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HPTLC Method Development and Validation: An Overview

Sonia K*, Beddi Bhavya shree, Dr.K.S.Lakshmi
Department of Pharmaceutical Analysis,
SRM College of Pharmacy, Kattankulathur.

Abstract:
This review article gives knowledge of about HPTLC-based analytical method development and validation parameter in accordance to practical evaluation. It meets standards and minimizes errors and investigation. This review article helps to choose best mobile phase and gives guidelines for the good validation practice and understand the steps of analytical procedure.
Keywords: method development, validation, HPTLC

INTRODUCTION:

High Performance Thin Layer Chromatography (HPTLC) is the most powerful advanced form of Thin Layer Chromatography (TLC) and consists of chromatographic layers of utmost separation efficiency and the application of sophisticated instrumentation for all steps in the procedure include accurate sample application, standardized reproducible chromatogram development and software controlled evaluation[1]. HPTLC is a concept that includes a widely standardized methodology based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis [2]. HPTLC meets all quality requirements for today's analytical labs, to increase the resolution and to allow more accurate quantitative measurements [3].

HPTLC method:

Stationary Phase:

HPTLC is the most advanced form of modern TLC. It uses HPTLC plates featuring small particles with a narrow size distribution which results in homogenous layers with a smooth surface to be obtained. HPTLC uses smaller plates (10 × 10 or 10 × 20 cm). HPTLC plates provide improved resolution, higher detection sensitivity, and improved in situ quantification and are used for industrial pharmaceutical densitometric quantitative analysis. Normal phase adsorption TLC on silica gel with a less polar mobile phase, such as chloroform– methanol, has been used for more than 90% of reported analysis of pharmaceuticals and drugs[4].

1. Simple and precise HPTLC methods were developed for the simultaneous estimation of two anti-inflammatory drugs (curcuminand galangin). The method was tailored to analyze both drugs in their commercial dosage form (capsules) with no interference fromingredients. Chromatographic separation was performed over precoated TLC plates (60 F254, 20 cm × 10 cm, 250µm thickness,Merck, Darmstadt, Germany) via a linear ascending technique using n-hexane, ethyl acetate, acetic acid, and methanol as the mobilephase. Detection and quantification was achieved at 404 nm through spectrodensitometricanalysis[5].
2. The report of TLC densitometric method, which has been developed and validated for quantification of stigmasterol from petroleum ether extract of leaves and stems of Bryophyllumpinnatum. The separation was performed on TLC aluminum plates precoated with silica gel 60 F254. Good separation was achieved in mobile phase using Chloroform:Ethanol (9.8:0.2 v/v). Determination and quantitation were performed by densitometric scanning at 490 nm in reflection/absorbance mode [6].
3. It describes a simple, precise and accurate HPTLC method for its estimation as bulk and in tablet dosage form. Thechromatographic separation was carried out on precoated silica gel 60 F254 aluminium plates using mixture of methanol and toluene (4:3%v/v) as mobile phase and densitometric evaluation of spots were carried out at 235nm[7].

Lipophilic C-18, C-8, C-2; phenyl chemically-modified silica gel phases; and hydrocarbon- impregnated silica gel plates developed with a more polar aqueous mobile phase, such as methanol–water or dioxane–water, are used for reversed-phase TLC.

1. A new high-performance thin-layer chromatographic (HPTLC) method has been established for determination of minocycline in human plasma. Chromatography was performed on aluminium plates coated with silica gel $60F_{254}$; the mobile phase was methanol:acetonitrile:isopropanol:water 5:4:0.5:0.5 (v/v). Densitometric analysis was performed at 345 nm [8].
2. A simple, precise, accurate and highperformance thin layer chromatographic method has developed and validated for the estimation of Olmesartanmedoxomil and hydrochlorthiazide simultaneously combined dosage forms. The stationary phase used isprecoated silica gel $60F_{254}$. the mobile phase was a mixture of acetonitrile: chloroform: glacial acetic acid (7:2:0.5, v/v/v). The detection of spots was carried out at 254nm [9].
3. A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of tenoxicam in the

microemulsion gels. Tenoxicam was chromatographed on silica gel 60 F254 TLC plate, as a stationary phase. The mobile phase was toluene: ethyl acetate: formic acid (6:4:0.3 v/v/v) used [10]

