RESEARCH ARTICLE Am. J. PharmTech Res. 2012; 2(3) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

RP-HPLC Method for Simultaneous Estimation of Escitalopram oxalate and Etizolam in Bulk and Tablet Dosage Form

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ABSTRACT

Present work describes a selective, precise and accurate Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for simultaneous estimation of Escitalopram Oxalate (ESC) and Etizolam (ETI) on Kromasil 100 C18, 5µ(150×4.6 mm) Column using Acetonitrile:0.005 M Hexane Sulfonic Acid pH 3.0 (adjusted with o-phosphoric acid) (40:60 v/v) as mobile phase, at a flow rate of 1.0 ml/min and the detection wavelength was 254 nm. The retention time for ESC and ETI was found to be 3.66 and 8.07 min, respectively. The ion-pairing reagent improved the retention of polar ESC on Reverse-phase column. The method was validated for linearity, precision, accuracy, LOD, LOQ robustness, solution stability and specificity. The method was linear in the concentration range of 20-160µg/ml for ESC and 2-16 µg/ml for ETI with a correlation coefficient of 0.9994 and 0.9993 for respective drugs. The percent recovery was found in the range of 98.14-101.72% and 98.83-101.12 % for ESC and ETI, respectively. The specificity of method was established based on peak purity data. The proposed method was successfully applied for quantitative determination of escitalopram oxalate and Etizolam in combined tablet dosage form for routine analysis.

Keywords: RP-HPLC, Simultaneous, Escitalopram Oxalate, Etizolam, Hexane Sulfonic Acid

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Received 30 April 2012, Accepted 15 May 2012

Please cite this article in press as: Patel VB *et al.*, RP-HPLC Method for Simultaneous Estimation of Escitalopram oxalate and Etizolam in Bulk and Tablet Dosage Form. American Journal of PharmTech Research 2012.

INTRODUCTION

Escitalopram oxalate (ESC) S-(+)-1-[3- (dimethyl-amino) propyl]-1-(p-fluorophenyl)- 5-phthalancarbonitrile oxalate), the S enantiomer of racemic citalopram, is a highly selective serotonin-reuptake inhibitor antidepressant used in treatment of depression and anxiety disorders (Figure 1)[1]. Etizolam(ETI) 4-(2-Chlorophenyl)-2-ethyl-9-methyl-6H-thieno[3,2-f] [1,2,4] triazolo [4,3-a] [1,4] diazepine[1] belongs to a new class of diazepines, thienotriazolodiazepines(Figure 2). Etizolam has anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant properties. It is 6-10 times more potent than diazepam[2].

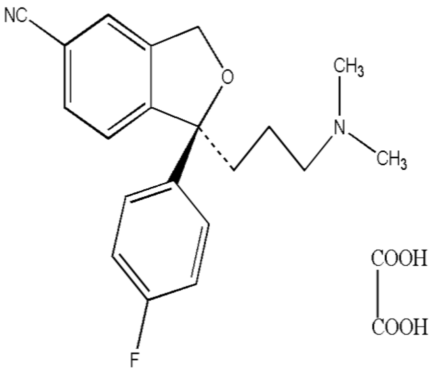

Figure 1: Structure of Escitalopram Oxalate

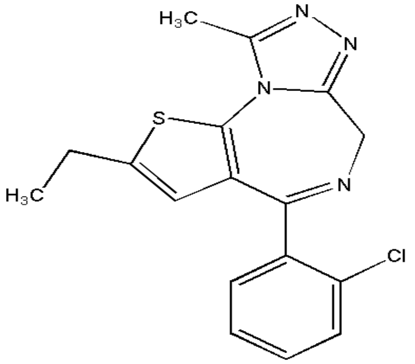

Figure 2: Structure of Etizolam

A literature survey revealed that ESC and its tablets are official in Indian pharmacopoeia and HPLC method has been reported for the assay [3]. Etizolam and its tablets are official only in Japanese Pharmacopoeia and potentiometric and HPLC method have been reported for the assay.[4] A literature survey for ESC revealed that several analytical methods have been reported for the determination of ESC alone and in combination with clonazepam in pharmaceutical formulations these include spectrophotometric[5,6], HPLC[7-9] and HPTLC[10] methods. A literature survey for ETI revealed that several analytical methods have been reported for the determination of ETI in biological fluids; these include HPLC[11], HPTLC[12], LC-MS,[13,14] GC-MS[15]. However no method has been reported for the simultaneous determination of ESC and ETI. Hence the objective of this work was to develop and validate suitable RP- HPLC method in accordance with ICH guidelines, which can be used successfully to determine ESC and ETI in tablet dosage form.[16]

