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HPTLC: A prominent tool for standardization of herbals

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ABSTRACT

Standardization of herbal drugs is not just an analytical operation for identification and assay of active principals rather it comprised total information controls to necessarily guarantee consistent composition of all herbals. The formulation standardized using modern scientific tools and with known markers has been accepted world wide. Chromatographic fingerprint analysis is a rational and practical analytical strategy to asses the authenticity quality consistency and stability of (tim)s as well as the information of other medicines. The information gathered from the fingerprint is more comprehensive then that provided from the typical approach. HPTLC is the principal method of monitoring the production process, HPTLC is mainly required for the complex or multi-herbal formulation. multiple chromatographic fingerprinting which consist of more than one chromatographic fingerprinting and represent the whole characteristics of the complex medicine.

KEYWORDS

HPTLC;
TIM (S);
Traditional indian medicines;
Standardization;
Fingerprinting;
Marker.

INTRODUCTION

There are certain instruments which shown there usefulness in the past. But now one have to develop newer methods and parameters to defend or reinstate our own tim (s) by HPTLC .This is the high performance thin layer chromatography.

Chromatographic fingerprint analysis is a rational and practical analytical strategy to assess. The authenticity quality and stability of times as well as other herbal medicines considered. The information gathered from the fingerprint is more comprehensive than that provided from the typical approaches. Chromatographic fingerprints sometimes exhibit variation in peak height and retention time of a given sample running through identical columns under the same separation condition. Because of this appropriate consideration must be given to proper normalization of chromatography.

For the complex or multi-herbal formulation, multiple chromatographic fingerprinting which consist of more than one chromatographic and represent the whole characteristics of chemical constitutions of the complex medicine is proposed as a potential strategy in this. Chemical characteristics of crude drug should be present in chromatogram of the analyte, when the analyte is the final product of herbal medicines. This procedure is called as authentification of multiple chromatographic fingerprints. After multiple chromatographic fingerprints of analyte were obtained, than compare the chromatographic fingerprint of analyte with the reference/standards fingerprints.

Chromatographic fingerprint is a chromatogram that represents the chemical characteristics of herbal medicines[1]

Chromatographic fingerprint has potential to determine the identity, authenticity and lot-to-lot consis-

tency of herbal formulation.

- It is inadequate to represent all chemical patterns of characteristics when the compositions of herbal medicines are too complex.
- Multi-herb botanical drug product

Advantages of HPTLC[2]

For analysis of constituents of herbals that belong to very different classes of chemical compounds can often create difficulties in detection. There is almost no limitation to the composition the mobile phase to maximize selectivity of the separation an enormous choice of stationery and mobile phase combination is available. The chromatographic condition can be changed with a few minutes and the chromatographic chamber requires very little time for equilibration.

Choice of detection-

(a) Multiple subsequent detection of the some chromatogram is possible beside densitometric evaluation of absorption of florescence using visible or ultraviolet light, several hyphenated detection techniques like as fourier transform, infrared, raman or mass spectroscopy are available.

(b) TLC plate stores the chromatography result.

(c) Partition of the chromatogram can be selectively evaluated allowing optimization of detector sensibility for specific compounds.

(d) Post chromatographic derivatization is employed substrate classes in particular can be cost and time efficiency multiple samples(upto 72) can be analyzed one plate .The result in low analysis costs per sample 10×10cm or 20×10cm plate in a twin-trough chamber. Densiometry evaluation of a plate can be accomplished with in 10min. Different samples must be analyzed using different methods flexibility of the system.

Requirements of HPTLC standardization-

The production of most herbal preparation includes some extraction process. It is essential for quality assurance that this extraction is standardization. The quality of marker compounds of their relative abundance assayed by HPTLC or HPLC is the principle method of monitoring the production process. When choosing marker compounds for a particular herb or herbal preparation. It is of critical importance that chemically well-characterized standards are available for their quantification. It is often impossible to separate all component of a plant extract completely.

HPTLC in pharmaceutical application

(1) Simple to learn to perform.

(2) Always available for use, since the precoated plates are usually employed without any further preparation.