4. A simple, precise, specific and accurate high performance thin layer chromatographic method has been developed for the simultaneous determination of Cinitapride and Omeprazole in pharmaceutical dosage form.The separation was carried out on Merck HPTLC aluminum plates of silica gel G60 F254, (20 × 10 cm) with 250µm thickness using chloroform: ethyl acetate: methanol (7.3: 2: 0.7, v/v/v) as mobile phase. HPTLC separation ofthe two drugs followed by densitometric measurement were carried out in the absorbance mode at 277 nm [11].
5. It describes developed and validated thin layer liquid chromatography (TLC) method for the simultaneous estimation of telmisartan and ramipril in a combined dosage form. Procedure does not require prior separation of components from the sample. Telmisartan and Ramipril were determined by High Performance Thin Layer chromatography method (HPTLC) in tablet dosage form. The method was carried out in TLC precoated silica gel on aluminum plate 60 F 254, (10 cm ×10 cm, prewashed by methanol and activated at 60° C for 5 min prior to chromatography). The solvent system was Acetone: Benzene: Ethyl acetate: Glacial acetic acid in the proportion of 5:3:2:0.03, (v/v/v/v)[12]

Other precoated layers that are used include aluminum oxide, magnesium silicate, magnesium oxide, polyamide, cellulose, kieselguhr, ion exchangers, and polar modified silica gel layers that contain bonded amino,cyano, diol, and thiol groups.Optical isomer separations that are carried out on a chiral layer produced from C-18.

MobilePhase:

The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyte. The mobile-phase systems are used based on their diverse selectivity properties are diethyl ether, methylene chloride, and chloroform combined individually or together with hexane as the strength-adjusting solvent for normal-phase TLC and methanol, acetonitrile, and tetrahydrofuran mixed with water for strength adjustment in reversed-phase TLC. Separations by ion pairing on C-18 layers are done with a mobile phase such as methanol–0.1 M acetate buffer (pH 3.5) containing 25 mM sodium pentanesulfonate (15.5:4.5).

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2.Simultaneous quantification of Lamivudine and Zidovudine in tablets by HPTLC method was developed and validated. The chromatograms were developed using a mobile phase of toluene:ethylacetate:methanol (4:4:2, v/v/v) on pre-coated plate of silica gel GF aluminum TLC plate and quantified by densitometric absorbance mode at 276 nm[13].

3. A new high-performance thin-layer chromatographic (HPTLC) method has been established for determination of minocycline in human plasma. Chromatography was performed on aluminium plates coated with silica gel $60F_{254}$; the mobile phase was methanol: acetonitrile: isopropanol: water 5:4:0.5:0.5 (v/v)[8].

4.A new and simple HPTLC method was developed and validated for the quantitative estimation of Eugenol in muscle and joint pain relaxant herbal oil. TLC aluminium plates precoated with silica gel 60F-254 (0.2 mm thickness) were used. The linear ascending development was carried out in twin trough glass chamber saturated with mobile phase Tolune: Ethyl acetate (9.3:0.7) ratio followed by densitometric determination was carried out by TLC scanner (CAMAG) at 560 nm in reflectance/absorbance mode [14].

5.A sensitive, fast, and reproducible high performance thin-layer chromatographic method has been developed for simultaneous analysis of diosgenin and quercetin from fenugreek seeds, using TLC aluminium plates precoated with silica gel G60F254. Among the different combinations of mobile phases used, best separation was achieved in Toluene-ethyl acetate-formic acid (5:4: 1, *v/v/v*). Densitometric scanning of the plates directly at 275nm was used for analysis of quercetin [15].

6. A new, simple, and rapid high-performance thin-layer chromatographic method was developed and validated for quantitativedetermination of Carbamazepine. Carbamazepine was chromatographed on silica gel 60 F254 TLC plate using ethyl acetate: toluene: methanol (5.0: 4.0 : 1.0 v/v/v) as mobile phase. Carbamazepine was quantified by densitometric analysis at 285 nm[16].

7. A new simple, precise, accurate, specific and selective high performance thin layer chromatographic (HPTLC) method has been developed for the simultaneous estimation of Terbinafine hydrochloride (TH) and Mometasonefuroate (MF) in cream dosage form. The chromatographic separation was achieved on Merck precoated silica gel aluminium plate 60 F254 using Toluene: Ethyl acetate: Glacial acetic acid (8: 4: 0.1 v/v) as mobile phase [17].

