DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD OF ESCITALOPRAM OXALATE IN BULK AND PHARMACEUTICAL FORMULATION.

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ABSTRACT

The present work discusses the development of a UV estimation method for Escitalopram Oxalate. A simple, accurate, cost effective and reproducible spectrophotometric method has been developed for the estimation of Escitalopram Oxalate in bulk and pharmaceutical dosage form. An absorption maximum was found to be at 239 nm. The percentage recovery of Escitalopram Oxalate ranged from 98.859% to 101.04% in pharmaceutical dosage form. The developed method was validated with respect to linearity, accuracy (recovery), precision, LOD, LOQ, Sandell's sensitivity, molar absorbtivity, molar extinction coefficient and specificity. Beer's law was obeyed in the concentration range of 5-30µg/ml having line equation y = 0.027x + 0.006 with correlation coefficient of 1.

Key words- UV spectrophotometry, Escitalopram Oxalate.

1. Introduction

Chemically Escitalopram Oxalate (EO) is (1S)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-3H-2-benzofuran-5-carbonitrile, having furancarbonitrile as parent nucleus and is the active S-enantiomer of the racemic selective serotonin reuptake inhibitor (SSRI) citalopram (RS-citalopram). It acts by selective inhibition of the serotonin transporter protein. It possesses a rapid onset of antidepressant activity and is an effective and generally well tolerated treatment for moderate-to-severe major depressive disorder (MDD). The drug is also effective in reducing ethanol uptake in alcoholics and depressed patients who suffer from tardive dyskinesia in preference to tricyclic antidepressants, which aggravate this condition (Murdoch David,2005).

Analysis is an important component in the formulation development of any drug molecule. A suitable and validated method has to be available for the analysis of drug in bulk, in drug delivery systems, from release dissolution studies and in biological samples. If a suitable method, for specific need is not available then it becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples.

The estimation of EO by Liquid chromatography – electrospray ionisation mass spectrometry method [LC-EIMS] (Singh, 2004), Flourometric and thin layer chromatography Densitometry (Elham, 2009) is reported in literature. Although Determination of EO and Clonazepam in combination by Spectrophotometric, RP-HPLC (Gandhi, 2008) and HPTLC (Dhavale, 2008) is reported, but estimation of this single drug has not been reported till date in bulk and pharmaceutical formulations. Thus the present study was undertaken to develop and validate a simple, sensitive, accurate, precise, and reproducible UV estimation method for EO.

2. Materials and method

2.1 Instrument and materials

UV/Visible spectrophotometer Schimadzu 1800 and analytical balance schimadzu AX200 were used for studies. EO pure drug was obtain from Aurobindo Pharma LTD., Hyderabad as gift sample with 99.99% w/w assay value and was used without further purification. All chemicals and reagents used were of analytical grade.

2.2 Preparation of standard stock solution

Standard drug solution of EO was prepared by dissolving 10 mg EO in 20 ml of 0.1N HCl and was transfered to 100ml volumetric flask and volume was made upto mark with 0.1N HCl to obtain stock solution of 100µg/ml concentration. Ultrasonication was done to obtain clear solution.

2.3 Preparation of calibration curve

Calibration curve was prepared in 0.1N HCl at λ_{max} 239 nm using UV/Visible spectrophotometer of stock solution of 100 µg/ml. Serial dilution of 5, 10,15,20,25 and 30 µg/ml were prepared and absorbance was taken at λ_{max} 239 nm. Average of such

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8 sets of values was taken for standard calibration curve and solutions were scanned in the range of 200-400 nm against blank. The calibration curve was plotted. The optical characteristics are summarized in table 1.

2.4 Preparation of sample solution

Ten tablets were weighed and powdered. The amount of tablet powder equivalent to 10 mg of EO was weighed accurately and transfered to 20 ml 0.1N HCl and kept for 15 min with frequent shaking and volume was made up to 100 ml mark with 0.1N HCl. The solution was then filtered through Whatmann filter paper # 41. This filtrate was diluted suitably with solvent (0.1N HCl) to get the solution of 15µg/ml concentration. The absorbance was measured against blank. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is given in table 3.

3. Results and discussion

3.1 Precision

Assay of method precision (intra-day precision) was evaluated by carrying out four independent assays of test samples of EO. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, systems and different days in the same laboratory. The percent relative standard deviation (%RSD) and assay values obtained by two analysts were 1.302827, 101.2087 and 0.823535, 98.66453 respectively (Table 4).

3.2 Accuracy (Recovery Test)

Accuracy of the method was studied by recovery studies. The recovery studies were performed by adding known amounts to tablet. The recovery was performed at three levels, 80,100 and 120% of EO standard concentration. The recovery samples were prepared as mentioned above. Three samples were prepared for each recovery level. The solutions were then analyzed and the percentage recoveries were calculated from the calibration curve. The recovery values for EO ranged from 98.859% to 101.04% (Table 3).