(3) Easily inspected the whole chromatogram is visible for inspection as if a video screen.

(4) There is no imperative necessity to elute the individual components.

(5) This is also true for substances, which remain at the origin because TLC and HPTLC are displayed.

(6) The method of detection does not place any restriction on the choice of mobile phase. It is possible to choose chromatographic condition yielding the best separation of the sample under investigation.

(7) There is normally no mobile phase present in the chromatogram at the time of detection.

(8) Acidic, basic or purely aqueous elutents can be employed.

(9) For the detection and determination of antibiotics, alkaloids, vitamins, bitter substances, saponins and pesticides.

DISCUSSION

TLC densitometry determination of plants and formulation is well accepted by ICH guideline and the developed finger print can be utilized further in assessing quality of the formulation[3]. Validation of protocol for the HPTLC of herbal products are necessary (birjnganineet al) stability indicating parameters can be achieve with discuss which is having efficacy to separate the drug from its degradation products[18] . Developing solvent system with an organic acids ensured in-situ color development of the complex for quantitation of drug[17]. choice of suitable marker or the compound present inside the plant can be used for the identification of the drug[46,47] . Quantitative determination can be achieve by using defferent absorbance measurement at various wavelength for photochemical ingredient and marketed formulation[27,51,52]. Quantitative estimation of

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plant species can be determine on the basis of bands at different RF value[37]. Developing method for determination of constituent from any plant can be achieved using detection agent[42,49,55,56]. The concentration, distribution, proportion of saponin differ between the species and each presents a unique fingerprint(peishan xie). 5 rapid method based on HPTLC and RPHPTLC with uv detection for quantitative determination of two major bioactive compounds[13]. Linearti and precision of method mostly playes important role for detection marker compound from the plant of herbal formulation as rutin in gink biloba in solid dosage form[28]. Developed HPTLC method should be rapid, simple and accurate for estimation of phytochemicalsas charentin in different marketed formulation[9]. HPTLC method should be validated for linearity, accuracy, interdayan dintraday precision, specificity, repetability of measurement peak area and repeatability of sample application[34]. Solid samples should applied after extraction with suitable solvent and filtration and liquid sample of herbal formulation can be apply after the effervancence ceases in the case of energy drinks[31]. Repeated quantification of the same species of plant can be different due to difffrent geographical location as in case of sennoside A and B[55]. Improved quality assessment method should be useful for establishing chromatographic fingerprint and quantification of marker compounds in the crude drugs. Method can be used for the authentification of non-phenolics and non polar compounds[16]. Investigation of two chromatographic method should be for describing the evaluation of the quality of any plant extract as in bacopamoniri and its commercial formulation methods are RPHPTLC and PC-SI DAD[6,14]. Method can be used for determination of varity of active and inactive ingredients like in L-argenin hydrochloride in nutrition supplements[8].

HPTLC studies are helpful to develop characteristic gross HPTLC fingerprint profile, which may be used as marker for quality evaluation and standardization of the drug[53]. Method are reproducible for the detection of teatree oil[30]. Method development is for investigation purposes to know the adulteration in edible oils as in case of argemopne oil adulteration in musterd oil[15].

Therefore HPTLC fingerprinting is proved to be alinear, precise, accurate method for herbal formulation and can be used further in quality control of not established herbals.

