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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE ESTIMATION OF ESCITALOPRAM OXALATE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new, simple high performance thin layer chromatographic method has been proposed for the determination of escitalopram oxalate in a tablet dosage form. Quantitative analysis was performed by densitometric scanning at 240 nm. The method was validated for linearity, accuracy, precision and robustness. The calibration plot was linear over the range 100-600 ng/band for escitalopram oxalate. The method was successfully applied to the analysis of drug in bulk and pharmaceutical dosage form.

KEYWORDS: ***High-performance thin-layer chromatography, escitalopram oxalate, tablets.***

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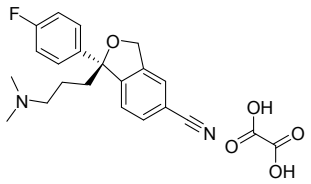
INTRODUCTION

Escitalopram is the pure S-enantiomer of the racemic bicyclic phthalene derivative, citalopram. Escitalopram is designated as S-(+)-1-{3-(dimethyl-amino) propyl}-1-) (p-fluoro phenyl)-5-phthalancarbonitrile.[1] Escitalopram was launched and marketed worldwide with success as oxalic acid salt. Escitaopram oxalate is an orally administered selective serotonin reuptake inhibitor with a molecularformula$C_{20}H_{21}FN_2O.C_2H_2O_4$.



Escitalopram oxalate

Literature survey revealed that various methods have been reported for determination of escitalopram in human plasma and its applications in bioequivalence study,[2] flourimetry quantitation plasma,[3] validation of capillary electrophoresis for simultaneous determination of impurities,[4] chiral LC method for separation of enantiomers,[5] simultaneous determinations of escitalopram and clonazepam in combined dosage form by UV, HPLC[6] and HPTLC methods.[7, 8] However the author felt it worthwhile to propose an alternative HPTLC method for determination of escitalopram oxalate in single dosage form for routine analysis. The aim of present study was to develop a simple, versatile, accurate, precise, and sensitive HPTLC method for determination of escitalopram oxalate in the pharmaceutical dosage form. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines.[9]

EXPERIMENTAL

Reagents and Chemicals

Methanol, acetic acid, acetone, toluene, ethyl acetate, hexane, ammonia and butanol (all are of analytical reagent grade) were obtained from Sisco Research Laboratories, Mumbai (India). Standard bulk drug sample of escitalopram oxalate (99.45% pure) was obtained as a gift sample from SMS Pharmaceuticals, Hyderabad (India). The pharmaceutical dosage forms used in this study were Nexito and Lexapro tablets with a declared content of 10 mg escitalopram oxalate.

Preparation of Standard Stock Solution

A standard stock solution of escitalopram oxalate was prepared by dissolving 10 mg drug in 100mL methanol in order to set a concentration of 0.1g L^{-1}.

Instrumentation and Chromatographic Conditions

Chromatography was performed on 10 cm x 10 cm aluminum plates precoated with 250-µm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). The plates were prewashed with methanol and activated at 110 ^oC for 5 min. Samples were applied to the plates as bands of 6 mm wide and 10mm apart by means of a Camag (Switzerland) Linomat V sample applicator equipped with a 100µL syringe (Hamilton, Bonaduz, Switzerland). Linear ascending development was performed in a 10 cm x 10 cm twin trough glass chamber (Camag), with butanol-acetic acid-water 3:1:1(v/v) as mobile phase, after saturation of the chamber with mobile phase vapour for 10 min. The distance traversed for development being 8.5 cm with a development time of 60 min approximately. After chromatography the plates were dried in a current of air by use of an air

drier. Densitometric scanning was performed with a Camag TLC Scanner 3 at 240 nm for all measurements with the use of UV detector. The scanner was operated by Wincats software Version 1.4.3 The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. The slit dimensions were 5 mm x 0.45 mm and the scanning speed was 20 mms^{-1}.

Selection of Wavelength

After chromatographic development, bands were scanned over the range 200–400 nm (spectrum scan speed 20 nm s^{-1}) and the spectra were recorded and thus inferred that the estimations can be done at 240nm (Fig.1).The standard stock solution of escitalopram oxalate (0.1g L^{-1}) was applied on top of each other on a TLC plate, in the range 1-6µL, by use of the Linomat V sample applicator and 100µL syringe. The plate was developed and scanned under the conditions described above. Each amount was analyzed five times and peak areas were recorded. Calibration plot of peak area against respective amount was established for escitalopram oxalate.

