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TLC-densitometric method for quantitation of Lurasidone hydrochloride in nanoemulsion,microemulsion, for equilibrium solubility and *ex vivo* diffusion studies

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ABSTRACT

A new TLC-densitometric method has been successfully developed and validated for quantitation of Lurasidone hydrochloride in pharmaceutical formulations. The Lurasidone hydrochloride was well separated from sample matrix and degradation product using TLC plate; precoated with silica gel G 60F254 on aluminium sheet as a stationary phase and mixture of hexane: ethyl acetate (6:4 v/v) as a mobile phase. Lurasidone hydrochloride was quantified by densitometric analysis at 323 nm. The method was found to give compact bands for the drug (Rf = 0.47 ± 0.01). Linear regression analysis data for the calibration plots showed a good linear relationship in the concentration range of 100–600 ng. Statistical analysis of the data showed that the method is specific, precise, accurate, reproducible, and selective for the analysis of Lurasidone hydrochloride. The TLC-densitometric method was successfully applied in analysis of LURAH in solution, nanoemulsion, mucoadhesive nanoemulsion and microemulsion; subjected to stability testing under accelerated conditions of alkali, acid, thermal, photolytic and humidity. The method was successfully used for the determination of equilibrium solubility of Lurasidone hydrochloride in various excipents and ex vivo diffusion study through sheep nasal mucosa from in-house developed formulations.

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1. Introduction

Lurasidone hydrochloride (LURAH), chemically [(3aR,4S,7R,7aS)-2-{(1R,2R)-2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-ylmethyl] cyclohexylmethyl} hexahydro-4,7-methano-2Hisoindole-1,3-dione hydrochloride is an azapirone derivative. LURAH was approved as an immediate release oral tablet (40, 80, 120 and 160 mg/tablet) for the treatment of schizophrenia [1,2]. LURAH is not official in any pharmacopoeia. Different analytical methods have been developed for quantitative estimation of LURAH in pharmaceutical dosage form and in biological fluids by UV [3-7], HPLC [8-12], LC-MS [13], LC-MS-MS [14] and HPTLC [15].

Literature survey has shown that no TLC- densitometric method has been reported for the estimation of LURHA in pharmaceutical formulation like nanoemulsion, mucoadhesive nanoemulsion, microemulsion, solution (developed in-house), for equilibrium solubility study and ex vivo diffusion study through sheep nasal mucosa from the formulations developed in house. Therefore, the new, TLC-densitometric method was developed and validated

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for estimation of LURAH in pharmaceutical formulation (i.e. nanoemulsion, mucoadhesive nanoemulsion, microemulsion, solution - developed in-house), for equilibrium solubility study and ex vivo diffusion study.

Taken together, this information has led our research group to evaluate the potential of TLC-densitometric method as an analytical tool for quantitative estimation of LURHA. The current study elaborates a new and simple TLC-densitometric method for quantitative determination of LURHA in house developed formulations, for determining equilibrium solubility of LURHA in various excipents as well as its ex vivo diffusion study through sheep nasal mucosa from the formulations developed in house.

2. Materials and Methods

2.1 Chemicals and reagents

Pure samples of LURHA were gifted by Astron Research Ltd. (Ahmedabad, India). Labrasol (caprylocaproyl polyoxylglycerides), plurol oleique CC (polyglyceryl oleate), transcutol P (diethylene glycol monoethyl ether), (Gattefosse Saint-Priest, France) were procured as gratis samples from Gattefosse Asia Ltd. (Mumbai, India). Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil) and Cremophor EL (polyoxyl 35 hydrogenated castor oil) were procured as gratis samples from BASF (Mumbai, India). Capmul MCM (glyceryl mono- and dicaprate) was procured as gratis sample from Abitec Corporation (Janesville, USA). Polycarbophil (AA-1, pharmagrade, molecular weight approximately 3.5 million) was procured as gratis sample from Lubrizol Advanced Material India Pvt Ltd. (Mumbai, India). Oleic acid, tween 80, potassium dihydrogen phosphate, sodium chloride, methanol and propylene glycol were purchased from SDfine Chemicals (Ahmedabad, India). Double distilled water was used throughout the study. All other chemicals and solvents were of analytical reagent grade and used as received without further purification.

2.2 Instrumentation

The HPTLC system (CAMAG, Muttenz, Switzerland) consisted of a Linomat 5 autosprayer, a twin-trough chamber for 10 × 10 cm plates, a derivatization chamber, and a plate heater. Precoated silica gel $60F_{254}$ TLC plates (10 × 10 cm, layer thickness 0.2 mm; E. Merck KGaA, Darmstadt, Germany) were used as the stationary phase. Densitometric analysis was carried out using a CAMAG TLC Scanner 3 with winCATS software [16-18.

2.3 Preparation of Lurasidone hydrochloride standard solution

Standard solutions of LURHA (100 µg/mL) was prepared in methanol. It was prepared by accurately weighing 10 mg LURHA then transferred into, a 100 mL calibrated volumetric flask, dissolved in 50 mL methanol and completed the void volume with methanol to produce standard solution of LURHA (100 µg/mL).