TABLE : 1 Recent research carried out

S.No	Plant and extract	Solvent system	Recent work done	Absorbance mode	Reference
1	A herbal medicinal product containing aesculus and vitis dry extractss.	Acetic acid/water/butanol (10/40/50 v/v/v)	Assay method is specific for aescin in the presence of Vitis dry extract and formulation excipients	535nm.	3
2	Dried extracts of fraxinus excelsior leaves, Ribes nigrum leaves and filipendula ulmaria aerial part	2-propanol–ethyl acetate–ammonia solution (8%) (10:10:10, v/v/v).	Quantitative determination of glucosamine in a herbal dietary supplement.	415nm	4
3	Leaves of melaleuca alternifolia, tea tree oil from cosmeceutical formulations	Toluene and ethyl acetate (85:15)	Determination of tea tree oil from cosmeceutical formulations	366nm	5
4	Gum-resin exudates of (Commiphora mukul) both as a bulk drug and in formulations	Toluene–acetone (9:1, v/v).	Quantitative determination of E- and Z-guggulsterone in herbal extract and pharma ceutical dosage form	250nm.	6
5	Plantago palmata hook. f. (Plantaginaceae) leaves 8-epi-loganic acid (0.13%), arborescoside (0.005%) and acteoside	Ethyl acetate:water:acetic acid:formic acid (67:18:7.5:7.5)	Determination of acteoside in leaves of Plantago palmata Hook. f.s.	334nm	7
6	L-arginine hydrochloride nutrition supplements	1-butanol–acetic acid water, 3:1:1	Quantification of arginine in dietary supplement tablets and capsules	495nm	8

TABLE 1 is countinue on next page

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S.No	Plant and extract	Solvent system	Recent work done	Absorbance mode	Reference
7	Momordica charantia Linn. Charantin	Benzene: methanol (80:20)	Estimation of charantin in herbal formulations	536nm	9
8	Trigonella foenum-graecum from the family Fabaceae,	n-propanol-methanol-water (4:1:4, v/v/v)	Determination of trigonelline in herbal extract and pharmaceutical dosage form	269 nm	10
9	ginseng (Panax ginseng, Panax quinquefolium, Panax noto-ginseng) and	Water-acetonitrile-isopropanol-citric acid (1000:200:30:4.92g); (B) water-acetonitrile-isopropanol-citric acid (1000:470:50:6.08g)	Quality assessment of traditional Chinese herbal medicine	366 nm.	11
10	Artemisia absinthium L.	Acetone/acetic acid (98%)/toluene/dichloromethane (10:10:30:50, v/v).	HPTLC analysis of the bitter principle absenthin	400-700nm	12
11	Compounds in andrographis paniculata,	Chloroform: methanol (8:2)	Determination of andrographolides and antioxidant potential of Andrographis aniculata	532nm.	13
12	Bacopa monnieri extract and its commercial formulations.	Chloroform-methanol-ethyl acetate, 7.5:2.5:2.0 (v/v)	Separation of bacoside a3 and bacopaside ii, major triterpenoid saponins in bacopa monnieri,	344nm	14
13	Sanguinarine (sanguinarine + dihydrosanguinarine) in argemone oil	Hexane:acetone:methanol, 80:15:5, v/v)	Quantitative evaluation of sanguinarine as an index of argemone oil adulteration in edible mustard oil	210nm	15
14	Radix Salviae Miltiorrhizae (Root of Salvia miltiorrhiza BGE.)	Chloroform-ethyl acetate-toluene-formic acid-methanol (15:20:10:10:1) petroleum ether-ethyl acetate-cyclohexane(5:3:2)	Quality Assessment of Radix Salviae Miltiorrhizae	366nm	16
15	Various species of Solanaceae) like S. khasianum, xanthocarpum, S. nigrum, S. gracile,	Chloroform-methanol (7.5:2.5 v/v). Glacial acetic acid0.1%v/v	Quantification Of Solasodine in Various Solanum Species, Market Samples and Formulations	254nm	17
16	Rhizome of curcuma longa Linn. (Family:Zinziberaceae)	Chloroform:methanol (9.25:0.75 v/v).	Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations	461nm	18
17	Triphala' terminalia chebula, terminalia belarica and emblica officinalis,	Toluene: ethyl acetate: glacial acetic acid: formic acid (20: 45: 20: 05) solvent system	Clinical Study of 'Triphala' -	430nm	19
18	Gymnema sylvestre	Chloroform : methanol (9:1)	Determination of gymnemagenin (1) in gymnema sylvestre	293nm	20
19	Polygonum cuspidatum root extracts	Chloroform-ethylacetate-formic acid (2.5 : 1 : 0.1	Estimation of trans-resveratrol in herbal extracts and dosages by HPTLC	313nm.	21
20	Valeriana jatamansi and Valeriana officinali	Hexane - ethyl acetate - acetic acid 160:40:1.	HPTLC of valerenic acid in Valeriana jatamansi and Valeriana officinali	700 nm.	22