MATERIALS AND METHODS

Instrumentation

Shimadzu HPLC LC 2010 CHT series equipped with quaternary constant flow pump, auto injector, SPDM 10 A VP Shimadzu Photo Diode array detector, LC Solution Version 1.22 SP1 software, Kromasil 100 C18, 5µ(150×4.6 mm) column as stationary phase, An analytical balance (Acculab ALC-210.4, Huntingdon Valley, PA); pH meter (Thermo Electron Corp., Pune India); and sonicator (EN 30 US Enertech Fast Clean, Mumbai, India) were used in study.

Materials

ESC was obtained from Apostle Remedies (Vadodara, Gujarat, India) and ETI was obtained from Macleods Pharmaceutical Limited (Valsad, Gujarat, India) as gift sample. Acetonitrile (HPLC grade), water (HPLC grade), Hexane sulfonic acid (HPLC grade), orthophosphoric acid (AR grade) were obtained from Rankem Pvt. Ltd. Delhi, India. The 0.45 µm membrane filter was used throughout the experiment. The tablets of ESC in combination with ETI were purchased from local market. Other chemicals used in the experiment were of analytical or HPLC grade.

Optimized Chromatographic Conditions for Escitalopram and Etizolam.

- **Column** : Kromasil 100 C18, 5µ(150×4.6 mm)
- **Mobile phase** : Acetonitrile:0.005 M Hexane Sulfonic Acid pH 3 (40:60 v/v)
- **Flow rate** : 1 ml/min
- **Detection** : 254 nm
- **Injection volume** : 10 µl
- **Column oven Temp** : 30˚C (± 2˚C)
- **Run time** **:**10.0 min

Preparation of standard stock solution

Accurately weighed quantity of 63.5 mg ESC (equivalent to 50 mg Escitalopram) and 5 mg ETI API were transferred into 50 ml volumetric flask and dissolved in acetonitrile using ultra sonication and diluted up to mark to give a stock solution having concentration of 1000 µg/ml ESC and 100 µg/ml ETI.

Preparation of sample solution

About 20 tablets were weighed and powdered, a quantity of tablet powder equivalent of 50 mg Escitalopram and 5 mg of Etizolam was weighed accurately and transferred to a 50ml volumetric flask. The tablet powder was extracted with about 30 ml acetonitrile with aid of ultra sonication and diluted to mark with same solvent and filtered through 0.45 µ filter to yield 1000 µg/ml Escitalopram and 100µg/ml Etizolam concentration.

Validation of the method

This optimized method was validated in terms of linearity, accuracy, precision, specificity, limit of detection, limit of quantification and solution stability as per ICH guidelines.

Linearity

Aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ml of the standard stock solution were transferred into a series of 10 ml volumetric flasks in three sets and diluted to the mark with mobile phase. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

Accuracy

To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 20, 40 and 60 %. Mean percentage recovery was determined.

Precision

Intraday and interday precision were evaluated by determining the corresponding responses for ESC and ETI (20+2, 80+8 and 140+14 µg/ml) standard mixture in triplicate on the same day and on different days. The repeatability was also performed using six replicate samples.

Specificity

The specificity of the method was checked by placebo sample and peak purity test using PDA detector.

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve.

System suitability test parameters

System suitability parameters were verified with respect to No. of theoretical plates, asymmetric factor and resolution of six replicate of injection of ESC (20µg/ml) and ETI (2µg/ml).

Robustness

The robustness was checked by changing in parameters of mobile phase flow rate (±0.1 ml/min), mobile phase composition (± 2.0 ml organic modifier), temperature of column oven (±2°C), pH (± 0.20 unit) and concentration of ion pair reagent (± 0.001 M). After each change sample and standard solution were injected and percent assay was calculated.

Solution stability study

Standard and sample solutions stability was evaluated at room temperature for 24 hours by analyzing same solution up to 24 hr at different time intervals.

