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Application of Stability Indicating HPTLC Method for Quantitative Determination of Escitalopram Oxalate in Pharmaceutical Dosage Form

Mahadeo V. Mahadik [a], Sunil R. Dhaneshwar [a], Mahesh J. Kulkarni [b]

[a] Department of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, India 411038.

[b] Centre for Material Characterisation, National Chemical Laboratory, Pune, Maharashtra, India 411008.

Abstract

A sensitive, selective, precise and stability indicating high-performance thin-layer chromatographic method of analysis of escitalopram oxalate both as a bulk drug and in formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene: acetone: ethanol: ammonia (5:1:1:0.2 v/v/v/v). This system was found to give compact spots for escitalopram oxalate (R_f value of 0.50±0.02). Escitalopram oxalate was subjected to acid and alkali hydrolysis, oxidation, dry heat treatment and photodegradation. Also the peaks of degraded products were well resolved from the pure drug with significantly different R_f values. Densitometric analysis of escitalopram oxalate was carried out in the absorbance mode at 239 nm. The linear regression analysis data for the calibration plots showed good linear relationship with concentration range of 200–1200 ng.spot^{-1}. The mean value of correlation coefficient, slope and intercept were 0.9987±0.236, 4.186 ±1.53 and 594.8 ±0.856, respectively. The method was validated for precision, robustness and recovery. The limits of detection and quantitation were 20 and 50 ng.spot^{-1}, respectively. Statistical analysis proves that the method is repeatable and selective for the estimation of the said drug. As the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one.

Keywords: Escitalopram oxalate; HPTLC; Stability indicating; Degradation

1. Introduction

Escitalopram is the *S*-enantiomer of racemic citalopram. It is chemically 1-(3-dimethyl-aminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile. The chemical structure

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of escitalopram oxalate is shown in Fig 1. It is highly selective serotonin re uptake inhibitor antidepressant, developed for the treatment of depression and anxiety disorders [1]. Preclinical studies have demonstrated that therapeutic activity of citalopram resides in escitalopram and the *R*-enantiomer is approximately 30-fold less potent than escitalopram [1]. Escitalopram exhibits linear pharmacokinetics and its half life in human is 27.0–32.0 h. It has a low potential for drug–drug interactions. Quantification of citalopram—the racemic form, has been performed in the past [2–10] using HPLC coupled with UV or fluorometric detection employing the two prevalent technique of sample pre-treatment i.e., liquid-liquid extraction and solid phase extraction.

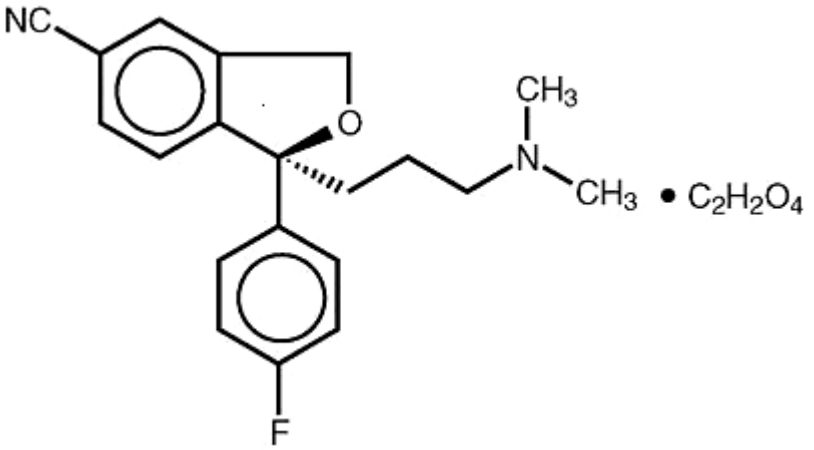

Fig. 1 Structure of Escitalopram Oxalate

To our knowledge, no article related to the stability indicating high-performance thin-layer chromatography (HPTLC) determination of escitalopram in pharmaceutical dosage forms has ever been mentioned in literature. The international conference on harmonization (ICH) guideline entitled *Stability Testing of New Drug Substances and Products* requires the testing to be carried out to elucidate the inherent stability characteristics of the active substance [11].

Now-a days HPTLC is becoming a routine analysis technique due to advantages of low operating cost, high sample throughput and need for minimum sample clean up. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis [12-14].