8. A Thin Layer Chromatography (TLC) method for the qualitative and quantitative analysis of diclofenac sodium tablets was developed and validated according to ICH and USP guidelines. The method was developed using a mobile phase prepared with environment-friendly solvents: toluene, acetone and glacial acetic acid (10:15:0.2 v/v/v), on pre-coated TLC silica gel 60 F254 glass plates with a saturation time of 25 min and a densitometer detection wavelength of 284 nm in the reflectance absorbance mode[18].

9. An accurate, sensitive, precise, reliable, and quick method for the determination of cholesterol content by high-performance thin layer chromatography is developed.

In this method, aluminum-backed precoated silica gel 60 F254 plates were used as the stationary phase and the samples were sprayed with the help of CAMAG sample applicator Linomat 5. The chromatogram was developed with the mobile phase consisting of chloroform: methanol (9.5:0.5, v/v)[19].

10. A new, simple, high-performance thin-layer chromatographic method for the determination of mycophenolate mofetil in bulk and tablet dosage form. The drug was separated on aluminum plates precoated with silica gel 60 F_{254} with toluene, acetone, and methanol in the ratio of 6:2:2 (v/v/v) as the mobile phase. Quantitative analysis was performed by densitometric scanning at 254 nm [20].

11. A simple, precise and accurate HPTLC method Duloxetine Hydrochloride for its estimation as bulk and in tablet dosage form. The chromatographic separation was carried out on precoated silica gel 60F254 aluminium plates using mixture of Chloroform: methanol (8:1 v/v) as mobile phase and densitometric evaluation of spots was carried out at 235nm [21].

Prewashing:

Plates are handled at the upper edge to avoid contamination. Plates are used without pretreatment unless chromatography produces impurity fronts due to contamination of the plate. For reproducibility and quantitative analysis, layers are often prewashed using 20 ml methanol. The methanol is used as a prewashing solvent, a mixture of methanol and ethyl acetate or even mobile phase is used, per trough in a 20 × 10 cm twin-trough chamber (TTC). The two 20 × 10 cm or four 10 × 10 cm plates can be developed back-to-back in each trough of the TTC. Remove the plate and dry it for 20 min in a clean drying oven at 120˚C. Equilibrate plate with laboratory atmosphere (temperature, relative humidity) in a suitable container providing protection from dust and fumes. [3]

Preparation of plate:

TLC plates can be made with suitable apparatus. such layers do not adhere well to the glass support. Precoated plates use small quantities of very high molecular weight polymer as binder overcomes most limitations of a home-made layer.Precoated layers are reasonably abrasion resistant, very uniform in layer thickness, reproducible, preactivated, and ready to use. They are available with glass or aluminum or polyester support. Aluminum foil plates are less expensive to buy, cheaper, can be cut, and therefore easy to carry around or transport or mail. Glass plates are the best for highest quality of results. Most often, layers containing a fluorescent indicator F 254 are used. This enables the visualization of samples in a UV cabinet very simply, instantly, and in a nondestructive manner.Commonly used size of plates in TLC is 20×20 cm and in HPTLC 20 × 10 cm or 10 × 10 cm is widespread.

Steps involved in HPTLC procedure

- Sample Application
- Chromatogram Development
- Derivatization
- Evaluation: Detection
- Evaluation: Documentation

Sample Application:

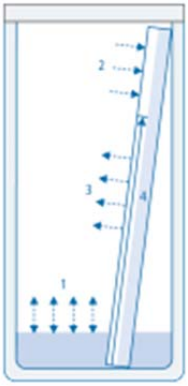
The sample application plays an important role and its techniques are spot application and Spraying – on samples.Sample application is the first step in chromatography and it affects the quality of the result at the end of the process.The choice of the application technique and the device depend on the requirements. Spot wise sample application using a fixed volume capillary is the simplest way. Sample volumes of 0.5 to 5 µL can be applied as spots onto conventional layers without drying, on HPTLC layers it is up to 1 µL per spot.

Spraying-on samples as narrow bands of larger volumes is the best resolution that can be achieved with the chromatographic system selected.Large sample volumes or samples with high matrix content can be sprayed-on in the form of rectangles and focused into narrow bands.

