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Application of HPTLC in the Standardization of Herbal Medicinal Products

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ABSTRACT:
In order to control and tackle the complex nature of various herbal medicines and other herbal products, the analysis, quality control and standardization of those herbal medicines and products is moving a step ahead towards an integrated and comprehensive direction. High-performance thin layer chromatography (HPTLC) is a very advanced and revolutionary instrumental technique for the quantitative and qualitative analysis of herbs, herbal medicines, and other herbal products. This article focuses on the HPTLC based analytical method development and evaluation of validation characteristics of the herbal medicinal products.

KEYWORDS: High-performance thin layer chromatography (HPTLC), herbal medicines, herbal products, standardization, analytical method.

INTRODUCTION:

Nowadays, universal trend has been shifted from synthetic to herbal drug i.e., Return to Nature.[1]Ayurveda is a time- tested, trusted worldwide plant based system of drugs [2] which is developed through daily life experiences with the collective relationship between humanity and nature.[3] As per WHO, there are three kinds of herbal drugs raw plant material, reused plant material and medicinal herbal products.[4] Herbal drugs are complex chemical fusions attained from a plant which is extensively used in health-care in both developed and developing countries.[5] It's no wonder that the world's one- fourth population is using traditional drugs for the treatment of colourful affections.[6] still, one of the impediments in the acceptance of the Ayurvedic or Herbal drugs is the lack of standard quality control biographies.[7] Due to the complex nature and essential variability of the chemical ingredients of the plant based medicines, it's delicate to establish quality control parameter. [8] Quality assurance of herbal drug is an important factor and introductory demand for herbal medicine assiduity and other medicine development association.[9] There are several problems which impact the quality of herbal medicines.

- Variable sources of the raw material.
- The chemical constituents of herbs and herbal products may vary depending on stage of collection, parts of the plant collected, harvest seasons, plant origins (regional status), drying processes and other factors. [10]
- Extracts are usually mixtures of many constituents.
- The active principle(s) is (are), in most cases unknown.
- Selective analytical methods or reference compounds may not be available commercial. [11-13]

All pharmacopoeias set norms for the quality, chastity, strength, and thickness of these products – critical to the public health. USP – NF contains roughly 4500 studies for medicine substances, lozenge forms, excipients, and other rectifiers. moment, USP proposes the first 23 constituents to be included in the new Herbal Medicines Compendium (HMC).[14] The IP 2007, which was made effective from last July, has formerly over 1600 studies.[15] The British Pharmacopoeia 2012 contains roughly 3375 studies for substances, medications and papers used in the practice of drug.[16]

Standardization is an important step for the establishment of a harmonious natural exertion, a harmonious chemical profile, or simply a quality assurance program for product and USP manufacturing of an herbal medicine.[17] It is the process of developing and agreeing upon specialized norms. Specific norms are worked out by trial and compliances, which would lead to the process of defining a set of characteristics

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displayed by the particular herbal drug. Hence standardization is a tool in the quality control process.[18]