RESULTS AND DISCUSSION

Both the drugs were resolved using a mobile phase consisting of acetonitrile:0.005 M Hexane Sulfonic Acid pH 3.0 (adjusted with o-phosphoric acid) (40:60 v/v). The flow rate was 1.0 ml/min and the effluent was monitored at 254 nm using PDA detector. Acetonitrile was used for enhancing elution of ETI, but this resulted in too instant elution of ESC near unretained peak. Strongly acidic ion-pairing reagent enabled retention of ESC on column and prolongation of elution time. The retention times for ESC and ETI were 3.66 min and 8.07 min, respectively. (Figure 3)

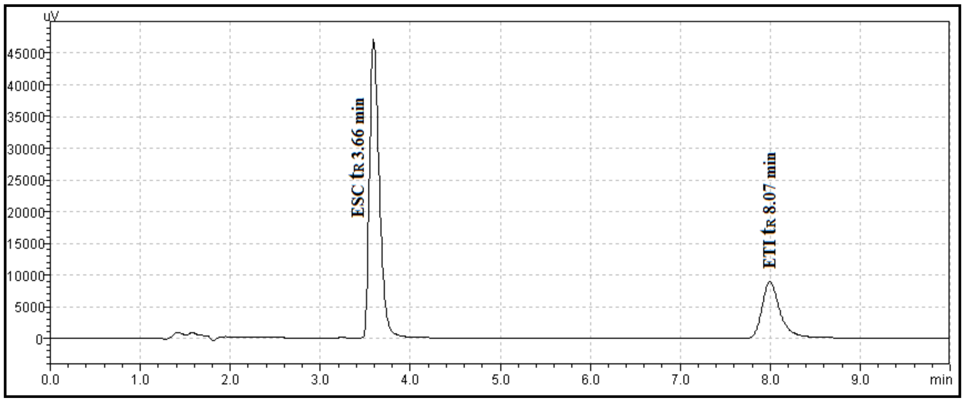

Figure 3: HPLC Chromatogram of ESC and ETI sample. (50and 5 μg/ml ;Tablet dosage form at 254 nm)

Table 1: Summary of System Suitability test and validation Parameters

Sr. No.	Parameter		ESC	ETI
1	Retention Time		3.659 ± 0.024	8.075 ± 0.006
2	Theoretical Plates		4454 ± 35	7893 ± 53
3	Tailing Factor		1.418 0.0025	1.298 ± 0.002
4	HETP		33.68 ± 0.27	19 ± 0.13
5	Resolution		-	15.07 ± 0.032
6	Linearity Range		20-160 μg/ml	2-16 μg/ml
7	Regression line equation		y = 5701.x + 8725	y = 20170x + 2820
8	Correlation coefficient (R^2)		0.9994	0.9993
9	Precision (%RSD)	Repeatability	0.32	0.35
		Intraday	0.16-0.35	0.23-0.48
		Interday	0.18-0.49	0.32-0.79
10	Accuracy (% recovery)		99.79-100.86	98.78-100.24
11	Limit of Detection		0.23 μg/ml	0.04 μg/ml
12	Limit of Quantitation		0.68 μg/ml	0.11 μg/ml
13	Robustness (% RSD of Assay)		0.52	0.41
14	Solution Stability		Up to 24 Hour	Up to 24 Hour

RSD- Relative Standard Deviation

Method Validation

Linearity

The method was linear in the concentration range of 20-160 µg/ml for ESC and 2-16 µg/ml for ETI with a correlation coefficient of 0.9994 and 0.9997 for respective drugs. The regression equations for ESC and ETI were $y = 5701.x + 8725$ and $y = 20170x + 2820$ for ESC and ETI respectively. (Table 1)

Accuracy

The results for accuracy study are shown in Table 2. The recovery was found in the range of 99.79-100.86% for ESC and 98.78-100.24% for ETI (Table 2), indicating the accuracy of method.