The aim of the present work is to develop an accurate, specific, repeatable and stability-indicating HPTLC method for the determination of escitalopram oxalate in presence of its degradation products and related impurities for assessment of purity of bulk drug and stability of its bulk dosage forms. The proposed method was validated as per ICH guidelines [15, 16] and its updated international convention [17].

2. Experimental

2.1. Materials

Mepro Pharmaceuticals Pvt. Ltd, Goa, India, kindly supplied pure drug sample of Escitalopram oxalate as a gift sample of Batch No.: EO-082105. It was used without further purification and certified to contain 99.65 % (w/w) on dried basis. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

2.2 Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60 F – 254, (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany, using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed by methanol and activated at 110^{o}C for 5 min prior to chromatography. A constant application rate of 0.1 µl/s was employed and space between two bands was 5 mm. The slit dimension was kept at 5mm × 0.45mm and 10 mm/s scanning speed was employed. The monochromator bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. The mobile phase consisted of toluene: acetone: ethanol: ammonia (5:1:1:0.2 v/v/v/v/v) and 15 mL of mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 ^{o}C ± 2) at relative humidity of 60% ± 5. The length of chromatogram run was 8 cm. Subsequent to the development; TLC plates were dried in current of air with the help of air dryer in wooden chamber with adequate ventilation. The flow rate in laboratory was maintained unidirectional (laminar flow, towards exhaust). Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 239 nm and operated by CATS software (V 3.15, Camag). The source of radiation utilized was deuterium lamp emitting continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of diffused light. Evaluation was via peak areas with linear regression.

2.3 Forced Degradation Studies

A stock solution containing 100 mg escitalopram oxalate in 100 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of proposed method. In all degradation studies the average peak area of escitalopram oxalate after application (1000 ng.spot^{-1}) of seven replicates was obtained. In order to study the degradation products of escitalopram oxalate using HPTLC

method most of the study was carried out by single development of TLC plate in order to prevent the movement of the non-polar degradates to extreme end of the plate.

2.3.1. Preparation of acid and base induced degradation product

Acid decomposition studies were performed by refluxing the solution of drug in 2M hydrochloric acid at 80°C for 24 h. The studies in alkaline conditions were carried out in 0.1M sodium hydroxide and the solution was refluxed for 2 h at 50°C. The resultant solutions were applied on TLC plate in such a way that final concentration achieved was 1000 ng.spot^{-1} for acid degradation product and 1200 ng.spot^{-1} for the base degradation product and the chromatograms were run as described in section 2.2.

2.3.2. Preparation of hydrogen peroxide induced degradation product

To study hydrogen peroxide induced degradation, initial studies were performed in 3% hydrogen peroxide at room temperature for 6 h. Subsequently drug was exposed to 30% hydrogen peroxide at room temperature for a period of 24 h and 72 h, and then heated in boiling water bath for 10 min to completely remove the excess of hydrogen peroxide. For HPTLC study resultant solutions were applied on TLC plate in such a way that final concentration achieved was 1000 ng.spot^{-1} and the chromatograms were run as described in section 2.2.

2.3.3. Dry heat and wet heat degradation product

The standard drug in solid form was placed in oven at 50°C for 30 days to study dry heat degradation and for wet heat degradation drug was kept in humidity chamber at 50°C, 75% relative humidity (RH) for 3 months.

2.3.4. Photochemical Degradation Product

The photochemical stability of the drug was studied by exposing the stock solution (1 mg.mL^{-1}) as well as solid drug to direct sunlight for 30 days on a wooden plank and kept on terrace. One microlitre of the solution (1000 ng.spot^{-1}) was applied on TLC plate and chromatograms were run as described in section 2.2.

2.3.5 Neutral Hydrolysis

To study degradation behavior of drug in neutral condition drug was dissolved in water and solution was refluxed at 80°C for 5 days and subsequently for 8 days.