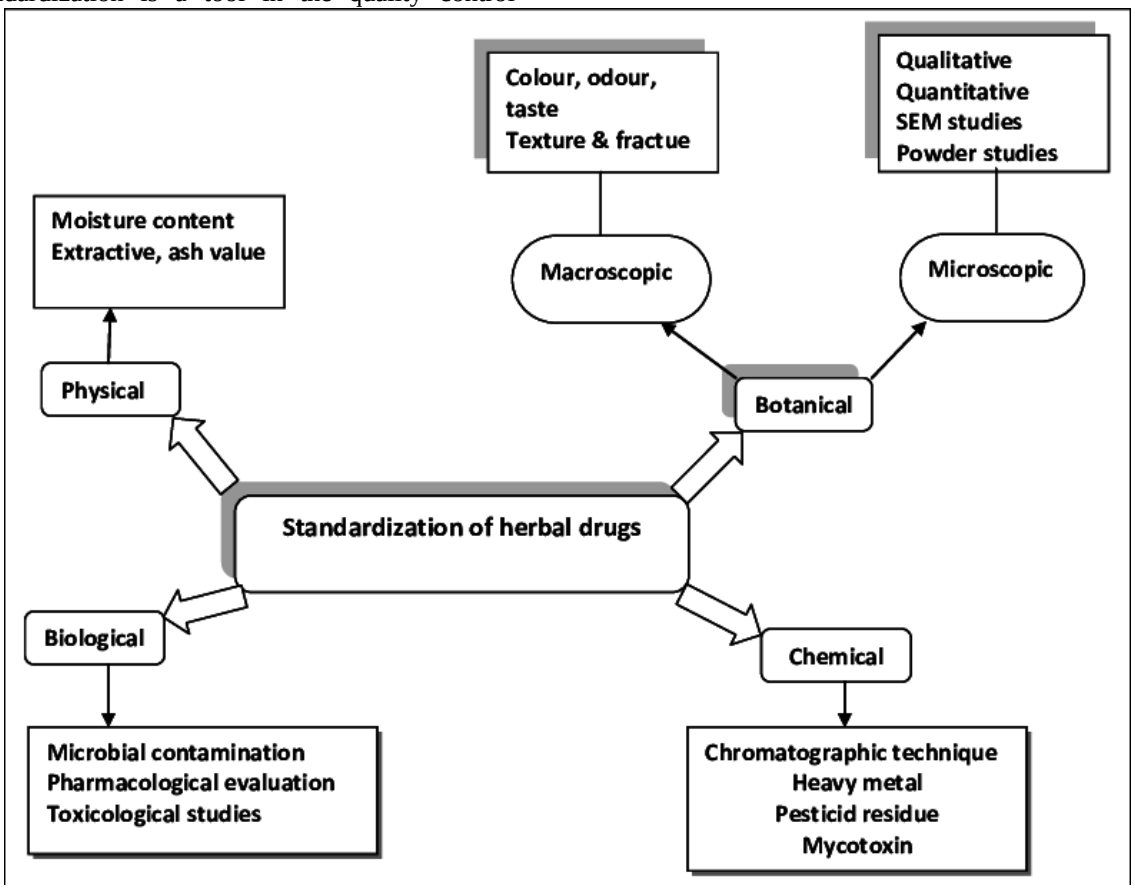

Figure 1: A schematic representation of standardization of herbal drugs.

HPTLC is a modern adaptation of TLC with better and advanced separation efficiency and detection limits. The table 1 compares HPTLC and TLC. [19-28]

Table 1: Difference between TLC and HPTLC

PARAMETER	TLC	HPTLC
Technique	Manual	Instrumental
Efficiency	Less	High (Due to smaller particle size)
Layer	Lab Made/ Pre-Coated	Pre-coated
Mean particle size	10-12 um	5-6 um
Layer Thickness	250 um	100 um
Plate Height	30 um	12 um
Solid Support	Silica Gel, Alumina, Kiesulguhr	Silica Gel- Normal Phase C8 and C18- Reverse phase

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Sample Spotting	Manual Spotting (Capillary/ Pipette)	Auto sampler (Syringe)
Sample Volume	1-5 ul	0.1-0.5 ul
Shape of Sample	Circular (2-4 nm Dia)	Rectangular (6 mm L × 1mm W)
Separation	10-15 cm	3-5 cm
Separation Time	20-200 Min	3-20 Min
Sample tracks per plate	≤ 10	≤ 36 (72)
Detection Limits (Absorption)	1-5 pg	100-500 pg
Detection limits (Fluorescence)	50-100 pg	5-10 pg
PC connectivity, Method Storage	No	Yes
Validation, Quantitative Analysis, Spectrum Analysis	No	Yes
Analysis Time	Slower	Shortage Migration Distance and the analysis time is greatly reduced
Wavelength Range	254 or 366 nm, Visible	190 or 800 nm, Monochromatic
Scanning	Not possible	Use of UV/ visible/ fluorescence scanner scans the entire chromatogram qualitatively and quantitatively and scanner is an advanced type of Densitometer

Some of the advantages of HPTLC are:

- Ability to analyse crude samples containing multi-components.
- The separation process is easy to follow especially with coloured compounds.
- 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.
- Choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step.
- Two-dimensional separations are easy to perform. Stability during chromatography should be tested using two-dimensional development.[28]
- Specific and sensitive colour reagents can be used to detect separated spots (Dragendroff reagent/Kedde reagent).[29]
- HPTLC can combine and consequently be used for different modes of evaluation, allowing identification of compounds having different light-absorption characteristics or different colours.
- Contact detection allows radiolabelled compounds to be monitored and microbial activity in spots to be assessed.
- HPTLC method may help to minimizes exposure risk of toxic organic effluents and

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significantly reduces its disposal problems, consequently, reducing environment pollution. [21-31]

CLASSIFICATION OF HPTLC:

HPTLC ways may be classified into four classes i.e., Classical, High performance, Ultra and Preparative thin-layer chromatography. They differ with classical TLC in the flyspeck size distribution and consistence of the sorbent layers. The mean patches sizes are 12, 5, 25 µm for classical, high-performance and preliminary thin- layer chromatography, independently, whereas Ultra-thin layer chromatography does not have patches but a monolithic layer with 1 – 2 um macropores.[32] Another difference is the consistence of the sorbent layers which is 250 um, 200 um, 10 um and 0.5 – 2 mm, for classical, high- performance, ultra-thin and preliminary sorbent layers, independently.[21]

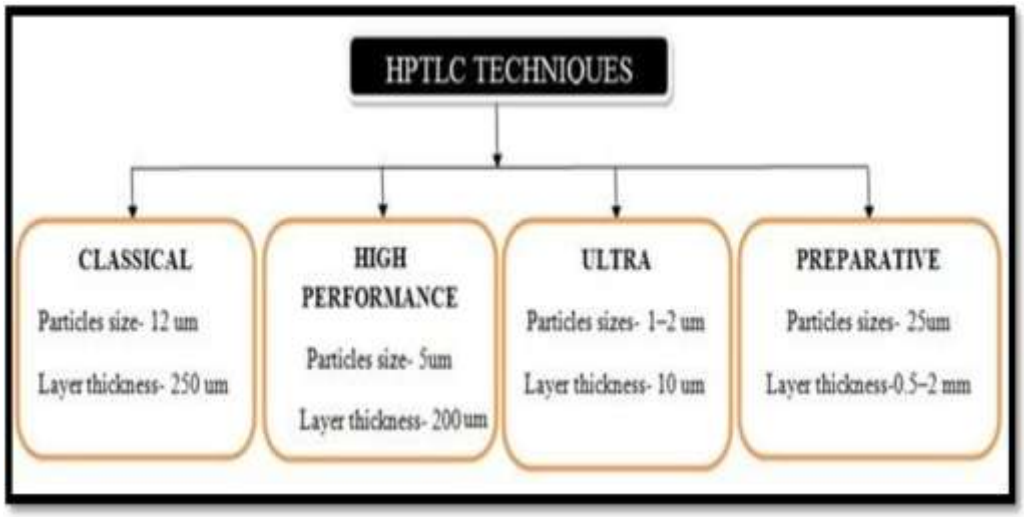

Figure 2: Classification of HPTLC techniques.

METHODOLOGY FOR HPTLC ANALYSIS:

Method development in thin- layer (planar) chromatography is one of the most significant ways for a qualitative and quantitative analysis. During establishing a new logical procedure, always starts with wide literature survey[33]i.e., primary information about the physicochemical characteristics of sample and nature of the sample (structure, opposition, volatility, stability, and solubility). It involves considerable trial and error procedures.[34] General ways involved in HPTLC system developments are as follows [23],[35]:

Basic Steps:

- Selection of the stationary phase
- Mobile phase selection and optimization
- Sample Preparation and Application
- Chromatogram Development (separation)
- Detection

QUANTITATION:

HPTLC method validation for pharmaceutical analysis:

- Specificity
- Linearity
- Range
- Accuracy
- Precision
- Detection Limit, Quantitation Limit
- Robustness

Basic Steps:

Selection of the stationary phase:

During system development, stationary phase selection should be based on the type of constituents to be separated.[36] HPTLC uses lower plates (10*10 or 10*20 cm) with significantly dropped development distance generally 6 cm) and analysis time (7–20 min). HPTLC plates give bettered resolution, advanced discovery perceptivity, and bettered in situ

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quantification and are used for artificial medicinal densitometric quantitative analysis.[31], [37]

Mobile phase selection and optimization:

The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical parcels of analyte.[28],[29]

The Table 2 gives the details of mobile phase generally used in discovery of some chemical constituents.