Table 2: Accuracy data for analysis of ESC and ETI

Amount sample taken (µg/ml)		Amount std added (µg/ml)		Total Amount found (µg/ml)		% Recovery* ± %RSD	
ESC	**ETI**	**ESC**	**ETI**	**ESC**	**ETI**	**ESC**	**ETI**
50	5	-	-	49.90	5.01	-	-
50	5	10	1	59.88	5.99	99.79 ± 0.33	98.78 ± 0.83
50	5	20	2	70.08	7.00	100.86 ± 0.43	99.72 ± 0.66
50	5	30	3	79.78	8.01	99.59 ± 0.92	100.24 ± 1.06

* Average of three determination

Precision

The results for intraday and interday precision studies and repeatability are listed in Table 1, indicating that the method was precise and reproducible.

Specificity

Peak purity graph of Escitalopram and Etizolam in sample solution were shown in Figure 4, Peak purity data suggested that there was no interference from excipients or impurities. Placebo did not reveal any signal at analytes retention time. Hence the developed method has been found to be specific.

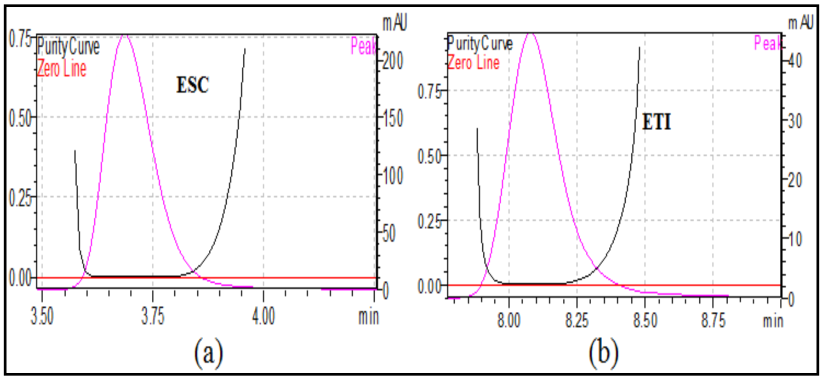

Figure 4: Peak purity graph of ESC and ETI in sample solution

LOD and LOQ

The LOD for ESC and ETI were found to be 0.23 µg/ml and 0.04 µg/ml, respectively, while LOQ were 0.68 µg/ml and 0.11 µg/ml, respectively. (Table 1)

System suitability test parameters

The system suitability test parameters are listed in Table 1.

Solution stability study

ESC and ETI sample and standard solution were stable for 24 hour.

The proposed method was successfully applied to the determination of ESC and ETI in their tablet dosage form. The results obtained for ESC and ETI were comparable with the corresponding labelled amounts (Table 3). Results for robustness evaluation for both the drugs (Table 4). % RSD of sample preparation assay was below 2 % under modified conditions indicating robustness of the method.

Table 3: Analysis of marketed formulation (ETILAAM-S-5 tablet)

Formulation	**Label claim (mg/tablet)**		**% Assay(Mean ± S.D, n=6)**	
	ESC	ETI	ESC	ETI
ETILAAM-S-5 tablet	5 mg	0.5 mg	98.85 ± 0.33	100.05 ± 0.36

n= number of replicates, S.D – Standard Deviation

Table 4: Robustness data of for analysis of ESC and ETI

Condition		**ESC**		**ETI**	
		% Assay	**% RSD**	**% Assay**	**% RSD**
As such		99.42		99.76	
Temperature	35° C	98.83		98.84	
	45° C	99.56		99.53	
Flow Rate	0.9 ml/ min	98.78		99.09	
	1.1 ml/min	98.85		98.96	
Mobile phase	Acetonitrile: Buffer (42:58)	98.59	0.52	99.69	0.41
	Acetonitrile: Buffer (38:62)	98.34		99.89	
pH	2.8	98.08		99.11	
	3.0	98.17		98.93	
Hexane sulfonic acid	0.004 M	97.74		99.76	
	0.006 M	98.76		98.65	

CONCLUSION

The developed RP-HPLC method was found to be rapid, sensitive, selective, precise, accurate and robust for ESC and ETI. The method was validated as per the guidelines laid by ICH. The results of the validated tests were found to be satisfactory and therefore this method can be applied successfully for routine quality-control analysis of escitalopram oxalate and etizolam in pharmaceutical preparations.

ACKNOWLEDGEMENT

The authors are thankful to Apostle Remedies (Vadodara, Gujarat, India) and Macleods Pharmaceutical Limited (Valsad, Gujarat, India) for providing gift sample of ESC and ETI for research. The authors are highly thankful to Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

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