Chromatogram Development

In this technique in addition to stationary and mobile phases, a gas phase is present. This gas phase can significantly influence the result of the separation.

Figure.1. process in developing chamber[1]



Processes :

The lower end of the plate should be immersed and act by capillary action the developing solvent moves up the layer until the desired distance is reached and chromatography is stopped. The following considerations primarily concern silica gel as stationary phase and developments, which can be described as adsorption chromatography.

Four types of Processes occur:

The four types of processes occurs they are

1. When it is dry, the stationary phase adsorbs molecules from the gas phase. This process, adsorptive saturation an equilibrium in which the polar components will be withdrawn from the gas phase and loaded onto the surface of the stationary phase.
2. The part of the layer which is already wetted with mobile phase interacts with the gas phase. Thereby especially the less polar components of the liquid are released into in the gas phase.

3. Unlike (1) this process is not as much governed by vapor pressure as by adsorption forces.
4. During migration, the components of the mobile phase can be separated by the stationary phase under certain conditions, causing the formation of secondary fronts.

The following aspects should be followed:
The developing solvent and mobile phase arenot the same. Their composition changes with chromatography. The term developing solvent, mobile phase are often used as same, the liquid in the chamber should be called developing solvent, while the liquid moving through the layer constitutes the mobile phase. Only the composition of the developing solvent at the time when it is placed into the chamber is positively known. The processes (1) and (2) can be experimentally affected by:

- Fitting the chamber more or less completely with filter paper soaked with developing solvent.
- Waiting a certain time between the introduction of developing solvent into the chamber and the beginning of chromatography – chamber saturation.
- Allowing the plate to interact with the gas phase prior to chromatographic development, i.e. without contact to the developing solvent – preconditioning.

The step 2 and 3 can be prevented by placing a counter plate at a distance of one or a few millimeters to the chromatographic layer. This is called sandwich configuration. Equilibriumexists for step 1 and 2 and the less different the components of the mobile phase are in respect to their adsorption behavior, for step 4 it is less pronounced in the formation of secondary fronts. In well-saturated chambers and on preconditioned layers secondary fronts are often not observed. During chromatography, components of the developing solvent, which have been loaded onto the dry layer via the gas phase according to (2), are pushed ahead of the true but invisible solvent front. Exceptions are very polar components such as water, methanol, acids, or bases. This results in RF values being lower in saturated chambers and particularly on pre-conditioned layers, than in unsaturated chambers and sandwich configurations Due to possible demixing of the solvents and possible beta fronts, development in sandwich configuration or in an unsaturated horizontal developing chamber works best with single component solvents or multi component solvents behaving like single component solvents.

Consequences:
Thin-layer Chromatography in most cases proceeds in a non-equilibrium between stationary, mobile, and gas phase. For this reason it is very difficult to correctly describe the conditions in a developing chamber. Reproducible chromatographic results expected when all parameters are kept as constant as possible. Chamber shape and saturation are playing a predominant role in this regard. Unfortunately this means that the chromatographic result is different in each chamber.

Choosing a developing chamber:
The selection of chamber is done during method development and follows considerations such as chamber is available, which one must be used.A focus should also be on economic aspects such as time requirement and solvent consumption. The Horizontal Developing Chambers have proven to be exceptionally economical, flexible and reproducible in operation. It is designed for applications where the plate is developed from two sides; they are also suitable for single-sided developments in unsaturated, saturated and sandwich configuration for preconditioning of HPTLC plates. For development a conventional 20 x 10 cm Twin Trough Chamber is used. This way chamber geometry and chromatographic conditions of already existing analytical procedures can be retained, but environmental and operational effects are standardized.

Derivatizer:
TheDerivatizer presents an automated spraying device which sets a new standard of reproducibility in the reagent transfer onto TLC plates by employing a unique "micro droplet" spraying technology (patent pending). The Derivatizer ensures homogeneous and reproducible application of all common reagents. To meet the diverging physicochemical properties of the different reagents, *e.g.* acidity or viscosity, four different color-coded spray nozzles are employed, and the user can select from six spraying levels.
In addition to the significantly increased homogeneous reagent distribution, the Derivatizer offers other advantages compared to manual spraying:

- Environmentally friendly and safe handling through a closed system
- Intuitive handling and easy cleaning
- Low reagent consumption through efficient operation (4 mL for 20 x 20 cm plates and 2 mL for 20 x 10 cm plates)
- Reproducible and user-independent results

Evaluation: Detection
The chromatogram is evaluated under white or ultraviolet light. Options range from visual inspection of electronic images to quantitative determinations using video or scanning densitometry.