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

SN	Chemical Compounds	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	Essential Oil	Toluene: Ethyl Acetate [93:7]
10	Lignans	Chloroform: Methanol: Water [70:30:4] Chloroform: Methanol [90:10] Toluene: Ethyl Acetate [70:30]
11	Pigments	Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water [100:11:11:26]
12	Pungent Testing	Toluene: Ethyl Acetate [70:30]
13	Terpenes	Chloroform: Methanol: Water [65:25:4]
14	Triterpenes	Ethyl Acetate: Toluene: Formic Acid [50:50:15] Toluene: Chloroform: Ethanol [40:40:10]

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SAMPLE PREPARATION AND APPLICATION:

A good Solvent system is one that moves all factors of the admixture off the birth, but does not put anything on the solvent front. The peaks of interest should be resolved between Rf 0.15 and 0.85. The elution power of the mobile phase depends on a property called eluent strength which is related to the opposition of the mobile phase factors.[23] The further nonpolar the emulsion, the briskly it will elute (or the lower time it will remain on the stationary phase) and the more polar the emulsion the slower it'll elute (or further time on the stationary phase). The following map is helpful in prognosticating the order of elution. [21, 28]

Table 3: Common Mobile Phases listed by Increasing Polarity.

S. No	Solvent	Eluent Strength
1	N- Pentane	0.00
2	Hexane	0.01
3	Cyclohexane	0.04
4	Carbon tetrachloride	0.18
5	Toluene	0.29
6	Chloroform	0.40
7	Methylene Chloride	0.42
8	Tetrahydrofuran	0.45
9	Acetone	0.56
10	Ethyl Acetate	0.58
11	Aniline	0.62
12	Acetonitrile	0.65
13	Ethanol	0.88
14	Methanol	0.95
15	Acetic Acid	Large

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Pharmaceutical medication with sufficiently high attention of analyte is simply dissolved in a suitable solvent that will fully solubilize the analyte and leave excipients undissolved to yield a test result that can be directly applied on HPTLC plate.[34, 38] It's a fact that operation of the sample is the most critical step to gain good resolution for quantification in HPTLC21.Sample operation technique depends on factors similar as the types of sample matrix, workload and time constraints. [39,23]

Chromatogram Development (separation):

Although chromatogram development is the most pivotal step in the HTLC procedure, important parameters are generally overlooked. [28] HPTLC plates are developed in twin- trough chambers, or vertical- development chambers. In general, impregnated twin- trough chambers fitted with sludge paper offer the stylish reproducibility. twin-through chamber avoids solvent vapor preloading and moisture. [40- 42]

Detection:

Discovery of separated composites on the sorbent layers is enhanced by quenching of luminescence due to UV light ranged typically at 200- 400 nm. This process is generally called luminescence quenching.

Visualization at UV 254 nm:

F254 should be described as phosphorescence quenching. F254 fluorescent index is agitated with UV wavelength at 254 nm and emits green luminescence.[43] Composites that absorb radiation at 254 nm reduce this emigration on the subcaste, and a dark violet spot on a green background is observed where the emulsion zones are located. [23, 44]
Anthraglycosides, coumarins, flavonoids, propylphenols in essential canvases, some alkaloid type similar as indole, isoquinoline and quinoline alkaloids etc. should be detected under 254 nm. [29, 45]

Visualization at UV 366 nm:

F 366 should be described as Fluorescence quenching. This quenching is shown by all anthraglycosides, coumarins, flavonoids, Phenolcarboxylic acids, some alkaloid types (Rauwolfia, Ipecacuanha alkaloids).[29]

Visualization at white light:

Zone containing separated compounds can be detected by viewing their natural color in daylight (White light).[37]

Derivatisation:

Derivatization can be defined as a procedural technique that primarily modifies an analyte's functionality in order to enable chromatographic separations. For better reproducibility, immersion is the preferred derivatization technique.