2.4 Preparation of stress test samples

2.4.1 Acid hydrolysis

10 mg of standard LURAH was accurately weighed and transferred to a 100 mL volumetric flask. 10 mL of 1 M HCl were added, kept at 80 °C for 24 hrs on water bath. After treatment, solution was neutralized with 1 M NaOH to *pH* 7 and volume was made up to the mark with water. The resulted solution was subjected to chromatograph to check the specificity. Similar methodology was followed for formulations.

2.4.2 Ikali hydrolysis

Alkali hydrolysis was carried out as per the procedure described for the acid degradation using 1 M NaOH instead of 1 M HCl (1 M HCl was used for the neutralization purpose to *pH* 7). Similar methodology was followed for formulations.

2.4.3 Oxidative stress degradation

Peroxide degradation was carried out as per the procedure described for the acid degradation using 6 % v/v H_2O_2 instead of 1 M HCl. Similar, methodology was followed for formulations.

2.4.4 Thermal (Dry heat) degradation

LURAH powder and formulations were heated at 100 °C for 48 hrs and from this 100 µg/mL solution containing LURAH was prepared. This solution was chromatographed to check the specificity.

2.4.5 Photochemical degradation

LURAH powder and formulation were exposed to UV light for 72 hrs and from this 100 µg/mL solution containing LURAH was prepared. This solution was chromatographed to check the specificity.

2.4.6 Moist degradation

LURAH API and developed formulations were exposed to 40 °C and 75 % relative humidity for 48 hrs, and from this 100 µg/mL solution containing LURAH was prepared. This solution was chromatographed to check the specificity.

All the solutions were passed through Whatman filter no. 41 before sample application. For each degradation study, blank solutions were also prepared without taking standard LURAH or formulations as per the same procedure described above, to check the interference of chemical reagents used.

2.5 TLC-densitometric method development and validation

The standards and test sample solutions of LURHA were applied on the TLC plate and developed by linear ascending development technique in a twin-trough glass chamber equilibrated with the mobile phase vapors for 20 min at 25 ± 2°C. All samples were permitted to travel a distance of 70 mm. The developed TLC plates were dried thoroughly before densitometric evaluation. Densitometric scanning was performed at 323 nm using the absorbance mode under control by winCATS planar chromatography software Version 1.3.4. The calibration curves (n = 6) for LURHA were constructed by plotting peak area versus concentration (100–600 ng) with the help of the winCATS software [19-22].
The developed TLC-densitometric method was validated as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) [23].

2.6 Application of developed TLC-demsitometric Method

2.6.1 Solubility study

The solubility of LURAH was determined in various oils, emulsifiers, and co-emulsifiers. In this experiment, excess amount of LURAH was added in 1 mL of each excipeints and mixed thoroughly using vortexer at ambient temperature. The mixed samples were kept for 72 h to allow equilibration. Then the samples were centrifuged at 5000 × g for 30 minutes to separate the un-dissolved drug. Supernatants were diluted and analysed by developed TLC-denisitometric method in order to estimate concentration of soluble LURAH [24,25].

2.7 Determination of pH-solubility profile

Solubility of LURAH in various buffers was determined by the method as described above. Acetate buffer pH 4.5, and phosphate buffer, *pH* 5.0 were used.

2.8 LURAH formulations

Nanoemulsion [LURAH (10 mg/mL), capmul MCM (3–11 % wt/wt), mixture (1:1) of tween 80 and propelyne glycol (23–37 % wt/wt) and water (51–74 % wt/wt)], mucoadhesive nanoemulsion (contain additional polycarbophil (0.5% wt/wt) as a mucoadhesive polymer), and microemulsion (LURAH (5mg/mL), Oleic acid (0.4% wt/wt), cremophore RH 40 (11.25% wt/wt), labrasol (11.25% wt/wt), transcutol P (7.5% wt/wt), and water (66% wt/wt)] formulation of LURAH were developed in-house. Nanoemulsion and mucoadhesive nanoemulsion formulations were prepared using high pressure homogenizer (Panda plus 2000, GEA Niro Soavi, Parma, Italy); while microemulsion formulation was prepared by spontaneous emulsification method. Solution was prepared by dissolving LURAH (10 mg/mL) in a mixture of propylene glycol and water (2:8 v/v) [24,25].

2.8.1 In vitro diffusion profile of LURAH formulation

Solution, nanoemulsion, mucoahdesive nanoemulsion and microemulsion formulations of LURAH for intranasal delivery were evaluated for in vitro release using a Franz (Orchid Scientifics, Nashik, India) diffusion cell with a diameter of 10 mm. The temperature of the receiver chamber containing 15 mL diffusion medium (Acetate buffer, *pH* 5.0) was controlled at 37 ± 1°C under continuous stirring with a Teflon coated magnetic bar at a constant rate, in a way that the nasal membrane surface just flushed the diffusion fluid. During the study, 2 mL aliquots were removed at 15, 30, 60, 90, 120, and 240 min and replaced with fresh buffer. The amount of drug released was determined using the developed TLC-densitometric method [24, 25].

