ISSN 0975-6299 Vol 2/Issue 1/Jan-Feb 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PHARMACEUTICAL ANALYSIS

DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHIC METHOD FOR ESTIMATION OF ESCITALOPRAM OXALATE IN TABLET DOSAGE FORMS

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ABSTRACT

A simple, specific, accurate and precise RP-HPLC method was developed and validated for the determination of escitalopram oxalate in tablet dosage forms. A hypersil BDS C8, 5µ column having 250x4.6mm internal diameter in isocratic mode with mobile phase containing methanol: disodium hydrogen phosphate: acetonitrile (28:44:28v/v, pH 7.0±0.05) was used. The flow rate was 1.5ml/min and effluents were monitored at 226nm. The retention time of escitalopram oxalate was 8.45 min. The linearity range is 250-1500µg/ml with coefficient of correlation 0.9999. The method was validated in terms of accuracy, precision, repeatability. The percentage recovery for escitalopram oxalate was found to be 99.0%. The proposed method was successfully applied for quantitative determination of escitalopram oxalate in single dosage form for routine analysis.

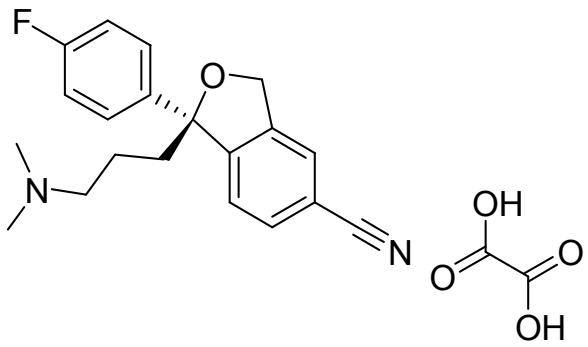
ISSN 0975-6299 Vol 2/Issue 1/Jan-Feb 2011

KEY WORDS

Escitalopram oxalate; anti-depressant; HPLC; validation.

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 molecular formula $C_{20}H_{21}FN_2O.\ C_2H_2O_4$.



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 authors felt it worthwhile to propose an alternative RP-HPLC method for the determination of escitalopram oxalate in single dosage form for routine analysis.

MATERIALS AND METHODS

Instrument:

The liquid chromatographic system consists of following components; WATERS liquid chromatograph equipped with a isocratic pump (WATERS 501), UV detector and rhoedyne injector (7125) with 20µl loop. The system was connected with help of millennium 32 software in a computer system for data collection and processing. The analytical column used was hypersil BDS C8 (250X4.6mm).

Chemicals and Reagents:

Standard escitalopram oxalate (purity 99%) was procured as a gift sample from SIBRA pharmaceuticals, Hyderabad. Analytical reagent grade methanol (E.Merck, INDIA) and disodium hydrogen phosphate (Qualigens) were used. LC grade water was obtained by double distillation and purification through milli-

Q water purification system. Pharmaceutical tablet formulations LEXAPRO (USA), NEXITO (INDIA) were obtained commercially from market.

Mobile phase preparation:

3.7gms of Na_2HPO_4 were dissolved in 800ml water the pH was adjusted to 7.0±0.05 with dilute H_3PO_4. The volume is made up to 1000ml proper care has been taken by filtering through 0.45µ membrane filter before use.

Chromatographic conditions:

Column	:	C8, 250x4.6mm
Flow rate	:	1.5ml/min
Detection	:	226nm
Injection volume	:	20mcL
Column temperature	:	Ambient
Mobile phase	:	Methanol: disodium hydrogen phosphate: acetonitrile (28:44:28v/v)

Standard stock solution:

About 250 mg of escitalopram oxalate was accurately weighed and transferred to 25 ml volumetric flask and it was dissolved in sufficient quantity of buffer. The solution was sonicated for 10 min and made up to volume with the same diluent (stock-1) contains 10 mg/ml of escitalopram oxalate.

Analysis of formulations:

Twenty tablets of escitalopram oxalate were weighed accurately and finely powdered. The powder equivalent to 100mg of escitalopram oxalate was taken in a 100ml standard volumetric flask containing mobile phase and kept for sonication for 10 min and made up to the mark with mobile phase and filtered through 0.45µ membrane filter

METHOD OF VALIDATION

As per ICH guidelines and USP the method is validated and following parameters were evaluated [9-11].

Linearity:

Different aliquots of standard escitalopram oxalate stock solution was taken into series of 10ml standard volumetric flasks and diluted up to the mark with mobile phase such that final concentration of escitalopram in the range of 250-1500µg/ml. Under optimized chromatographic conditions a steady base line was recorded. After stabilization for 10 min all the solutions were injected separately and the chromatograms were recorded.