Quantitation:

Generally quantitative evaluation is performed by measuring the zones of samples and norms using a densitometer or scanner with a fixed sample light ray in the form of a blockish tear. The chromatogram can be scrutinized in reflectance or in transmittance mode by absorbance or by fluorescent mode; surveying speed is used up to 100 mm/s. Due to surveying spectrum estimation of single and multiple situations of linearity, direct and nonlinear retrogression equations are possible. Scanning has been done by two styles i.e., Slit Scanning and Video Scanning.[21]

HPTLC method validation for pharmaceutical analysis:

Specificity:

Particularity is the capability to assess unequivocally the analyte in the presence of factors which may be anticipated to be present. generally, these might include contaminations, degradants, matrix, etc. It includes identification, chastity tests and Assay.[46]

Linearity:

The linearity of an logical procedure is its capability within a given range) to gain test results which are directly commensurable to the attention(quantum) of analyte in the sample.[46]

Range:

The range of an logical procedure is the interval between the upper and lower attention (quantities) of analyte in the sample (including these attention) for which it has been demonstrated that the logical procedure has a suitable position of perfection, delicacy and linearity.[46] In the Pharmaceutical Industry generally a range from 80 to 120 of the target attention was tested.[17]

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Accuracy:

The delicacy of a logical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value set up.[46] For bio-analytical system, delicacy should be performed for at least three quality control (QC) samples (low, medium, and high) in triplet, and the delicacy was expressed as recovery.[47]

Precision:

Precision of the logical system can be divided into three orders, i.e., repetition, intermediate perfection, and reproducibility. For bioanalytical system, it's recommended to test the perfection using a minimum of five determinations per attention. A minimum of three situations of attention in the anticipated range is recommended; RSD isn't permitted further than 15, and at the outside limit, lower attention RSD of 20 is respectable. [48, 49]

Detection Limit, Quantitation Limit:

The detection limit of an individual logical procedure is the smallest amount of analyte in a sample which can be detected but not inescapably quantitated as an exact value. The quantitation limit (QL) of an individual logical procedure is the smallest amount of analyte in a sample which can be quantitatively determined with suitable perfection and accuracy48. Generally, QL can be estimated as 2–3 times of DL. DL and QL for necessary chromatographic) logical styles can be defined in terms of the signal- to- noise rate (21 – 31 for DL and 101 for QL) or in terms of the rate of the standard divagation of the blank response, the residual standard divagation of the estimation line, or the standard divagation of intercept(s) and pitch (s) can be used. [50, 51]

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in system parameters and provides a suggestion of its trust ability during normal operation.[46] Some important parameters for testing of the robustness evaluation for HPTLC methods are mobile phase composition, pH of the mobile phase, temperature, development distance, spot shape, spot size, batch of the plates, volume of samples, drying condition (temperature, time) and condition of spot visualization (spraying reagents, dipping reagents, UV detection). [52]

REFERENCES:

[1]. Sharma A., Shanker C., Tyagi L.K., Singh M., Rao V. Herbal Medicine for Market Potential in India: An Overview. Academic J. Plant Sci. 2008; 1 (2): 26-36.

[2]. Kadam P.V., Yadav K.N., Shivatare R.S., Pande A.S., Patel A.N., Patil M.J. Standardization of Gomutra Haritaki Vati: An Ayurvedic Formulation. Int. J. Pharm. Bio. Sci. 2012; 3(3): 181 – 187.

[3]. Mukharjee P.K., Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J. Ethanopharmacol. 2006; 103 (1): 25-35.

[4]. Choudhary N., Sekhon B.S. An overview of advances in the standardization of herbal drugs. J. Pharm. Educ. Res. 2011; 2 (2): 55-70.

[5]. Amit J., Sunil C., Vimal K., Anupam P. Phytosomes: A revolution in herbal drugs. The Pharma Review 2007; 11- 13.

[6]. Jena A., Saha D., Biswal B., Jana S.B., Koley A., Sur D., Battacharya A. Pharmacognostic Studies of leaves of Pterospermum Suberifolium. Int. J. Res. Pharmaceutical Biomed. Sci. 2011; 2(1): 2229-3701.

[7]. Bagul M.S., Rajani M. Phytochemical evaluation of classical formulation: A case study. Indian Drugs 2005; 42:15-19.