2.4. Optimization of stability indicating HPTLC method

The HPTLC procedure was optimized with a view to develop stability indicating assay method. Both the pure drug and degraded drug solution were spotted on TLC plates and run in

different solvent systems. Initially, toluene and acetone were tried in different ratios. Toluene was used to impart the necessary polarity to escitalopram oxalate to get significantly good R_f value. Initially, toluene, acetone and ethanol in the ratio of 5:1:1 v/v/v was selected but it was found that spot was over run. Then volume of acetone was decreased and R_f was found to be 0.50, but compactness of spot was lacking, as a result considerable amount of peak tailing was observed. Therefore to reduce the tailing and improve compactness of the spot 0.2 mL ammonia solution was added. Ultimately the mobile phase consisting of toluene: acetone: ethanol: ammonia in the ratio of 5:1:1:0.2, v/v/v/v was optimized (Fig 2). In order to reduce the neckless effect TLC chamber was saturated for 20 min using saturation pads. The mobile phase was run upto distance of 8 cm; which takes approximately 20 min for development of TLC plate.

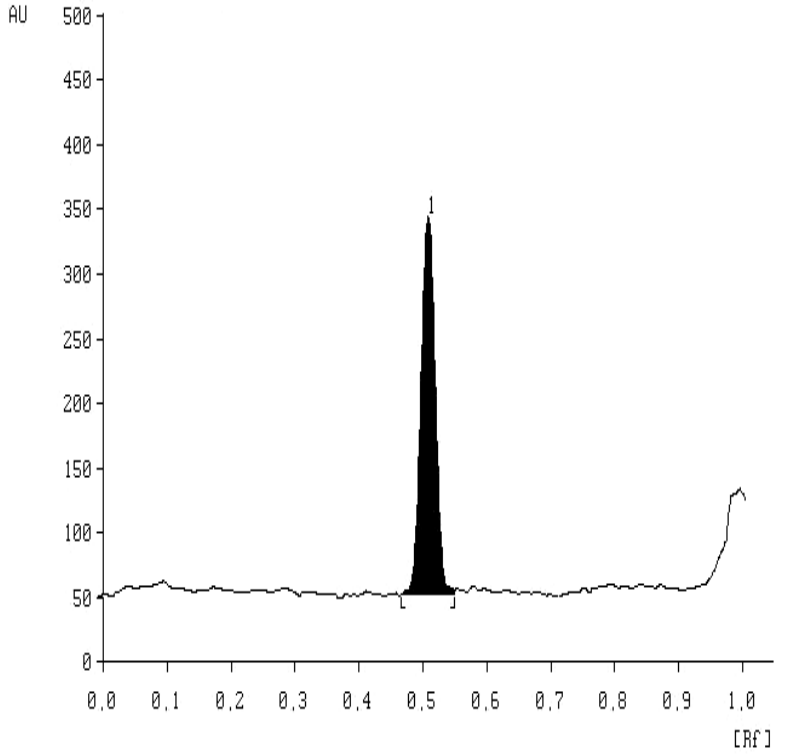

Fig. 2 Densitogram of standard escitalopram oxalate 1200 ng.spot^{-1}; Peak 1 (R_f : 0.50 ± 0.02), mobile phase toluene-acetone-ethanol-ammonia (5:1:1:0.02 v/v/v/v).

2.5. Validation of the method

Validation of optimized HPTLC method was done with respect to following parameters.

2.5.1. Linearity and range

The standard solutions of escitalopram oxalate were prepared to reach a concentration range of 200-1200 ng.µl^{-1}. One micro liter from each standard solution was spotted on the TLC plate to obtain final concentration 200-1200 ng.spot^{-1}. Each concentration was spotted six times

on the TLC plate. The plate was developed on previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.5.2. Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of three different concentrations (200, 600, 1000 ng.spot^{-1}) of the drug in hexaplicate on the same day. Intermediate precision of the method was checked by repeating studies on three different days. Additionally, the developed HPTLC method was checked through separation studies on the mixture of reaction solutions on a different chromatographic system on a different day.

2.5.3. Limit of detection and limit of quantitaiton

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted six times following the same method as explained in section 2.2. The signal to noise ratio was determined. An LOD was considered as 3:1 and LOQ as 10:1. The LOD and LOQ were experimentally verified by diluting known concentrations of standard solution of escitalopram oxalate until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

2.5.4. Robustness of the method

By introducing small changes in the mobile phase composition (± 0.1 ml for each component) the effects on the results were examined. Mobile phases having different composition like toluene: acetone: ethanol: ammonia (5.1:1.0:1.0:0.2,v/v/v/v), (5.0:1.1:1.0:0.2, v/v/v/v), (5:1.0:1.1:0.2,v/v/v/v), (5.0:1.0:1.0:0.3,v/v/v/v) and so on were tried and chromatograms were run. The amount of mobile phase was varied in the range of ±5%. The plates were prewashed by methanol and activated at 60°C for 2, 5, 7 min respectively prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied by ± 10 min. Robustness of the method was done at three different concentration levels 200, 600, 1000 ng.spot^{-1} for escitalopram oxalate.