Chromatogram evaluation with classical densitometry
The instrumental Thin-Layer Chromatography should be able to resort to both classical densitometry and electronic image acquisition. Densitometry uses monochromatic light and a slit of selectable length and width to scan the tracks of a chromatogram, measuring the diffusely reflected light. The TLC Scanner uses the entire spectral range from 190 to 900 nm with high spectral selectivity for data acquisition. Absorption spectra for substance identification and for selection of the most suitable measurement wavelength can be recorded within this range.
The strengths of classical densitometry are the spectral resolution of the light source and the higher reproducibility of quantitative determinations.

Requirements for high precision of evaluation

- Use of HPTLC plates. Small layer thickness, narrow particle size distribution and the homogenous packing of the HPTLC layer result in less fraction broadening and low background noise.
- Automatic spray-on sample application technique. Only by spraying the size of the starting zone does remain independent of the application volume and the sample is homogenously distributed across the application position. Data acquisition can be based on larger substance amounts.
- Use of a chamber providing good reproducibility of chamber conditions.
- Choosing a working range for calibration according to the absorption/fluorescence behavior of the substances. The evaluation software offers suitable calibration functions.
- Optimization of light and measurement parameters, such as slit dimensions, measuring wavelength, scanning speed for the substances to be analyzed.
- Suitable baseline correction to maximize the signal to noise ratio.
- Derivatization can contribute to the overall error of the determination. The more homogenous the reagent is applied the smaller the error.

Evaluation: Documentation, TLC-MS, Bioluminescence:

The electronic images are easy to capture and to archive for documentation. They can be reproduced on screen without changes over time and thus compared with current images. TLC-MS and bioluminescence expand the capability of TLC.

Application:

1. Pharmaceutical applications

- Quality control
- Content Uniformity Test (CUT)
- Identity- and purity checks
- Stability tests, etc.

2. Clinical applications

- Lipids
- Metabolism studies
- Drug screening
- Doping control, etc.

3. Cosmetics

- Identity of raw material
- Preservatives, colouring materials, etc.
- Screening for illegal substances, etc.

4. Herbal medicines and botanical dietary supplements

- Identification
- Stability tests
- Detection of adulteration
- Assay of marker compounds, etc.

5.Food and feed stuff

- Quality control
- Additives (*e.g.* vitamins)
- Pesticides
- Stability tests (expiration), etc.

6. Industrial applications

- Process development and optimization
- Process monitoring
- Cleaning validation, etc.

7. Forensics

- Detection of document forgery
- Investigation of poisoning
- Dyestuff analyses, etc.