[8]. Jeganathan N.S., Kannan K. HPTLC Method for Estimation of Ellagic Acid and Gallic Acid in Triphalachurnam Formulations. Res. J. Phytochemistry 2008; 2 (1): 1-9.

[9]. Saraswathy A., Rukmani S., Ariyanathan S. Analysis of HinguvacadiCurna. Indian J. Trad. Knowledge 2009: 8(3):319-325.

[10]. Ahirwal B., Ahirwal D., Ram A. Evaluation of standards and quality control parameters of herbal drugs, Souvenir, recent trends in herbal therapy 2006: 25-29.

[11]. Wani M.S.. Herbal medicine and its standardization. Pharma info. 2007; 5(6): 1-6.

[12]. Ekka N.R., Namdeo K.P., Samal P.K. Standardization stratergies for herbal Drugs- an Overview. Res. J. Pharm. And Tech. 2008; 1(4):310-312.

[13]. Bele A.A., khale A. Standardisation of herbal drugs: an overview. Int. j. pharmacy 2011; 2(12): 56-60.
[14]. US Pharmacopoeia National Formulary, USP 36/NF 31, United States Pharmacopoeial Convention. Inc., Rockville, MD, 2013.
[15]. Indian Pharmacopoeia, Volume-1, published by The Indian Pharmacopoeia Commission, Central Indian pharmacopoeia Laboratory Govt. Of India, Ministry of Health & Family Welfare Sector-23, Raj Nagar, Ghaziabad-201 00, 2007.
[16]. British Pharmacopeia (BP). Vol. II, Her Majesty"s Stationary Office, London. 2001.
[17]. Patra K.C., Pareta S.K., Harwansh R.K., Kumar J.K. Traditional approaches towards standardization of herbal medicines -A review. J. Pharm. Sci.Technol. 2010; 2 (11): 372- 379.
[18]. Folashade K.O., Omoregie E.H., Ochogu A.P. Standardization of herbal medicines - A review. Int. J. Biodiversity Conserv. 2012; 4(3): 101-112.
[19]. Attimarad M., Mueen Ahmed K.K., Aldhubaib B.E., Harsha S. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Symposium HPTLC 2011: 2 (2)7: 1-75.
[20]. Reich E., Schibli A. Stationary Phases for Planar Separations - Plates for Modern TLC. LC GC 2005; 23: 58-69.
[21]. Shewiyo D.H., Kaaleb E., Rishab P.G., Dejaegherc B., Verbekec J.S., Heydenc Y.V. HPTLC methods to assay active ingredients in pharmaceutical formulations: A review of the method development and validation steps. J. Pharm. Biomedical Anal. 2012; 66: 11–23. 22.
[22]. Shuijun L., Gangyi L., Jingying J., Xiaochuan L., Chen Y. Liquid chromatography – negative ion electrospray tandem mass spectrometry method for the quantification of ezetimibe in human. Plasma J. Pharm. Biomed. Anal. 2006: 40(4): 987-992.
[23]. Patel R., Patel M., Dubey N., Dubey N., Patel B. HPTLC Method Development and Validation: Strategy to Minimize Methodological Failures. J. Food Drug Anal. 2012; 20(4): 794-804.
[24]. Sherma J. Modern thin layer chromatography. J. AOAC Int. 2008; 91: 1142-1144.
[25]. Ravali R., Phaneendra M., BhanuJyothi K., RamyaSanthoshi L., Sushma K. Recent Trends in Analytical Techniques for the Development of Pharmaceutical Drugs. J. Bioanal. Biomed. 2011: 1-7.
[26]. Renger B. Planar chromatographic procedures in pharmaceutical analysis. J. AOAC Int. 2001; 84: 1217.
[27]. Patel R.B., Patel M.R. An introduction to analytical method development for pharmaceutical formulations. Pharma Rev. 2008; 6 (4): 1-5.
[28]. Srivastava M.M. High-Performance Thin-Layer Chromatography (HPTLC).Springer Verlag Berlin Heidelberg 2011: 32-60.
[29]. Wagner H. Plant Drug Analysis: A Thin Layer Chromatography Atlas 2nd ed. Springer 1996.
[30]. Kalasz H., Bathori M. Pharmaceutical applications of TLC. LC–GC Eur 2001; 10: 311–321.
[31]. Sethi P.D. Quantitative Analysis of Pharmaceutical Formulations, High Performance Thin Layer Chromatography. CBS Publishers and Distributors, New Delhi, India, 1996: 1-30.
[32]. Gocan S. Stationary phases for thin-layer chromatography. J. Chromatogr. Sci. 2002; 40: 538–549.
[33]. Nyiredy S. Planar chromatographic method development using the PRISMA optimization system and flow charts. J. Chromatogr. Sci. 2002; 40: 553–563.
[34]. Koll K., Reich E., Blatter A., Veit M. Validation of standardized high-performance thin-layer chromatographic methods for quality control and stability testing of herbals. J. AOAC Int. 2003; 86: 909-915.
[35]. Sherma J. Review of HPTLC in drug analysis: 1996-2009. J. AOAC Int. 2010; 93: 754-764.
[36]. Scott R.M. The stationary phase in thin layer chromatography. J. Chromatogr Sci. 1973; 2: 129–135.
[37]. Sherma J. Chromatographic Methods of Analysis: Thin Layer Chromatography. In Encyclopedia of Pharmaceutical