TABLE 1 is countinue on next page

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S.No	Plant and extract	Solvent system	Recent work done	Absorbance mode	Reference
21	Piper longum	Hexane-ethyl acetate 3:1.	Standardization of Piper longum-an ayurvedic medicinal plant.	260nm	23
22	Panax quinquefolium L.	Methanol - chloroform	Analysis of ginsenosides from panax quinquefolium	540nm.	24
23	Phyllanthus amarus	Toluene - ethyl acetate-formic acid 6:2:1	Simultaneous quantitation of phyllanthin, hypophyllanthin, gallic acid, and ellagic acid in phyllanthus amarus.	280nm.	25
24	Swertiaspecies	Ethyl acetate: methanol: water 77:8:8	Determination of swertiamarin and amarogentin in swertia species	235nm.	26
25	Fruits of Mimusops elengi Linn	Toluene - ethyl acetate - chloroform - acetic acid 35:35:28:2	Phytochemical investigation and antimicrobial activity of fruits of Mimusops elengi Linn. TLC/HPTLC fingerprint profile	254nm, 366nm	27
26	Ginkgo biloba	n-butanol - n-propanol-chloroform - acetic acid-water 4:1:2:1:1	Determination of rutin in Ginkgo Biloba from a solid dosage	254 nm.	28
27	Colchicum autumnale (meadow saffron) extracts	Chloroform - acetone - diethyl amine 5:4:1	Fast determination of colchicine	350nm.	29
28	Melaleuca alternifolia oil	Toluene - ethyl acetate 93:7	Characterization of tea-tree Melaleuca alternifolia oil	243nms	30
29	liquid samples (coca cola)	Ethyl acetate - methanol 17:3.	determination of caffeine in stimulant herbal products and power drinks.	275 nm.	31
30	Oldenlandia corymbosa L. wholeplant	Dichloromethane-toluene - acetone- methanol-30:40:15:3.	Quantitation of oleanolic acid in Oldenlandia corymbosa L. wholeplant powder	529 nm.	32
31	Humulus lupulus L.	Oldenlandia corymbosa L Toluene - dioxane - acetic cacid 77:20:3	Determination of xanthohumol in hops (Humulus lupulus L.) and hop products	368 nm	33
32	For Shankhpushpi	Toluene – diethyl ether 1:1	Establish a distinct chemical profile for Shankhpushpi and for quantification of scopoletin in Convolvulus pluricaulis Choisy and in commercial formulations of Shankhpushpi	366nm	34
33	Stephania tetrandra	Toluene-ethyl acetate-water-formic acid20:10:1:1	Quality control of Stephania tetrandra.	210nm	35
34	Rhododendron arboreum flowers	Methanol-water-formic acid (40:57:3, v/v/v)	Simultaneous quantitative determination of three biologically active phenolic compounds i.e. quercetin, rutin and coumaric acid in flowers of rhododendron arboreum	280nm	36
35	Roots of S.cordifolia and S.rhombifolia whole plant of S.cordifolia	Methanol-water-formic acid (40:57:3, v/v/v)	Quantitative estimation of ephedrine	366 nm	37
36	GarlicandGarlic preparation	Chloroform/methanol/water (6:4:1)	Separation of Garlic or identifying Garlic in complex herbal formulation or dietarysupplement	254nm	38
37	Sphaeranthus indicus linn	Tolune-ethyl acetate 7:3, ethyl acetate-methanol-water 200 :27 :20,n-butanol-glacial acetic acid3 :1	Protective effect of bioactive fraction of Sphaeranthus indicus linn	254 nm, 366nm	39

