49±0.02, 0.59±0.02 and 99.84%, 100.10%
Valsartan and Hydrochlorothiazide	Tablet Dosage Form.	Stationary phase: precoated silica gel 60F(254). Mobile phase: chloroform: methanol: toluene: glacial acetic acid (6:2:1:0.1 v/v/v/v). Densitometric scanning at 260 nm.
Etoricoxib and Thiocolchicoside	Combined tablet dosage form	Stationary phase: silica gel 60F254. Mobile phase: ethyl acetate:methanol (8:2 v/v). Densitometric scanning at 290 nm.
Ambroxol hydrochloride	Bulk drug and pharmaceutical dosage form.	Stationary phase: aluminium plates precoated with silica gel 60F-254. Mobile phase: methanol:triethylamine (4:6 v/v). Densitometricanalysis at 254 nm. Rf value of 0.53 +/- 0.02.
Almotriptan Malate	Tablet Dosage form.	Stationary phase: aluminum plates precoated with silica gel 60 GF(254). Mobile phase: butanol:aceticacid:water (3:1:1.v/v/v). Densitometric scanning at 300 nm.
OlmesartanMedoxomil	Tablet formulation	Stationary phase: aluminium plates precoated with silica gel 60F254. Mobile phase: toluene:acetonitrile: methanol: ethyl acetate: acetic acid (5:3.5:0.3:1:0.3 v/v/v/v). Densitometric detection at 264 nm.

Aceclofenac	Tablet formulation	Stationary phase: Aluminium backed silica gel 60 F254 plate . Mobile phase: toluene: ethyl acetate: glacial acetic acid, (6:4:0.02v/v. Densitometry analysis at 282 nm.

Differences between HPLC and HPTLC

Parameters	HPLC	HPTLC
Type	Reverse Phase Chromagraphy	Straight Phase Chromagraphy
Stationary phase	Liquid	Solid
Conditioning phase	None	Gas
Separation by	Partition	Adsorption
Analysis	On-line	Off-line
Resolution	Very High	Moderate to high
Strongly Retarded Fractions Seen As	Broad Peaks	Sharp Peaks
Sensitivity	High to Ultra	High Moderate to ultrahigh
Detectors	UV, Fluor, electrochem Light scatter , MS	UV - Vis, bioluminescence , MS
Fraction collection / micro preparative chromatography	Requires prep. scale chromatograph & fraction collector	Simple. No special requirements
Chromatographic fingerprint	Yes but limited	Yes. Comprehensive
Post chromatography derivatisation	Limited possibilities. Cumbersome.	Simple. Possible for every sample. Gives additional information

CONCLUSION

It can be conclude that proven applications of HPLC and HPTLC are widely used in the pharmaceutical testing include: manufacturing (the manufacturing units of bulk drugs, process monitoring, fermentation broth analysis, residue analysis and in process materials testing); quality control (for raw material assays, multi-component formulations, uniformity of content testing, impurity profiling and methods validations); the analyses of formulations, stability, sustained release and bio-availability studies. HPLC and HPTLC is an ideal tool for identification of herbal materials. It is used for semi-quantitative comparison to provide quantitative results. HPTLC use for screening pharmaceutical compounds for the antimicrobial activities is emerging. The uses in validation of new incoming products and its introduction into the regulatory systems are of much importance towards the future of HPLC and HPTLC. In marine invertebrates, HPTLC has been utilized to separate new promising pharmaceutical therapeutants which could be used in pharmaceutical industries.

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