2.5.5. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for escitalopram oxalate in sample was confirmed by comparing the R_f and spectra of the spot with that of standard. The peak purity of escitalopram oxalate was assessed by comparing the spectra at three different levels i.e. peak start (S), peak apex (M) and peak end (E) position of the spot.

2.5.6. Accuracy

Accuracy of the developed method was tested by fortifying a mixture of decomposed reaction solutions with three concentrations of drug corresponding to 80, 100 and 120% and determining the recovery of added drug. At each level of the amount six determinations were performed.

2.6. Analysis of marketed formulation

To determine the content of escitalopram oxalate in conventional tablets (Brand name: C-Pram, label claim: 10 mg escitalopram oxalate per tablet), the contents of twenty tablets were weighed, their mean weight determined and finely powdered. An equivalent weight of the drug was transferred into a 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to 100 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for drug content (1000 $\mu g.mL^{-1}$). One microlitre of the above filtered solution was diluted to produce 100 $\mu g.mL^{-1}$. Two microlitre of the above solution (200 $ng.spot^{-1}$) was applied on the TLC plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

2.7. Detection of the related impurities

The related impurities were determined by spotting higher concentrations of the drug so as to detect and quantify them. Escitalopram (2000 mg) was dissolved in 100 mL of methanol, and this solution was termed as sample solution (20 $mg.mL^{-1}$). 1 millilitre of the sample solution was diluted to 100 mL with methanol and this solution was termed as standard solution (0.2 $mg.mL^{-1}$). 1 microlitre of both the standard (200 $ng.spot^{-1}$) and the sample solution (20,000 $ng.spot^{-1}$) was applied on TLC plate and the chromatograms were run as described in Section 2.2.

3. Results and discussion

3.1. Stability indicating property

HPTLC studies of samples obtained on stress testing of escitalopram oxalate under different conditions using toluene: acetone: ethanol: ammonia (5:1:1:0.2, v/v/v/v) as the mobile solvent system suggested the following degradation behaviour.

3.1.1. Acid induced degradation product

The rate of degradation in acid was slower as compared to that of alkali. Initially 0.1M and 1M hydrochloric acid used at 80°C for 24 h but no degradation was observed hence the

strength of acid was increased, 10-20% degradation was observed by heating drug solution with 2M hydrochloric acid at 80°C for 24 h, whereas the densitogram of acid degradation product was observed at R_f 0.28 (Fig 3).

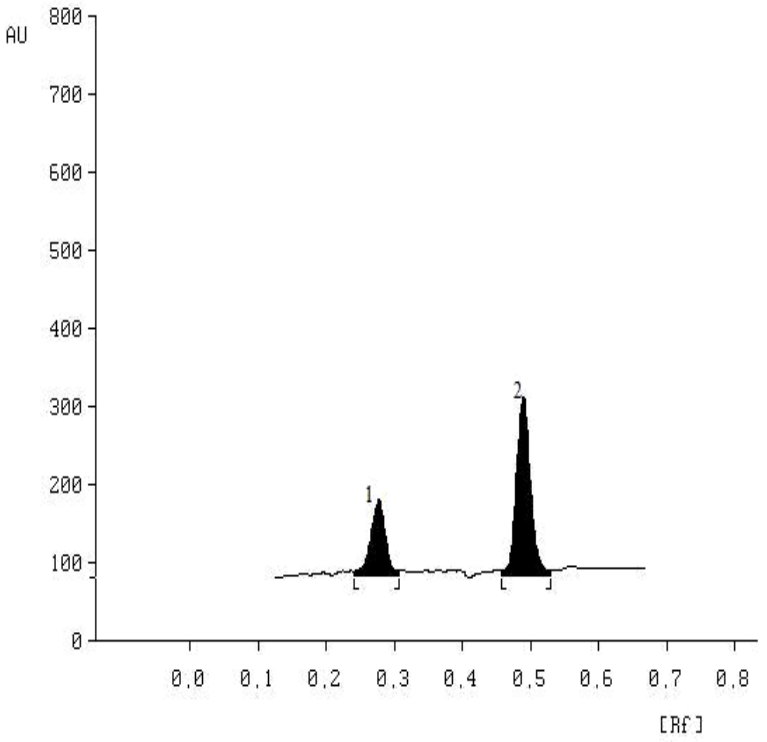

Fig.3 Densitogram of acid degradation product 1000 ng.spot $^{-1}$; Condition: 2 M HCl at 80°C for 24 h; Peak 1(degraded, R_f : 0.28), Peak 2 (escitalopram, R_f : 0.50).