3. Results and Discussion

3.1 Selection and optimization of chromatographic conditions to develop TLC-densitometric method

To develop the TLC-densitometric method for quantitative estimation of LURAH, selection of stationary phase was quite easy. Commercially available precoated TLC plates with Silica Gel 60 F_{254} on aluminum backing were selected and used as a stationary phase, which is reasonable and nearly suits all kind of drugs as reported in most of the research papers [16-22]. Selection of the mobile phase was carried out on the basis of methodology suggested by CAMAG laboratory [17]. A mobile phase that would give a dense and compact band with an appropriate Rf value (0.2<Rf<0.8), for LURAH was desired [17]. CAMAG laboratory suggested four level techniques for mobile phase selection and optimization. First level involves the use of neat solvents (seven to twelve) and then by finding such solvents which can have average separation power for the desired drugs. Accordingly, various neat solvents such as acetone, methanol, chloroform, toluene, ethyl acetate, hexane, acetonitrile, were evaluated as mobile phase. Further, binary mixtures of solvents with suitable proportion were selected and evaluated on the basis of results obtained in first level of optimization. As a result, combination of hexane: ethyl acetate (6:4 v/v) was found suitable as a mobile phase which gave good separation of LURAH from formulation matrix. It was also observed that chamber saturation time and solvent migration distance were crucial in the chromatographic separation as chamber saturation time of less than 15 min and solvent migration distances greater than 70 mm resulted in diffusion of the analyte band. Therefore, combination of hexane: ethyl acetate (6:4 v/v) as mobile phase with a chamber saturation time of 20 min at 25°C and solvent migration distance of 70 mm was used [18-20]. These chromatographic conditions produced a well-defined, compact band of LURAH with optimum migration at Rf = 0.47 ± 0.01 at 323 nm (Fig. 1). They also gave a good resolution of the analyte from excipients used in various nanoemulsion and microemulsion formulations.

3.2 Method validation

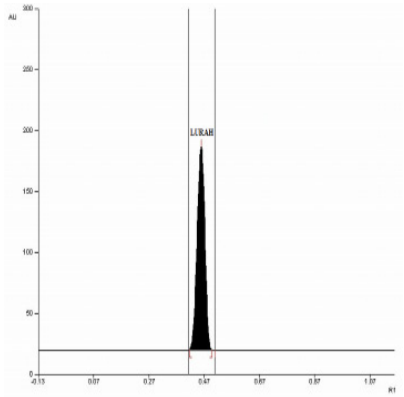

Figure 1 Chromatogram of standard solution containing 400 ng/band Lurasidone hydrochloride using proposed TLC-densitometric method.

The stress testing studies resulted that the method was highly specific for LURAH from its degradation products. The drug was found to be degraded in all stress condition studied. Acid stress led to 82.59 % recovery; alkali stress led to 80.04 % recovery; peroxide stress led to 87.09 % recovery; thermal stress led to 90.73 % recovery; and photolytic stress led to 93.97 % recovery with no unknown degradation peak (Table 1; Fig. 2A-E). The peak for degradation product was not appeared in densitogram. However, prominent peak of LURAH was found stable at Rf 0.47 ± 0.01. The force degradation studies in moisture degradation conditions of LURAH resulted in an insignificant decrease of the peak area and no any detectable degradation products (Fig. 2F). Table 1 outlines the results of degradation study of LURAH at each stress condition.

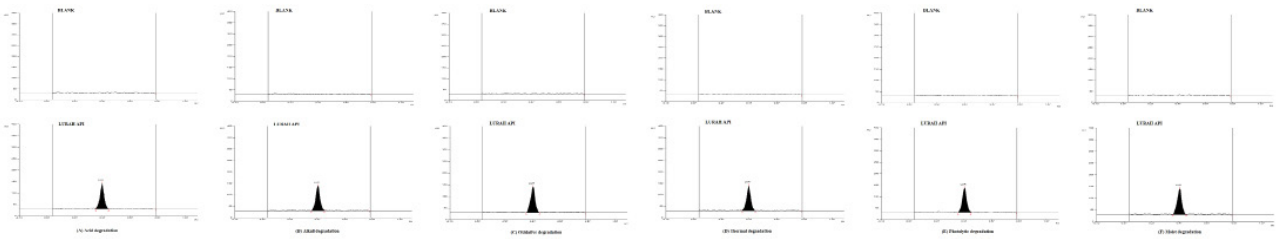

Figure 2 Chromatograms for blank, and Lurasidone hydrochloride after forced degradation study, using (A) Acid (B) Alkali (C) Peroxide (D) Thermal (E) Photo and (F) Moisture, respectively, using proposed TLC-densitometric method.

Specificity is a measure of the degree of interference from other active ingredients, excipients, impurities, and degradation products [8-21]. Specificity in a method ensures that a peak response is due to a single component only. In the present study, the ability of the method to separate the drug from its degradation products and the non interference of the excipients indicate the specificity of the method. Values of peak purity index were higher than 0.9999. These results indicated that the proposed method is specific and stability-indicating, and can be applied for stability studies and QC analysis of LURAH in pharmaceutical products.