REFERENCE

1. CAMAG, 2010-2011. Basic equipment for modern thin layer chromatography. Switzerland: Camag. Available from: camag.com/downloads/free/brochures/CAMAG-basic-equipment-08.pdf
2. CAMAG, 2010-2011. Instrumental thin layer chromatography. Switzerland: Camag. Available from: camag.com/downloads/free/brochures/CAMAG_TLC10-11_E.pdf
3. Patel, R.B. and Patel, M.R. and Patel, B.G. (2011) Experimental Aspects and Implementation of HPTLC. In: Shrivastava, M.M. HPTLC. New York: Springer, pp. 41- 54.
4. Shrivastava, M.M. (2011) An Overview of HPTLC: A Modern Analytical Technique with Excellent Potential for Automation, Optimization, Hyphenation, and Multidimensional Applications. In: Shrivastava, M.M. HPTLC. New York: Springer, pp. 3- 24.
5. Sharad Kharat., Ajay Namde., Piyush Mehta.,Journal of Taibah University for Science xxx (2016) xxx–xxx, 1-7.
6. AnjooKamboj ., Ajay Kumar Saluja.,Development of validated HPTLC method for quantification of stigmasterol from leaf and stem of Bryophyllumpinnatum. Arabian Journal of Chemistry (2013) xxx, xxx–xxx
7. Dhandapani, B., Somasundaram, A., Shaik Harun Raseed., Raja, M and Dhanabal, K., Development and Validation of HPTLC Method for Estimation of Quetiapine in Bulk Drug and in Tablet Dosage form.International Journal of PharmTech Research.2009,1(2),139-141.
8. Jain, G. K., Jain, N., Iqbal, Z., Talegaonkar, S., Ahmad, F. J., and Khar, R. K.,Development and validation of an Hptlc method for determination of Minocycline in Human Plasma.ActaChromatographica. 2007, 19, 197-205.
9. Shah,N.J., Suhagia, B.N., Shah,R.R., and Patel, N.M., Development and validation of Simultaneous HPTLC method for the estimation of Olmesartanmedoxomil and Hydrochlorothiazide in tablet dosage form.Indian Journal of Pharmaceutical Sciences. 2007, 69, 834-836.
10. Chandel, S., Barhate, C. R., Srivastava, A. R., Kulkarni, S. R. and Kapadia, C. J., Development and validation of HPTLC method for estimation of Tenoxicam and its formulations.Indian Journal of Pharmaceutical Sciences. 2012, 36-40.
11. Hitesh, J., Vekari., Makani Yogesh., Kinesh Patel.,Development and validation of HPTLC method for simultaneous estimation of Cinitapride and Omeprazole in combined dosage form. Der Pharmacia Lettre, 2012, 4 (5):1467-1474.
12. Patel, V. A., Patel, P. G., Chaudhary, B. G., Rajgor, N. B., Rathi, S. G.Development and validation of HPTLC method for the simultaneous estimation of Telmisartan and Ramipril in combined dosage form, International Journal on Pharmaceutical and Biological Research. 2010, 1(1), 18-24.
13. PalaniVenkatesh., MaheshDaggumati. , Development andvalidationofanormal-phaseHPTLC method forthesimultaneousanalysisofLamivudine and Zidovudineinfixed-dosecombinationtablets.Journal of Pharmaceutical Analysis. 2012, 2(2):152–155
14. Charde, M. S., Chakolkar,M. D., Welankiwar, A. S., Keshwar, U., and Shrikande, B. K.,Development of validated HPTLC method for the estimation of eugenol in marketed herbal formulation of muscle and joint HRX pain relieving oil,International Journal of Phytopharmacy Research Article.2014,4 (1), 28-32.
15. Omi Laila., ImtiyazMurtaza.,Abdin, M. Z., Ahmad, S.,Nisar Ahmad Ganai, andMajid Jehangir.Development and validation of HPTLC method for Simultaneous Estimation of Diosgenin and Quercetin in Fenugreek Seeds (Trigonellafoenum-graceum),ISRN Chromatography.2014,1- 8.
16. Rashmin B. Patel,Mrunali R. Patel, Kashyap K. Bhatt, and Bharat G. Patel.Development and Validation of HPTLC method for estimation

of Carbamazepine in formulations and Its In Vitro Release Study.Chromatography Research International. 2011, 1-8 .
17. Mehul M. Patel,,Heta D. Patela. Development and Validation of Hptlc method for Simultaneous Estimation of Terbinafine Hydrochloride and MometasoneFuroate in combined dosage form, J. Chil. Chem. Soc., 2016, 2958-2962.
18. EliangiringaKaale., Bugusu C Nyamweru., Vicky Manyanga., MhinaChambuso and Thomas Layloff.The Development and Validation of a Thin Layer Chromatography Densitometry Method for the Analysis of Diclofenac Sodium Tablets.Pharmaceut Anal Act.a 2013, 4:1
19. Jinu John., Ankit Reghuwanshi., Usha K. Aravind., Aravindakumar, C.T.Development and validation of a HPTLC method for thedetermination of cholesterol concentration.Journal of food and drug analysis.2015, 2 3.219 -224.
20. Kathirvel,S., Rajendra Prasad,K., and MadhuBabu,K.Development and validation of HPTLC method for the determination of mycophenolate mofetil in bulk and pharmaceutical formulation. Pharm Methods. 2012. 3(2): 90–93.
21. Suneela S. Dhaneshwar., Deshpande, p., Patil,M., Vadnerkar,G., Dhaneshwar, S.R., Development and validation of HPTLC method for the estimation of Duloxetine Hydrochloride in bulk drug and in tablet dosage form.Indian Journal of Pharmaceutical Sciences. 2008, 233-238.