3.1.2. Base induced degradation product

The drug was found to be highly labile to alkaline degradation. The reaction in 0.1M sodium hydroxide at 80°C was so fast that around 80% of the drug was degraded in 1 h with evolution of ammonia which was confirmed by smell. Subsequently, studies were performed by reducing the temperature to 50°C. Drug showed degradation around 20% within 2 h at 50°C associated with rise in a major degradation product. Whereas in densitogram of base degradation, it was observed at R_f 0.25 (Fig 4). Complete degradation of the drug was observed in 12 h when refluxed with 0.1M sodium hydroxide at 50°C.

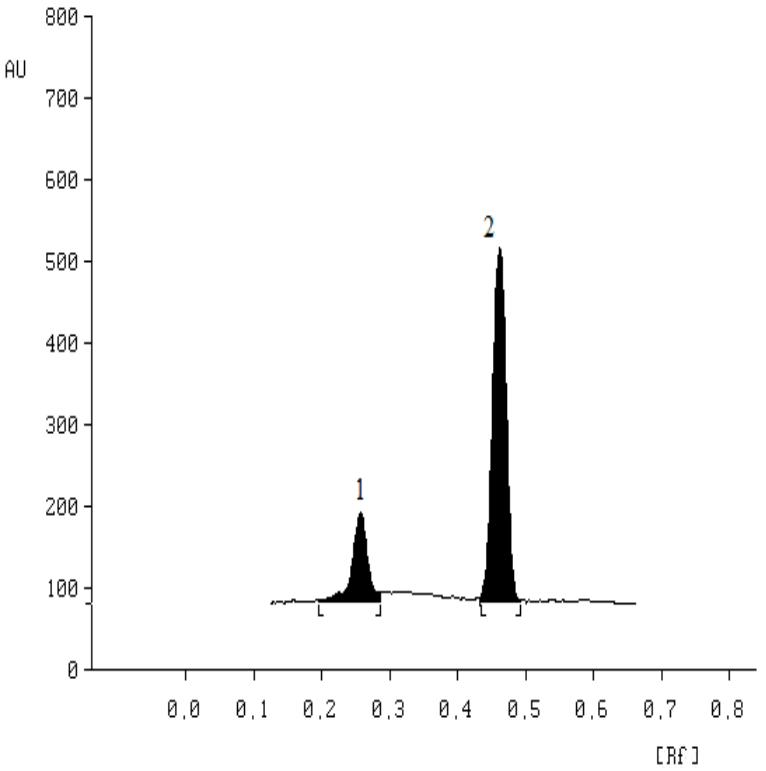

Fig.4 Densitogram of base treated escitalopram 1200 ng.spot $^{-1}$; condition: 0.1 M NaOH 50°C for 2 h; Peak 1(degraded, R_f : 0.25), Peak 2 (escitalopram, R_f : 0.48).

3.1.3. Hydrogen peroxide induced degradation product

In 3% hydrogen peroxide for 6 h drug was found to be very stable at room temperature showing no degradation. Hence drug was exposed to 30% hydrogen peroxide degradation for 72 h at room temperature. In densitogram peak for degradation product was observed at R_f 0.18 (Fig 5).