Table 1 Degradation study of Lurasidone hydrochloride at different stress conditions using the proposed HPTLC method.

Stress condition	Recovery [%]		
	API	Microemulsion	Nanoemulsion
Acidic	82.59 ± 0.56	83.11 ± 0.27	83.42 ± 0.49
Alkaline	80.04 ± 0.72	80.31 ± 0.77	80.59 ± 0.73
Oxidative	87.09 ± 0.58	87.18 ± 0.67	87.38 ± 0.82
Thermal	90.73 ± 0.74	91.17 ± 0.45	92.04 ± 0.57
Photo	93.97 ± 0.41	93.08 ± 0.39	94.09 ± 0.34
Moist	99.79 ± 0.53	99.56 ± 0.88	99.87 ± 0.59

Under the experimental conditions used, the lowest amount of drug that could be detected was found to be 13.11 ng, and the lowest amount of drug that could be quantified was 39.71 ng (Table 2).

Table 2 Regression analysis of calibration graphs for Lurasidone hydrochloride by proposed HPTLC method

Parameter	LURAH
Concentration range	100-600 ng/band
Slope	7.42
Residual SD	73.86
intercept	445
SD of the intercept	30.75
Correlation coefficient	0.997 ± 0.002
LOD	13.11 ng
LOQ	39.71 ng

The linearity of a method is defined as its ability to provide measurement results that are directly proportional to the concentration of the analyte [17]. The linearity of the detector was obtained by diluting the analyte stock solution and measuring the associated responses, while the linearity of the analytical method was determined by making a series of concentrations of the analyte from independent sample preparations (weighing and spiking). The linearity data described in the present study demonstrated the acceptable linearity for LURAH over

the range of 80 to 120 % of the target concentration. Linear correlation was obtained between peak areas and concentrations of LURAH in the range of 100–600 ng. The following regression equation was found by plotting the peak area (y) versus the LURAH concentration (x) expressed in ng; y = (7.42 ± 0.078) x + (445 ± 30.74). The coefficient of determination (r2: 0.997 ± 0.02) obtained for the regression line demonstrated the excellent relationship between peak area and concentration of LURAH (Fig. 3 and 4).

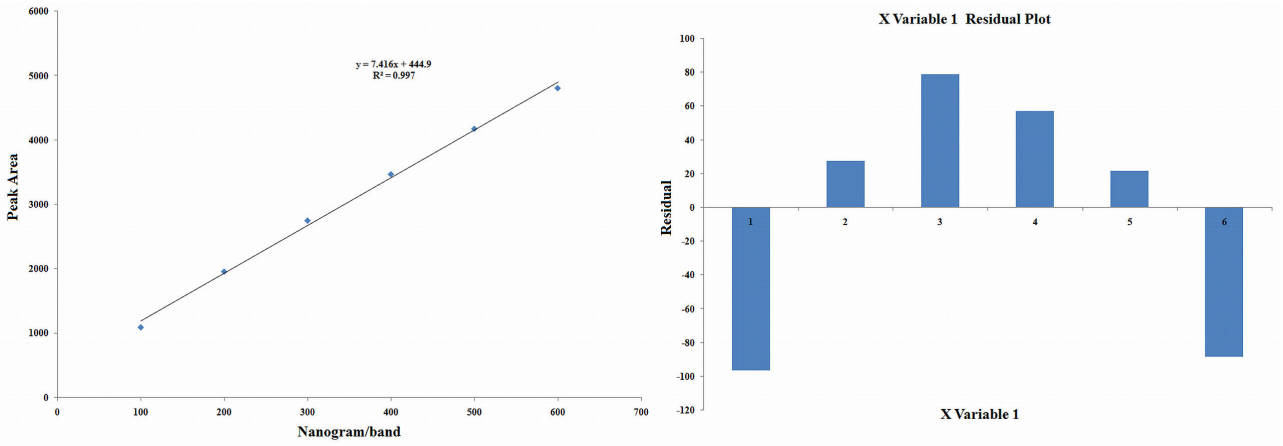

Figure 3 Calibration curve (A) and residual plot (B) of Lurasidone hydrochloride using proposed TLC-densitometric method.

Data of regression analysis are summarized in Table 2. The calibration can be accepted as linear; if it is statistically proved. However, declaring method linearity on the basis of value of coefficient of determination as a sole proof of linearity is inappropriate [22]. Different approaches for checking linearity objectively are reported and should be used. Residual plot is the simplest method of linearity test for TLC method [21]. Residuals are smallest possible differences, which may be obtained by minimizing the distance between the experimental points and regression line. Residuals are distributed randomly at both sides of the straight-line. Positive and negative deviations would cancel each other and square of residuals will be summarized and minimized giving its name least square regression. In this study, linearity was confirmed since residuals are randomly distributed around the regression line (no lack of fit) (Fig. 3). Further, CAMAG described parameter such as 'sdv' (residual standard deviation of the standard point), for fit of calibration curve for TLC-densitometric method by using WinCATS software [17-21]. The lower the value of sdv means, the closer the points to the curve. The acceptance limit of sdv is not more than 5.0 %. In present study, sdv value was found to be 2.77 %, which is lower than specified limit. In present study, the regression results reveal a good linear relationship over the concentration range studied, demonstrating the suitability of the method for analysis of LURAH. Three-dimensional overlay of calibration band and spectral comparison of various aliquots of LURAH during linearity studies are presented in Fig. 4.