Analysis of Tablet Formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg escitalopram oxalate was weighed and transferred to a 100-mL volumetric flask containing approximately 50mL methanol. The mixture was ultra sonicated for 5 min then diluted to volume with methanol.The solution was filtered using Whatmann filter paper 41 and 1-6µL of the filtrate was applied to a TLC plate. After chromatographic development the peak area of the band was measured at 240 nm and the amount of each drug in each tablet was determined from the respective calibration plot. The analytical procedure was repeated six times for the homogenous powder sample.

VALIDATION

Limit of Detection and Limit of Quantitation

The limit of detection and limit of quantification for escitalopram oxalate was calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve for escitalopram oxalate . The limit of detection of a compound is defined as the lowest concentration that can be detected. The limit of quantification is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy.

Precision

To study intra-day and inter-day variation, three different concentrations of sample solutions were prepared and applied to the TLC plates. All the solutions were analyzed in triplicate on the same day and on three different days to record intra-day and inter-day variations in the results respectively.

Recovery Studies

To check the accuracy of the method, recovery was measured by the addition of standard drug solution at three different levels (80, 100, and 120%) to pre-analyzed sample solution (200ng $band^{-1}$ for escitalopram oxalate, so that after standard addition samples would be in the linear range).

Robustness Studies

The effect of small, deliberate variation of the analytical conditions on the peak areas of the drugs was examined. The robustness of the method was checked for 100 and 600 ng $band^{-1}$ for escitalopram oxalate.

RESULTS AND DISCUSSION

Different mobile phases containing butanol, toluene, methanol, acetic acid, hexane, acetone, ammonia, ethyl acetate in different proportions were examined. Butanol-acetic acid-water 3:1:1 (v/v) was found to be most suitable for the studies. The R_F value of escitalopram oxalate was 0.61 ± 0.01. The densitogram obtained from a standard solution of escitalopram oxalate is shown in Fig. 2.The calibration plot was found to be linear over the

range 100–600 ng band^{-1} for escitalopram oxalate, with correlation coefficient of 0.9993 ± 0.0001 (Fig.3). The LOD and LOQ were 22.93 and 76.44 ng band^{-1}, respectively, for escitalopram oxalate. Intra-day variation was found to be 0.29 as RSD (%), and Interday variation was found to be 1.35 as RSD (%), for escitalopram oxalate, which indicates that the method is precise. The results are incorporated in Table 1. To study the accuracy of the method, recovery was determined for escitalopram oxalate. Recovery ranged from 99.58 to 101.02%, with RSD ranging from 0.14 to 0.51% (Table 2). Study of the robustness of the method revealed that the peak areas were unaffected (RSD < 2%) by small changes of the operating conditions. Hence the method is more robust. The method was also evaluated by assay of commercially available tablets containing escitalopram oxalate. Six replicate analyses were performed on accurately weighed amount of the tablets. The densitogram obtained from sample solution of escitalopram oxalate is shown in Fig 4. The assay (%) was found to be 99.57 ± 0.65 for escitalopram oxalate (Table 3). The HPTLC method appears to be more sensitive (detection limit, ng band^{-1}) than UV spectrophotometric method. Three dimensional representation of the HPTLC chromatogram has been depicted in Fig 5 showing the absorbance, R_F values and the distance traversed by the solutions at different concentrations taken for these studies in order to have better presentation of the results obtained.

Table 1
Intra and Inter – day precision by HPTLC Method

Amount (ng/spot)	Intra day precision		Inter day precision	
	Mean area* ± SD	%RSD	Mean area* ± SD	%RSD
100	863.71±3.892	0.45	778.07±4.132	0.97
300	2324.4±5.537	0.23	2147.9±7.612	1.92
500	3669.5±7.630	0.20	3555.7±9.126	1.16

Mean value of three determinations

Table 2
Results from recovery studies of Escitalopram oxalate

Sample	Excess of drug added to the analyte (%)	Theoretical content (ng)	% of recovery*	% of RSD
Escitalopram oxalate	50	300	99.54	0.21
	100	400	99.82	0.35
	150	500	101.1	0.61

**** Average of three determinations***

Table 3.
Mean (±SD) amount of Escitalopram oxalate in tablet dosage forms by proposed HPTLC method

Formulations	Labeled amount	Amount found*	%Purity*
Lexapro	10mg	9.957mg	99.57 ± 0.65
Nexito	10mg	9.948mg	99.48 ± 0.45