3.3 Linearity

The linearity of the response of the drug was veriüed at 5 to 50 µg/ml concentrations, but linearity was found in between 5-30 µg/ml concentration range. The calibration curve was obtained by plotting the absorbance versus concentration data and was treated by linear regression analysis (table 2). The equation of the calibration curve for EO was obtained as y = 0.027x + 0.006 and the calibration curve was found to be linear as mentioned above. The correlation coefficient (r^2) of determination was 1.

3.4 Limit of Detection(LOD) and Limit of Quantiûcation(LOQ)

The LOD and LOQ of EO were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines .The LOD and LOQ is shown in table 2.

3.5 Determination of Active Ingredients in Tablets

The validated method was applied for the determination of EO in Tablets. Six tablets were assayed and the results are shown in Table 3, indicating that the amount of drug in tablet samples were within required range (99–103% of the label claim).

4. Conclusions

The developed method was found to be simple, sensitive, accurate, precise, reproducible, and can be used for routine quality control analysis of EO in bulk and pharmaceutical formulation.

5. Acknowledgement

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Figure 1 - Chemical structure of EO

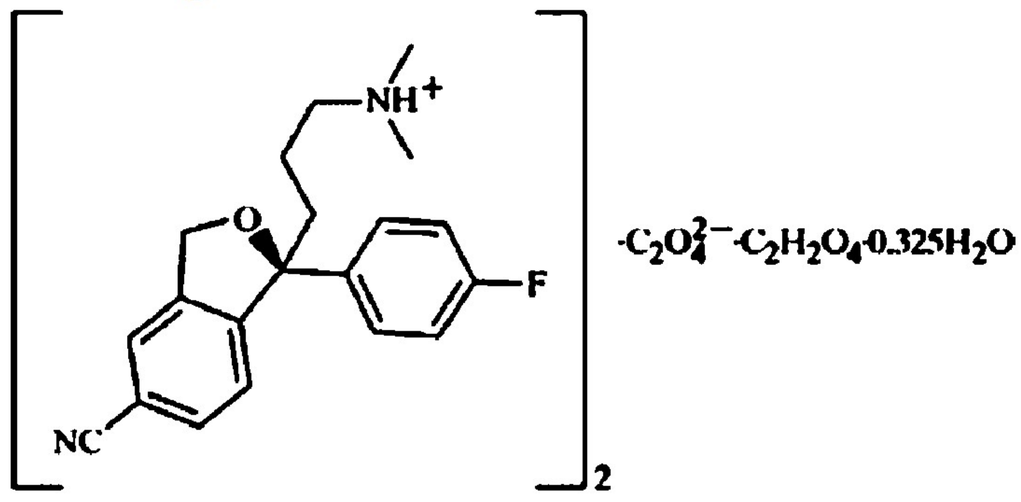

Figure 2 - Determination of λ_{max} of EO by UV scanning

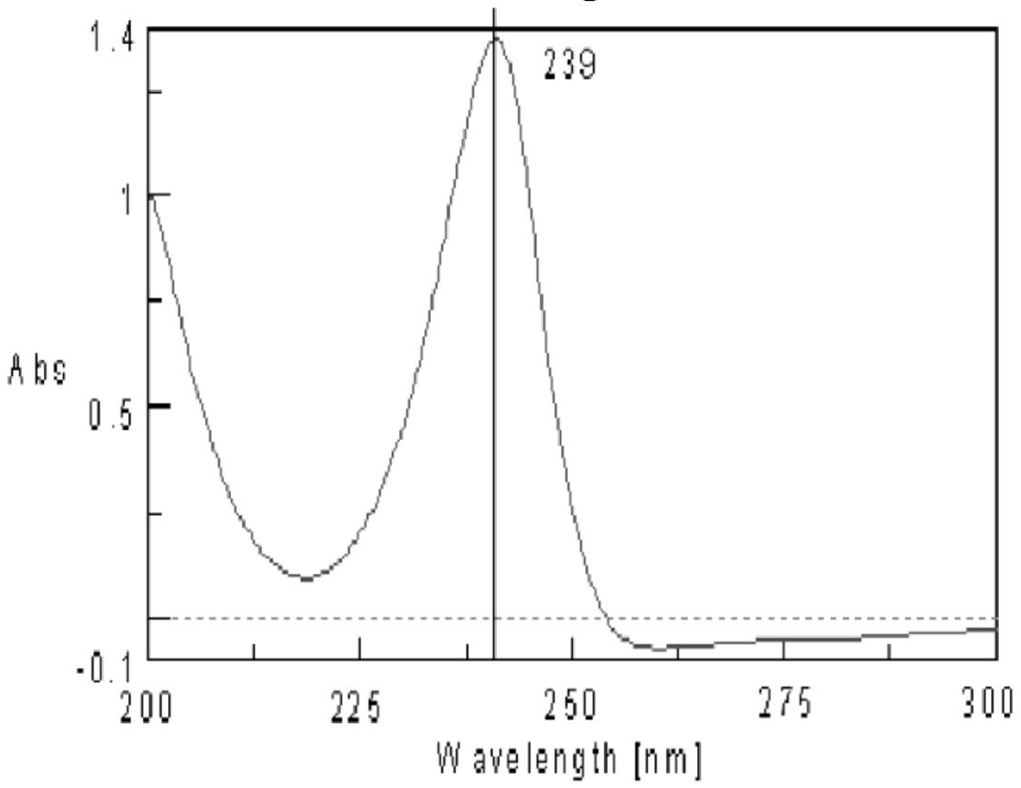

Figure 3- Calibration curve of EO

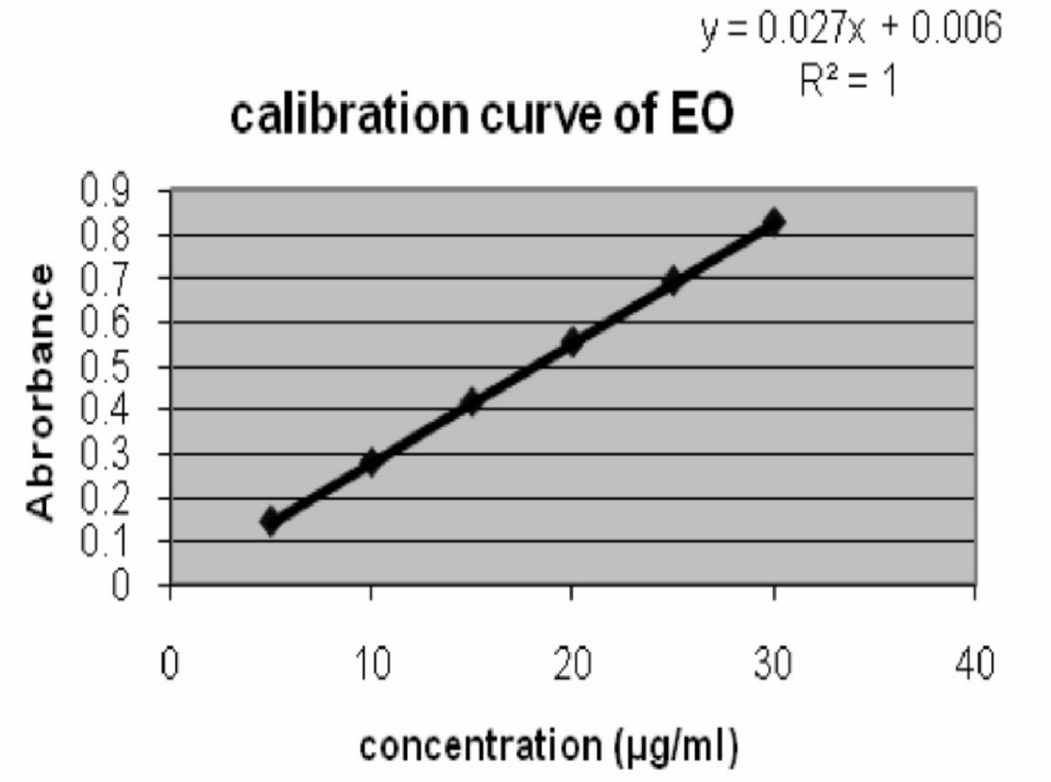