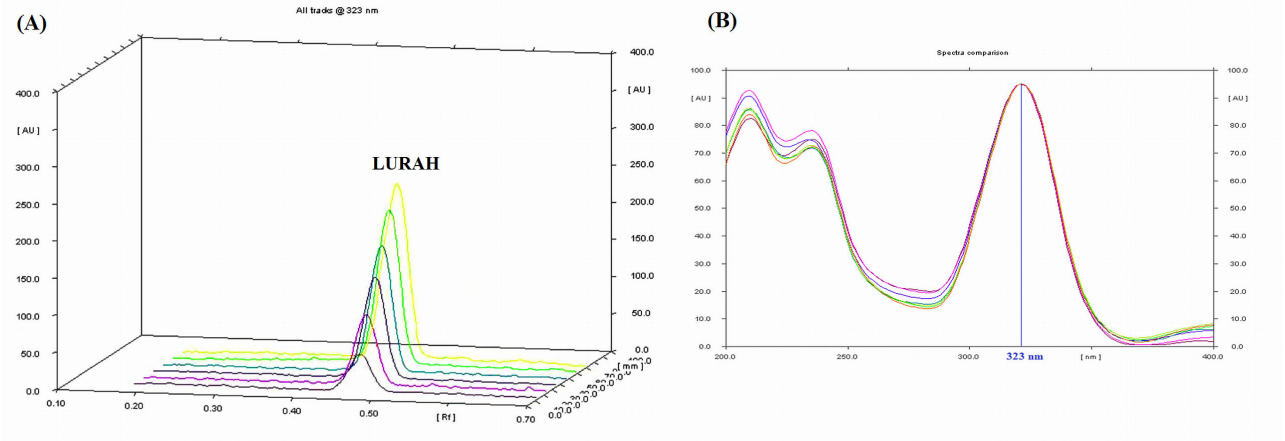

Figure 4 Three-dimensional overlay of densitograms of calibration bands (A) and spectral comparison of various aliquots of Lurasidone hydrochloride of linearity study (B).

Accuracy of an analytical method is given by the extent to which the value obtained deviates from the true value. It shows the proximity of measured results to the actual value. It was determined by the application of analytical procedure to recovery studies, where a known amount of standard is placebo. Results of the accuracy studies from excipient matrix (i. capmul MCM, tween 80, propelyne glycol, water and/or polycarbophil; ii. Oleic acid, cremophore RH 40, labrasol, transcutol P, and water) are shown in Table 3. The LURAH recoveries were obtained in a range of 99.30 to 101.02 % for in house developed formulations, using the proposed TLC-densitometric method. Recovery values demonstrated the accuracy of the method in the desired range.

Table 3 Summary of validated parameters of the proposed HPTLC method for Lurasidone hydrochloride.

Parameter	LURAH
Accuracy, (%, n=3)	99.30 ± 0.86 (400 ng/band)
	101.02 ± 0.52 (500 ng/band)
	99.43 ± 0.62 (600 ng/band)
Repeatability(RSD, %, n=6)	0.42
Precision (RSD, %) Interday (n=3)	0.29-0.45
Intraday (n=3)	0.44-0.76
Specificity	Specific
Solvent suitability	Suitable

The precision of an analytical method expresses the degree of scatter among a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Intraday precision refers to the use of an analytical procedure within a laboratory over a short period of time by the same operator with the same equipment, whereas interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts. The results obtained are shown in Table 3. In all instances, RSD values were less than 5%, confirming the precision of the method. Aliquots of 10 µL of samples containing 200, 400, and 600 ng LURAH were analyzed by the proposed method. In order to control scanner parameters, i.e., repeatability of measurement of peak area, one band was analyzed without changing the position of the plate (n = 7). By applying and analyzing the same amount seven times, precision of the automatic spotting device was evaluated. RSD was consistently less than 5% (Table 3), which was well below the instrumental specifications.

The results and the experimental range of the selected variables evaluated in the robustness study are given in Table 4. There were no significant changes in the chromatographic pattern when the modifications were made in the experimental conditions, thus showing the method to be robust.

System suitability study require asymmetry of analyte peak should not be more than 1.5, theoretical plates of analyte peak should not be less than 2000, and relative standard deviation for five replicates of standard preparation should not be more than 2.0 % [19-22]. As shown in Table 5, the results indicated that the system was suitable for the analysis intended.

Table 4 Chromatographic conditions and range investigated during robustness study for proposed HPTLC method.