Accuracy:

Accuracy of the method was demonstrated at three different concentration levels (80-120%) of specification limit in triplicate as per ICH guide lines. The recovery studies were carried out by spiking a known quantity of escitalopram oxalate into a previously analyzed sample (1mg/ml).

Precision:

Intraday and inter day precision was determined by analyzing sample of escitalopram oxalate at a concentration of 1.0mg/ml. Repeatability of the method was demonstrated by analyzing escitalopram oxalate sample for a minimum six determinations. Intermediate precision as demonstrated by analyzing the same sample of escitalopram oxalate at three different time periods on the same day and on three different days over a period of one week.

Limit of detection (LOD) and limit of quantification (LOQ):

The limit of detection and limit of quantification for escitalopram oxalate was calculated from the linearity data using relative

standard deviation of the response and slope of the calibration curve for escitalopram oxalate. LOD value was found to be 0.023mg/ml for escitalopram oxalate and LOQ value was found to be 0.072mg/ml for escitalopram oxalate.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by ±0.05 ml/min, change in pH of the buffer ±0.1 unit and change in the ratio of mobile phase (±2% absolute). The method was demonstrated to be robust over an acceptable working range of HPLC operational parameters.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by liquid chromatography has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The objective of this study was to develop a rapid, sensitive, highly accurate and precise HPLC method for the analysis of escitalopram oxalate in its tablet formulations using C_8 column with UV detection.

The retention time of escitalopram oxalate was found to be 8.45min (Fig.1). The repeatability of retention time was found to be excellent for escitalopram oxalate. The method was linear in the concentration range from 0.25 to 1.5 mg/ml of escitalopram oxalate. A good linear relationship (r = 0.9999) was observed from the linearity curve (Fig.2). The calculated LOD and LOQ values were found to be 0.023 and 0.072 mg/ml.

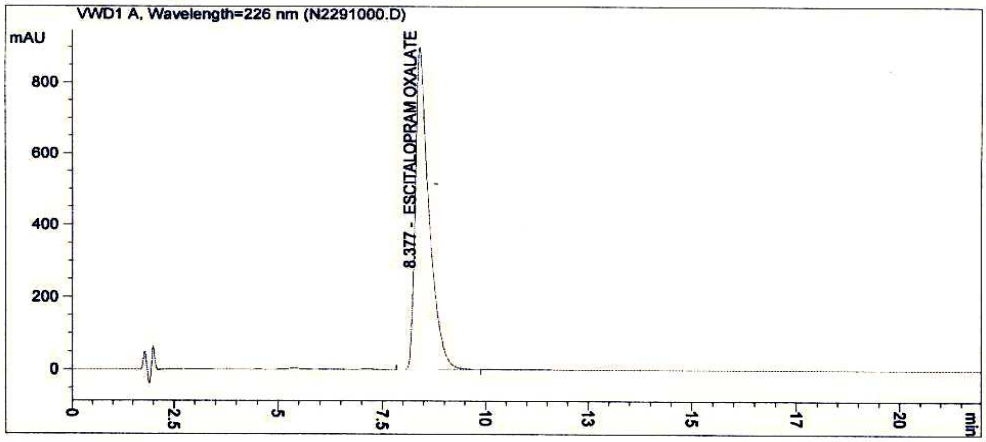


Fig 1.0

Typical chromatogram of pure escitalopram oxalate (1mg/ml)

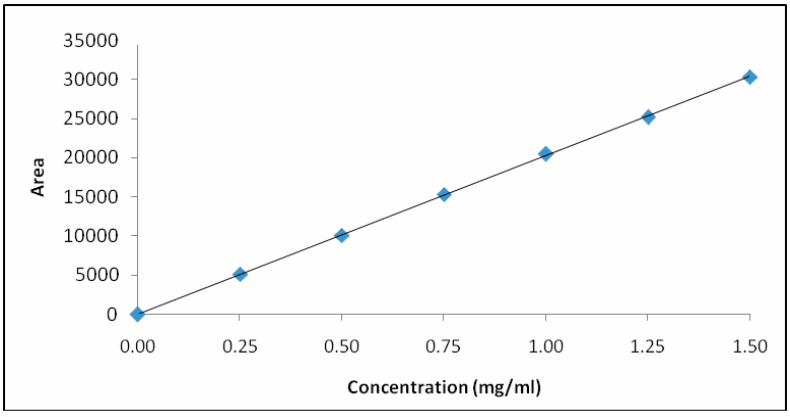


Fig -2.0
Linearity plot of escitalopram oxalate (RP-HPLC)