TABLE 1 is countinue on next page

S.No	Plant and extract	Solvent system	Recent work done	Absorbance mode	Reference
38	Grewia tiliaefolia vahl. bark	Tolune-ethyl acetate 9:1	Determination of betulin in Grewia tiliaefolia vahl. bark	540nm	40
39	Ocimum sanctum Linn.	Tolune-ethyl acetate-formic acid 35:15;1	Quantification of euginol, lutolin,urosolic acid, andoleanoic acid in blak (Krishna tulsi),green(sri tulsi)	280nm, 350nm, 530nm	41
40	Oscimum sanctum (Tulsi) leaves and its formulations	Toluene - ethyl acetate - acetic acid 30:3:1.	Determination of ursolic acid from Oscimum sanctum Linn (Tulsi) leaves and its formulations.	580nm.	42
41	Gymnema sylvestre leaves	Chloroform-methanol 4:1	Extraction andquantification of gymnemic acids through gymnemagnin from callus culture of Gymnema sylvestre	205nm.	43
42	CGX chinee s formulation	Ethylacetate- formic acid – aceticacid-water 15:1:1:2	HPTLC method development fo water extracts of CGX, Artemisia capillaris Herba, Aurantii Fructus, Glycyrrhizae Radix, Poria cocos and their standard components	366nm	44
43	Matricaria chamomilla L.	Tolune methanol , 5:1-	Determinatin of apigeninin some Iranian liquid products of Matricaria chamomilla L.	343nm	45
45	Nelumbo nucifera seed extract	Chloroform-methanol 7:1and Hexane -ethyl acetate,7:3	HPTLC study of Nelumbo nucifera seed extract	254nm	46
46	Rhizomes of gingiber officinalis	n-hexane-diethyl ether 2:3	Determination in 6- gingerol in ginger	325nm	47
47	Actacea racemosa extract	Tolune-ethyl acetate-formic acid 5:3:2	Rapid identification of Black gohosh	366nm	48
49	Phyllanthus amarus	Hexane ,acetone,-ethyl acetate 37:6:4	Quantitative Determinatin of phyllanthin and phypophyllanthin in phyllanthus species	580nm	49
50	amaryllis belladonna L. bulbs	Chloroform-methanol 9:1	Amarbellisine from amaryllis belladonna L.	254nm.	50
51	ethanolic extracts of rhizomes from curculigo orchioides	Toluene - ethyl acetate - acetic acid 25:15:1.	Standardization of curculigo orchioides rhizomes and its marketed formulations using gallic acid as standard	260nm.	51
52	Methanolic extract of A. paniculata, Methanolic extract of *P. amarus*, Petroleum extract of *B. aristata*	Chloroform:methanol (70:10) n-propanol:formic acid:water (90:1:9) solvent systemP. amarus with a hexane:ethyl acetate (2:1) solvent system	Development of t marker profile of Andrographis paniculata, Berberis aristata, and Phyllanthus amarus,	366nm., 223nm, 254nm.	52
53	Cassia angustifolia Vahl (Leguminosae) powdered seed samples,	Toluene:ethyl acetate:methanol (85:15:0.5).	De velopment of HPTLC method Cassia angustifolia Seeds	254nm	53
54	Lagerstroemia speciosa L. (Family Lythraceae)	Chloroform: methanol (9 : 1).	Quantitative Determination of Corosolic acid	210nm.	54
55	Ayurvedic formulation	Toluine- ethyl acetate-methanol 18:2:1	Quantification of curcumin, piprine,thymol	420nm 333nm 277nm	55

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CONCLUSION

A decade back the usefulness of HPTLC was not so often for research purposes but as soon as the economy of the instrument Comes ease, and its availability to research institute for establishing quality control parameters and its application in herbal drug technology proved its potential. even though its 100% potential is still to come according to WHO the problems stands with the tim are their authenticity and characterization of the active ingredients present in various tim,(s) the Ayurvedic literature revels only the pharmco theraptic effect of the drug but unfortunately not speak about its quality determination of it. The global market is asking for the quality as well as its safety .until and unless we will try to establish modern parameters for quality and safety of our own traditional drugs, it is worthless to stand in a war of global herbal market.. Among all the quality control system fingerprint has gained more andmore attention due to its ability to identify a particular herb and moreover a distinguish it from closely related species. The attempt for making this review is look in to this area of concern and make a universal approach

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