Table 1 - Calibration Curve parameters

Sr. No.	Concentration of Solution (µg/ml)	Mean Absorbance Value*	± % RSD
1.	5	0.145 ± 0.002	0.016
2.	10	0.280 ± 0.003	0.012
3.	15	0.417 ± 0.002	0.004
4.	20	0.554± 0.004	0.008
5.	25	0.693 ± 0.001	0.002

*n=8

Table 2 - Validation parameters

Sr. No.	Parameter	Result
1.	Absorption maxima(nm) (λ_{max})	239
2.	Standard Regression Equation	y = 0.027x + 0.006
3.	Regression Coefficient (r^2)	1
4.	Accuracy (% recovery ± SD)	99.93 ± 1.091
5.	Precision (% CV)	101.209 and 98.665
6.	Specificity	A 15 µg/ml solution of EO in 0.1N HCl at UV detection λ of 239 nm will show an absorbance value of 0.417±0.002
7.	Range (µg/ml)	5 to 50
8.	Linearity (µg/ml)	5 to 30
9.	LOD (µg/ml)	0.339
10.	LOQ (µg/ml)	1.027
11.	Molar absorptivity ($LMol^{-1}cm^{-1}$)	110357.9
12.	A(1% , 1cm)	279.406
13.	Sandell's sensitivity (µg/cm^2/0.001 absorbance units)	0.036

Table 3 - Determination of Accuracy by percentage recovery method

Ingredient	Tablet amount (µg/ml)	Level of addition (%)	Amount added (mg)	Drug found (µg/ml)	% Recovery	Average % recovery
EO*	15	80	12	26.692	98.859	99.93± 1.091
	15	100	15	29.967	99.891	
	15	120	18	33.343	101.04	

***EO having brand name Lexapro (10mg)**

Table 4 - Determination of Precision

Sample number	Assay of EO as percent of labeled amount	
	Analyst-I (Intra-day precision)	Analyst-II (Inter-day precision)
1	103.271	97.738
2	101.652	97.545
3	101.328	99.216
4	100.948	98.866
5	100.853	99.373
6	99.20	99.25
Mean	101.209	98.665
% RSD	1.303	0.824

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