**Mean value of six determinations*

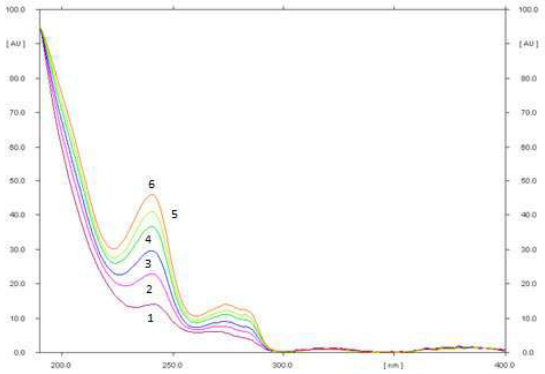


Figure 1.
Absorption spectra of escitalopram oxalate (100 to 600 ng/ band) scanned in the range 200 to 400 nm, recorded in situ on the plate

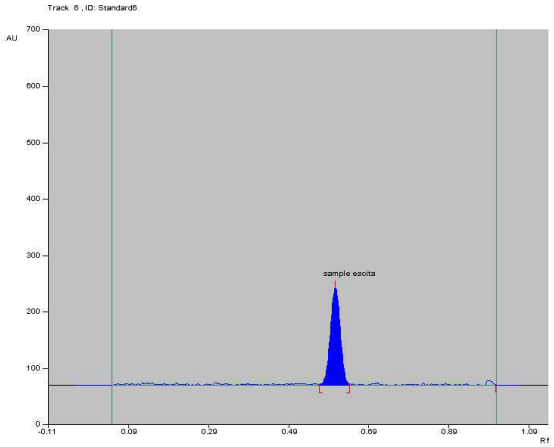


Figure. 2.
Densitogram obtained from a standard solution of escitalopram oxalate (600ng band^{-1}; RF = 0.61 ± 0.01)

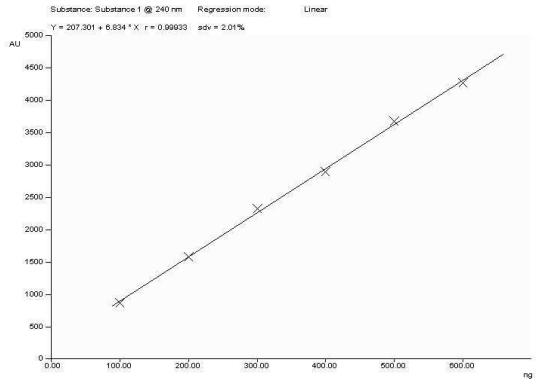


Figure 3.
Linearity plot of escitalopram oxalate

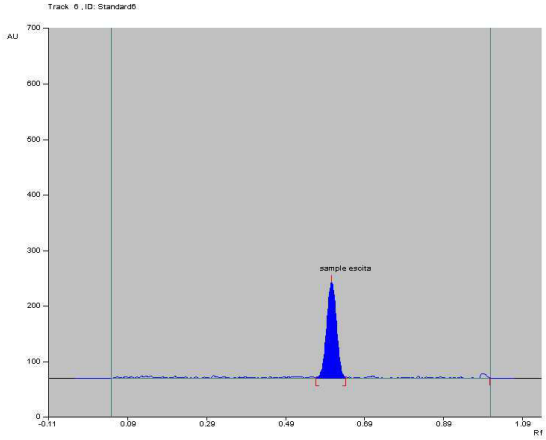


Figure. 4.
Densitogram obtained from a sample solution of escitalopram oxalate (600ng band^{-1}; RF = 0.61 ± 0.01)

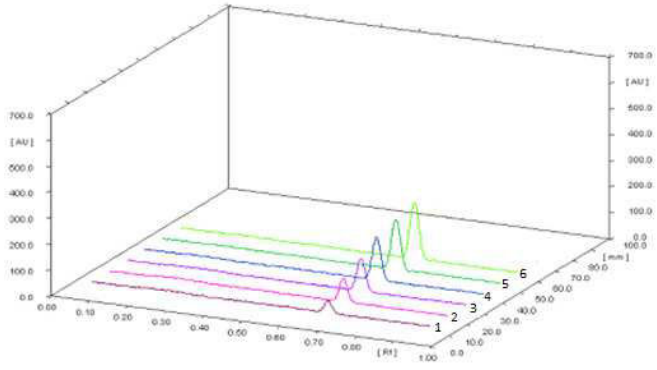


Figure. 5.
Chromatogram (three dimensional) showing standard peaks (100-600) ng/spot of escitalopram oxalate

CONCLUSION

The validated HPTLC method proposed for determination of escitalopram oxalate in bulk and pharmaceutical dosage form was simple, rapid, accurate, precise, sensitive, and robust and can thus be used for routine analysis of escitalopram oxalate in a tablet dosage form.

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