Variables	Range	Peak area±SD (n=3)	RSD %	Optimized Value
Chamber saturation time (min)	15	2748.00 ± 10.72	0.39	20
	20	2745.38 ± 9.06	0.33	
	25	2736.12 ± 15.87	0.58	
Distance of solvent front (mm)	65	2750.30 ± 12.65	0.46	70
	70	2743.98 ± 8.78	0.32	
	75	2747.52 ± 14.01	0.51	
Composition of mobile phase (mL)	6.2:3.8	2759.08 ± 21.52	0.78	6:4
	6:4	2746.91 ± 11.53	0.42	
	5.8:4.2	2738.56 ± 23.55	0.86	
Scanning time duration (min)	1	2751.82 ± 13.21	0.48	1
	5	2742.10 ± 14.53	0.53	
	10	2747.40 ± 16.21	0.59	
Detection wavelength (nm)	320	2746.12 ± 12.59	0.45	323
	323	2747.98 ± 11.54	0.42	
	326	2730.46 ± 17.75	0.65	

Table 5 System suitability test parameters for Lurasidone hydrochloride for the proposed HPTLC method.

Parameter	LURAH
Retardation factor (R_f)	0.47 ± 0.01
Peak Area	4176.5 ± 8.47
Tailing factor	1.00 ± 0.01
Number of theoretical plates	3462 ± 75
Peak purity	r (S, M)-0.9999 and
	r(M, E) 0.9999
Repeatability (%RSD)	0.20

Application of Developed Method

LURAH has very low water solubility (0.224 mg/mL at pH 3.6) [1,2], which limits the development of new pharmaceutical formulations aimed to improve its delivery. Therefore, solubility studies were performed to identify suitable oily phases, surfactants, and cosurfactants that are generally used (Table 6). In spite of, being lipophilic in nature, LURAH exhibited very low solubility in oily phases such as captex 200P, captex 355 EP and different grades of miglyols (data not shown). It was found that oily excipients like capmul MCM EP, capmul MCM C8 EP, oleic acid, castor oil and capmul MCM PG8 NF exhibited good solubility for LURAH. Further, LURAH exhibited good solubility in cremophor RH 40, and cremophor EL (surfactants), and in plurol oleique, transcutol P, and propylene glycol (cosurfactants).

An understanding of the *pH*-solubility profile of a drug candidate is regarded as one of the most important aspects of preformulation testing for poorly soluble compounds and has been found to be useful for topical, intranasal, oral, and parenteral formulation development. For example, nasal secretion exhibits a pH in the range of 4–6, and the solubility profile of a drug can help in predicting the absorption behavior of that drug molecule in the nasal mucosa. Our study indicated that LURAH has adequate aqueous solubility, and that it depends on the pH of the medium (Table 6).

Table 6 Solubility of Lurasidone hydrochloride in various excipients and buffers using proposed HPTLC method.

Excipients	Solubility[a]
Oily phases	
Campul MCM EP (Glyceryl mono & dicaprate)	17.53 ±1.92
Capmul MCM PG8 NF (Glyceryl mono & dicaprate)	4.45 ± 0.17
Capmul MCM C8 EP	22.80 ± 2.23
Oleic acid	14.00 ± 1.51
Castor Oil	7.83 ± 0.29
Surfactants	
Tween (Polysorbate) 80	4.58 ± 1.79
Cremophor EL (Polyoxyl hydrogenated castor oil)	15.53 ± 1.22
Cremophor RH 40 (Polyoxyl 40 hydrogenated castor oil)	15.34 ± 1.52
Solutol HS 15 (Macrogol 15 hydroxystearate)	5.50 ± 0.41
Cosurfactants	
Plurol Oleique CC (Polyglyceryl oleate)	13.27 ± 1.61
Transcutol P (Diethylene glycol monoethyl ether)	17.93 ± 2.25
Propylene glycol	18.03 ± 0.92
Aqueous Phases[b]	
Acetate buffer pH 4.5	5.68 ± 0.45
Phosphate buffer pH 5.0	0.11 ± .0.02

[a]Data expressed as mg/g, mean ± SD, n = 3; bData expressed as mg/mL, mean ± SD, n = 3.

The applicability of the method was verified by the determination of LURAH in solution, nanoemulsion, mucoadhesive nanoemulsion and microemulsion formulations (developed inhouse), and no interference from the excipient matrix was observed. The LURAH contents were found to be within the required limits (±5% of the theoretical value) and are shown in Table 7.

Table 7 Assay results for the different pharmaceutical dosage forms using the proposed HPTLC.

Parameter	LURAH ± SD (n = 5), %
In house developed formulations	
LURAH solution	99.71 ± 0.47
LURAH Nanoemulsion formulations	
Batch 1	99.84 ± 1.06
Batch 2	99.56 ± 0.88
Batch 3	99.91 ± 0.75
LURHA Mucoahdesive nanoemulsion formulation	99.97 ± 0.68
LURHA Microemulsion formulation	98.65 ± 1.28

Results indicated the suitability of developed TLC-densitometric method for routine analysis of LURAH in various formulations. During ex vivo diffusion study, LURAH concentration released from the formulations into the receptor fluid was analyzed by proposed TLC-densitometric method. The amount of LURAH released was expressed as a percentage, which was plotted against time in Fig. 5. Thus, the method developed was successfully applied and found very useful for elucidation of the release profile of LURAH through sheep nasal mucosa from the microemulsion, nanoemulsion and mucoadhesive nanoemulsion.