The proposed HPLC method has been used to quantify escitalopram oxalate in tablet dosage forms (Fig 3). The average drug content was found to be 99.8% of the labelled amount. Results are presented in Table.1.The mean absolute recovery of escitalopram oxalate was found to be more than 99.05% indicating that the proposed method is highly accurate (Table 2). The proposed HPLC method was also validated for intra and inter-day variations. The percentage coefficient of variance in the peak area was found to be less than 0.33% and 0.14% respectively. The results are presented in Table.3. The results showed that the proposed HPLC method is highly reproducible. Study of the robustness of the method revealed that peak areas were unaffected (RSD < 2%) by small changes of the operating conditions. The system suitability studies were carried out to determine the theoretical plates, tailing factor, HETP etc. The results are given in Table.4. The proposed method was successfully used for the analysis of two different brands of pharmaceutical dosage forms and the results were found to be quite encouraging.

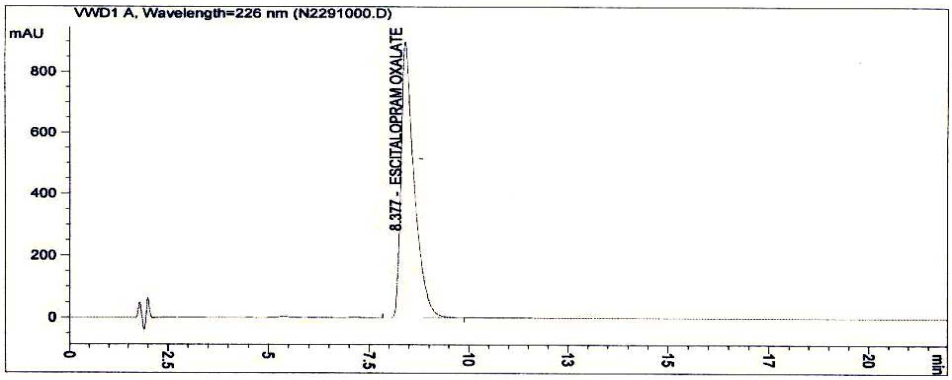


Fig 3.0
Sample chromatogram of escitalopram oxalate (1mg/ml)

Table – 1.0
Mean (±SD) amount of escitalopram oxalate in tablet dosage forms by proposed HPLC method

Formulations	Labelled amount	Amount found*(mg)	%Purity*
Lexapro	10mg	9.98± 0.06	99.8 ± 0.55
Nexito	10mg	9.94± 0.03	99.4 ± 0.24

*+*Average of six determinations*

Table- 2.0
Recovery of escitalopram oxalate using the proposed HPLC method

S. No	Amount of drug added (mg)	Mean(±SD)*amount found (mg)	Mean(±SD)* % of recovery
1	0.8	0.790 ± 0.03	98.80
2	1.0	0.985 ± 0.05	98.50
3	1.2	1.189 ± 0.24	99.09

** Average of three determinations*

Table- 3.0
Intra and Inter day precision for escitalopram oxalate assay in pharmaceutical dosage forms by the proposed HPLC method

S. No	Concentration (mcg/ml)	Injection Volume (µL)	Area*	Mean	%RSD
1.	Intraday	20	20484.79		
	1000		20553.12	20552.65	0.329
			20620.04		
2.	Inter day	20	20623.64		
	1000		20642.10	20616.96	0.140
			20585.16		

** Average value of six determinations*

Table 4.0
Performance calculations, detection characteristics and precision of the HPLC method for escitalopram oxalate

Parameter	Observation

Retention time (t)	8.45 ± 0.05 (min)
Theoretical plates (n)	3751
Plates per meter (N)	15004
Hight equivalent to theoretical plates (HETP)	0.66 x 10^{-4}
Peak asymmetry (T)	0.40
Linearity range (mg/ml)	0.5 – 1.5
Detection limit (mg/ml)	0.023
Quantitation limit (mg/ml)	0.072
Regression equation (Y = a+bx)	
Slope (b)	20228.91
Intercept (a)	41.64
Correlation Coefficient	0.9999
Relative standard deviation (%)	0.512

CONCLUSION

The validated HPLC method employed here proved to be simple, accurate, sensitive precise and robust and can be successfully applied for routine quality control analysis of escitalopram oxalate in tablet dosage forms.

ACKNOWLEDGEMENTS

We are grateful to Sibra Pharmaceuticals, Hyderabad (India), for providing a gift sample of drug for research work. One of us (A.S) is thankful to Management, Hindu College of Pharmacy, Guntur for providing the necessary facilities and constant encouragement.

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