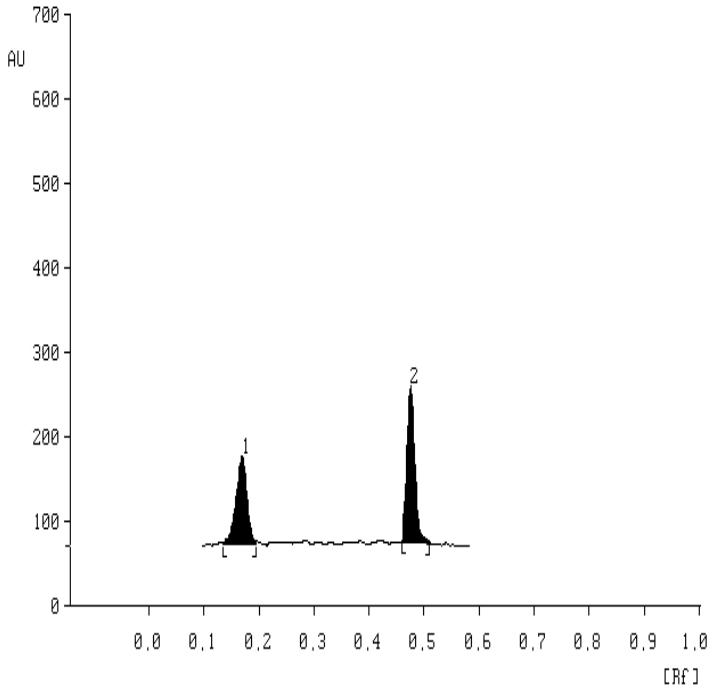

Fig.5 Densitogram of H_2O_2 treated escitalopram, condition: 30% H_2O_2 at room temperature for 72 h; Peak 1(degraded, R_f : 0.18), Peak 2 (escitalopram, R_f : 0.50).

3.1.4. Neutral hydrolysis product

When drug was exposed to neutral hydrolysis at 80°C for 5 days no significant degradation was observed, then time of exposure was increased. Ten percent degradation was observed after 8 days with the generation of three peaks at R_f 0.28, 0.36 and 0.42 (Fig 6).

3.1.5. Photochemical degradation product

Escitalopram was found to be stable to photochemical degradation as negligible (less than 2%) degradation was seen after exposing drug to sunlight for 30 days.

3.1.6. Dry and wet heat degradation product

Drug was also found to be stable to these conditions showing negligible degradation when subjected for dry and wet heat degradation.

3.2. Validation of the Stability Indicating Method

The results of validation studies on the stability indicating method developed for escitalopram oxalate in the current study involving toluene: acetone: ethanol: ammonia (5:1:1:0.2, v/v/v/v) as the mobile phase for HPTLC are given below.

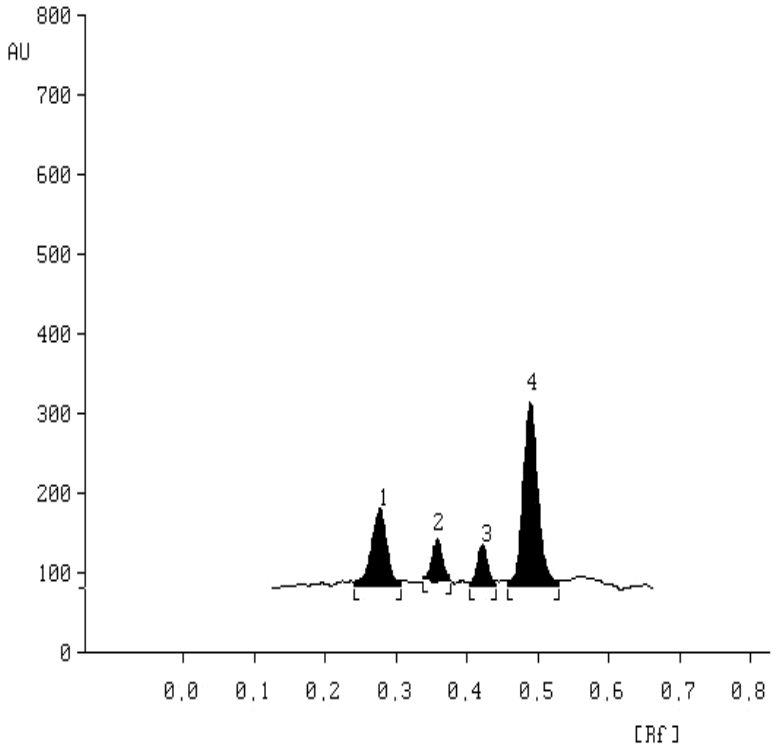

Fig.6 Densitogram of neutral hydrolysed escitalopram; Peak 1, 2, 3(degraded, R_f :0.18, 0.27, 0.43), Peak 4(escitalopram, R_f :0.50).