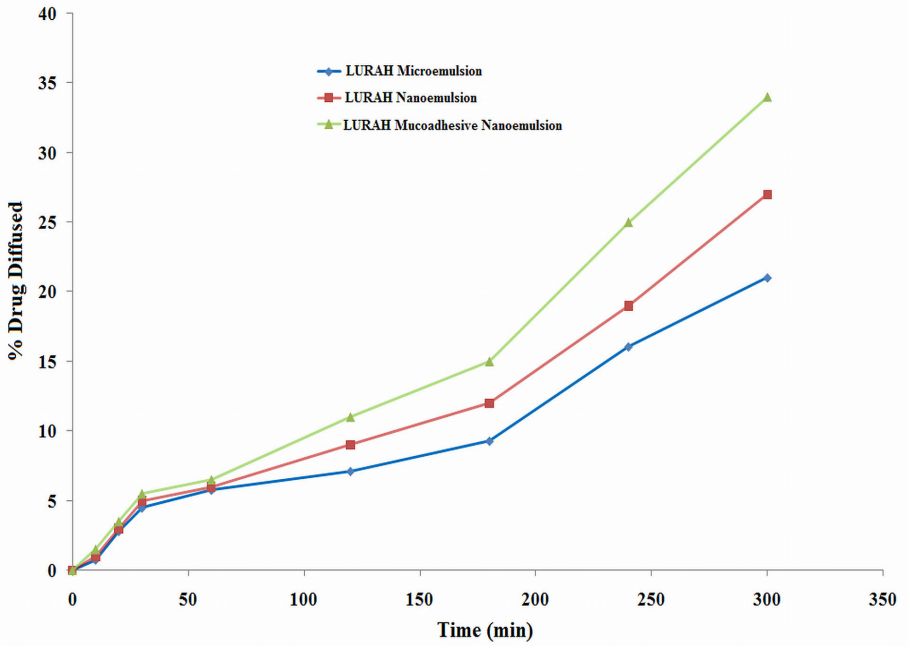

Figure 5 Diffusion profiles of Lurasidone hydrochloride from various formulations in *pH* 4.5 buffer using proposed TLC-densitometric method.

3.3 Comparison of methods

The comparison of the results obtained from analysis of LURAH sample by the developed TLC-densitometric method and reported HPLC8-12 and HPTLC methods [15] are reported in Table 8. The specificity of the detection and the selectivity of the separation for LURAH using TLC-densitometric and HPLC methods are comparable. For developed TLC-densitometric, the LOD and LOQ were better than reported HPLC methods. In TLC-densitometric, as depicted from results repeatability and reproducibility were better than in methods reported. The total analysis time per sample required for a determination by reported HPLC and HPTLC methods was almost 1.2 to 5.6 times higher than developed TLC-densitometric (time required for sample preparation, column washing, chamber saturation, and drying of plate were not considered for this calculation). Usually, it is observed that in TLC/HPTLC the total operating costs for a determination and sample preparation are less than in HPLC, although labor is higher [18,22]. As evident, the assay results of LURAH formulation for HPLC, HPTLC and TLC-densitometric methods are comparable. In a nutshell, advantages of using TLC-densitometric method for this analytical task are direct determination of the LURAH in sample matrix without prior extraction or clean up, high sample throughput and low operating cost.

Further, in reported HPTLC method, detection and estimation of LURAH was performed at 231 nm, which was selected on the basis of spectrum obtained for LURAH solution in methanol [15]. Mostly, in HPTLC method drugs are detected in a pure dry form, adsorbed over silica [16-20]. In view of this fact, our research team selected 323 nm as a suitable wavelength for estimation of LURAH (Fig. 6).

Table 8 Comparison of developed TLC-densitometric method with the reported HPTLC and HPLC methods.

Parameters	TLC-densitometry	HPTLC [24]	HPTLC 1 [10]	HPTLC 2 [11]	HPTLC 3 [12]	HPTLC 4 [13]	HPTLC 5 [14]
Comparison of validation							
Linearity	100-600 ng/band	200-1200 ng/band	30-225 µg/mL	5-25 µg/mL	4-32 µg/mL	10-80 µg/mL	10-50 µg/mL
Limit of detecttion (µg)	0.01	0.02	0.07	0.62	0.72	0.23	0.07
Limit of quantitation (µg)	0.04	0.05	0.23	1.89	2.19	5.6	0.20
Repeatability (% RSD)	0.42	N/R	N/R	N/R	1.25	N/R	N/R
Precision (% RSD)							
Intra day	0.29-0.45	1.39	0.18	1.47	0.63-1.54	0.69	0.10
Intra day	0.44-0.76	1.38	0.29	1.53	0.83-1.96	0.75	0.13
Comparison of analysis time per sample							
Application	33s	33s	-	-	-	-	-
Chromatography	100s	130s	900s	480s	360s	600s	480s
(Run time)			(15min)	(8 min)	(6 min)	(10 min)	(8 min)
Evaluation	33s	33s	33s	33s	33s	33s	33s
Total analysis time per sample	166s	196s	933s	513s	393s	633s	513s
Assay of formulation (Application of method)							
LURAH	99.97	97.20[a]	100.032[b]	N/R	100.96[a]	99.84[c]	99.91[c]