Technology. Informa Healthcare, Swarbrick, 3rd edition 2007: 538–550.
[38]. Sherma J. Planar chromatography, review: planar chromatography. Anal. Chem. 2008; 80: 4243–4267.
[39]. Jaenchen D.E, Reich E. Planar chromatography: instrumentation. In "Encyclopedia of separation science" Academic Press, New York, USA 2000: 839-847.
[40]. Renger B. Quantitative planar chromatography as a tool in pharmaceutical analysis. J. AOAC Int. 1993; 76: 7-13.
[41]. Renger B. Contemporary thin layer chromatographyin pharmaceutical quality control. J. AOAC Int. 1998; 81: 333-339.
[42]. Wall P.E. Thin-layer chromatography : a modern practical approach. chromatography : a modern practical approach, Cambridge : Royal Society of Chemistry, 2005. 100-150.
[43]. Sherma J. Basic Techniques, Materials and Apparatus in Handbook of thin-layer chromatography. Marcel Dekker, New York, USA; 1991: 3-41.
[44]. Brainthwaite S., Smith F.J. Chromatographic Methods, 5th ed, Kluwer Academic Publishers, London, 1999.
[45]. Andola H.C., Purohit V.K. High Performance Thin Layer Chromatography (HPTLC): A Modern Analytical tool for Biological Analysis. Nat. Sci.2010; 8(10): 58-61.
[46]. International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use: Validation of Analytical Procedures: Methodology, ICH-Q2B, Geneva 1996.
[47]. Shah V.P., Midha K.K., Findlay J.W.A., Hill A.M., Hulse J.D., Mc-Gilveray I.J., McKay G. Bioanalytical method validation – a revisit with a decade progress. Pharma Res. 2000; 17 (12):1551–1558.
[48]. Garofalo F. BioAnalytical Method Validation. In: Chan C.C., Lam H., Lee Y.C., Zhang, X-U. (Eds.). Analytical Method Validation and Instrument Performance Verification, published by Wiley-Interscience, Hoboken, NJ. 2004
[49]. International Organization for Standardization, Accuracy (Trueness and Precision) of Measurement Methods and Results, ISO:DIS 5725-1 to 5725-3, Geneva, 1994.
[50]. Yuwono M., Indrayanto G. Validation of Chromatographic Methods of Analysis. In: Brittain G. (ed.) Profiles of Drugs Substances, Excipients and Related Methodology vol. 32, published by Academic Press Elsevier Inc, San Diego 2005.
[51]. Lee Y.C. Method validation for HPLC analysis of related substances in Pharmaceutical Products. In: Chan C.C., Lam H., Lee Y.C., Zhang, X-U. (eds.) Analytical Method Validation and Instrument Performance Verification, published by Wiley-Interscience, Hoboken, NJ 2004.
[52]. Van der Hyden Y., Nijhuis A., Smayers-Verbeke J., Vandeginste B.M.G, Massart D.L. Guidance for robustness/ruggedness test in method validation. J Pharm. Biomed. Anal. 2001; 24: 723–753.