3.2.1. Linearity

The response for the drug was linear (r^2= 0.9987) in the concentration range between 200-1200 ng.spot^{-1}. The mean (±RSD) values of slope, intercept and correlation coefficient were 4.186 (±1.53) and 594.8 (±0.856) and 0.9987 (±0.236), respectively.

3.2.2. Precision

The results of the repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guideline. Separation of the drug and different degradation products in stressed samples was found to be similar when analyses were performed on different chromatographic system on different days.

3.2.3. LOD and LOQ

The signal: noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. The LOD and LOQ were found to be 20 ng.spot^{-1} and 50 ng.spot^{-1} respectively.

Table 1. Precision studies

concentration ($ng.spot^{-1}$)	Measured concentration ± S.D. ($ng.spot^{-1}$), RSD (%)	
	Repeatability (n= 6)	Intermediate precision (n= 6)
200	197.21 ± 1.45, 0.736	198.39 ± 0.76, 0.38
600	598.86 ± 1.3, 0.217	599.5 ± 1.5, 0.25
1000	997.4 ± 0.81, 0.081	998.315 ± 1.8, 0.180

3.2.4. Robustness of the method

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2%. The low values of % RSD as shown in Table 2. indicated robustness of the method.

Table 2. Robustness testing (n = 6)

Parameter	SD of peak area	% RSD
Mobile phase composition (± 0.1 ml)	2.64	0.192
Amount of mobile phase (± 5%)	3.5	0.25
Time from spotting to chromatography (± 10 min.)	1.52	0.102
Time from chromatography to scanning (± 10 min.)	1.36	0.098

3.2.5. Specificity

The peak purity of escitalopram was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9994 and r (M,E) =0.999. Good correlation (r=0.9996) was also obtained between standard and sample spectra of escitalopram.

3.2.6. Recovery Studies

As shown from the data in Table 3. good recoveries of the drug in the range from 98.5 to 99.1% were made at various added concentrations, despite the fact that the drug was fortified to a mixture that contained drug as well as degradation product formed at various reaction conditions.

Table 3. Recovery studies (n = 6)

Actual concentration (ng.spot^{-1})	Measured concentration (ng.spot^{-1}) ± S.D, RSD (%)	Recovery (%)
800	788.6 ± 0.457, 0.057	98.5
1000	985.86 ± 1.025, 0.1039	98.5
1200	1189.2 ± 1.211, 0.102	99.1

A summary of validation parameters is given in Table 4.

Table 4. Summary of validation parameters

Parameter	Data
Linearity range	100-1000 ng.spot^{-1}
Correlation coefficient	0.9987
Limit of detection	20 ng.spot^{-1}
Limit of quantitation	50 ng.spot^{-1}
% Recovery (n=6)	98.72 ± 0.32
Precision (%RSD)	
Repeatability (n=6)	0.344
Inter day (n=6)	0.27
Robustness	Robust
Specificity	Specific

3.3. Analysis of marketed formulation

Experimental results of the amount of escitalopram in tablets, expressed as percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any excipients, which are normally present in tablets. The drug content was found to be 97.5% ± 0.56. Two different lots of commercially available escitalopram oxalate tablet were analyzed using the proposed procedures and the results are summarized in Table 5.

3.4. Detection of the related impurities

The spots other than the principal spot (escitalopram) from the sample solution (Peak area = 86610.94) were not intense than principal spot from the standard solution. The sample solution showed one additional spot at R_f 0.54 (Fig 7). However the peak area of the additional

spot (854.55) was found to be much less as compared to the peak area of the principal spot (3546.34) from the standard solution. The λ_{max} of impurity was found to be 235 nm (Fig 8).

Table 5. Analysis of commercial formulation

Commercial formulation C-PRAM S (10 mg)	escitalopram oxalate found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	9.75 ± 0.569	97.5
2nd Lot	9.74 ± 0.247	97.4