N/R – not reported; asynthetic mixture/ blend; bLatuda tablet 120 mg; cLatuda tablet 40 mg

Further, significant variation in Rf values of LURAH were found using reported HPTLC method, in view of the fact that 'neckless' effect was observed (Fig. 6); which may be due to higher proportion of toluene present in the mobile phase [15]. Therefore, the new reliable, specific, accurate, precise and robust TLC-densitometric method

was developed and successfully validated which is very useful for the detection and quantitative estimation of LURAH in formulations such as solution, nanoemulsion, mucoadhesive nanoemulsion, microemulsion as well as for equilibrium solubility and ex vivo diffusion studies.

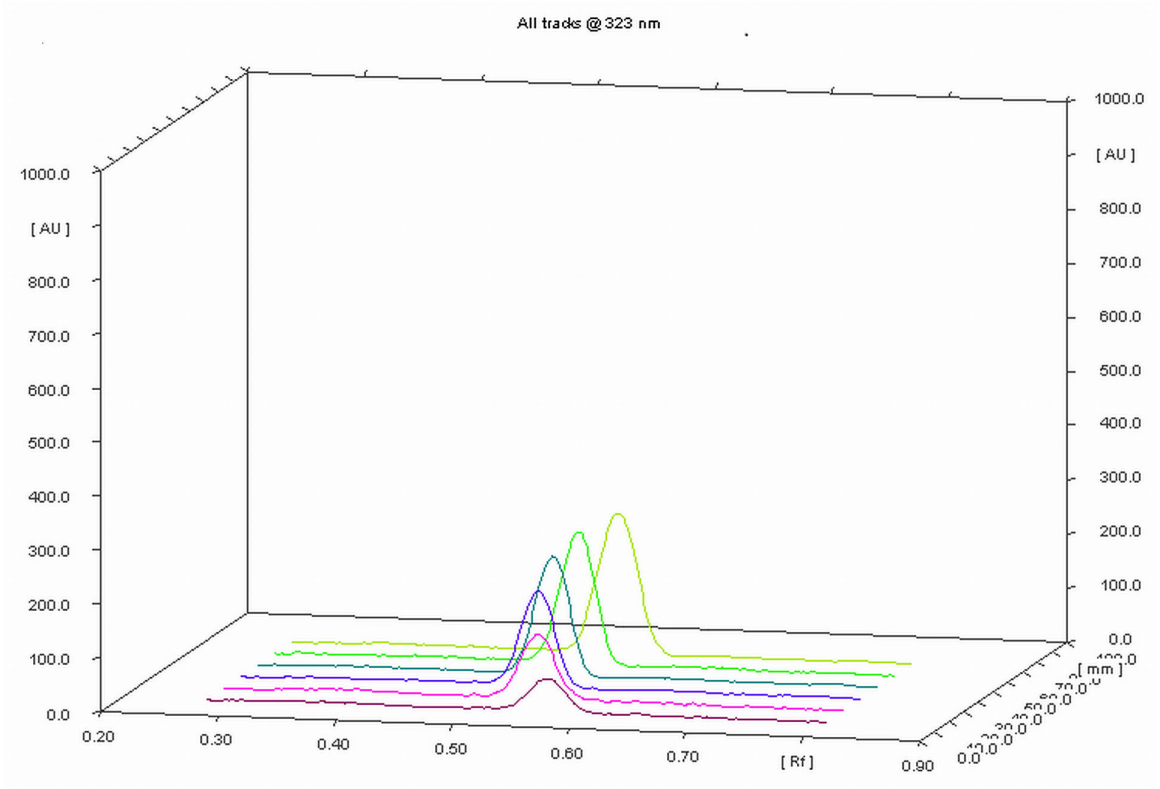

Figure 6 Three-dimensional overlay of HPTLC densitograms of calibration bands of Lurasidone hydrochloride using reported methodology [15].

4. Conclusion

A new TLC-densitometric method has been developed for quantification of LURAH. Low cost, fast speed, satisfactory precision, and accuracy are the main features of this method. The method was successfully validated in accordance with ICH guideline, and statistical analysis proved that the method is sensitive, specific, and repeatable. The results were statistically compared to those obtained by the reported HPLC and HPTLC methods, where no significant difference was found; indicating the ability of proposed TLC-densitometric method to be used conveniently for analysis LURAH as a bulk drug and in in-house developed formulations (solution, nanoemulsion and microemulsion; with or without mucoadhesive polymer) without any interference from excipients. The developed TLC-densitometric method was successfully applied for the estimation of the equilibrium solubility of LURAH in various excipients and exvivo diffusion study.

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