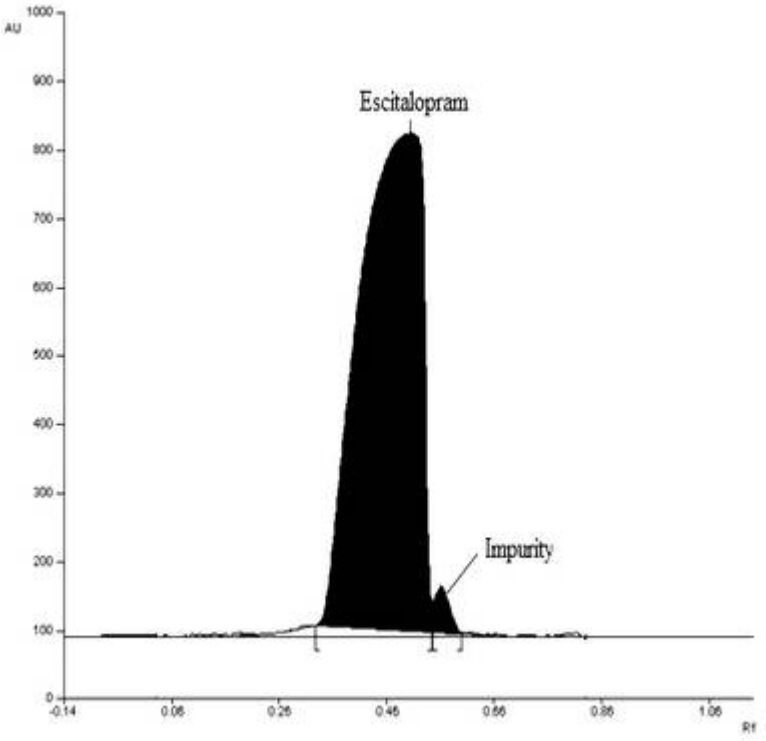

Fig. 7 Densitogram of escitalopram and its related impurity

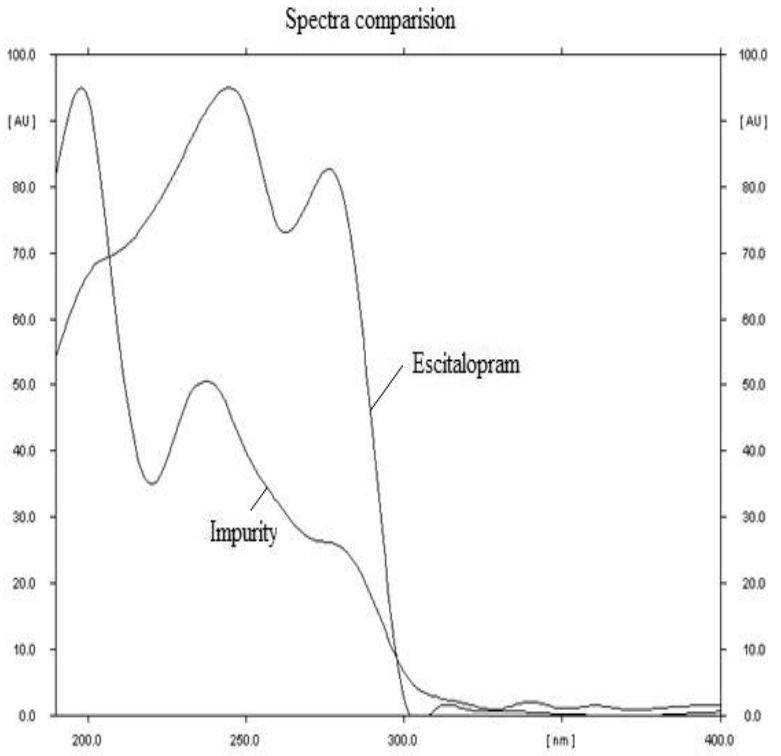

Fig.8 In situ overlain spectrum of escitalopram (λ_{max} = 239 nm) and its unknown related impurity (λ_{max} = 235 nm) measured from 200 to 400 nm

4. Conclusion

Introducing HPTLC in pharmaceutical analysis represents a major step in terms of quality assurance. The developed HPTLC technique is precise, specific, accurate and stability indicating. Statistical analysis proves that the method is suitable for the analysis of escitalopram as bulk drug and in pharmaceutical formulation without any interference from the excipient. This study is a typical example of stability indicating assay, established following the recommendations of ICH guidelines. The method can be used to determine the purity of drug available from various sources by detecting the related impurities. It is proposed for the analysis of drug and degradation products in stability samples in industry.

Acknowledgement

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Corresponding author:
Dr. S. R. Dhaneshwar, Professor,
Department of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, India 411038.
Phone: +912025437237, **Fax:** +912025439383,

E-mail: sunil.dhaneshwar@gmail.com