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Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Escitalopram oxalate and Etizolam in their Combined Tablet Dosage Form

Bhumika D. Sakhreliya*, Dr. Priti D. Trivedi, Darshana K. Modi

K .B. Institute of Pharmaceutical Education and Research, Sector-23, Gandhinagar, India-382023

ABSTRACT:

Three simple, rapid, accurate, precise and cost-effective UV spectrophotometric methods have been developed and validated for simultaneous estimation of Escitalopram oxalate and Etizolam in tablet dosage form. The UV methods have been developed utilizing concept of standard addition utilizing 0.1 N HCl as a solvent. Method I is estimation using simultaneous equation method at 238.2 nm (λmax of Escitalopram oxalate) and 251.6 nm (λmax of Etizolam). Method II is Q ratio (absorbance ratio) method utilize absorbance measurement at 238.2 nm and 248.8 nm (isoabsorptive point). Method III is absorbance correction method utilize absorbance measurement at 238.2 nm for Escitalopram oxalate and 292.8 nm for Etizolam. Linearity was observed in range of 10-60 µg/ml and 5-30 µg/ml for Escitalopram oxalate and Etizolam respectively for all three methods .The correlation coefficient value was found to be 0.9989-0.9998. All methods were statistically validated as per ICH guidelines and can be successively applied for analysis for tablets formulation.

For Correspondence:

Ms. Bhumika D. Sakhreliya

Department of Quality Assurance

K .B. Institute of Pharmaceutical Education and Research,

Sector-23, Gandhinagar, India-382023.

Email: bhumikasakhreliya83@gmail.com

(www.jpsbr.org)

INTRODUCTION:

Escitalopram oxalate (ESC) is the **(Figure 1)** selective serotonin reuptake inhibitor, antidepressant agent, chemically it is *S*-(+)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile[1,2].Etizolam (ETI) **(Figure 1)** belongs to an original chemical class of diazepines, namely thienotriazolodiazepines with antianxiety activity and chemically it is 4-(2-Chlorophenyl)- 2–ethyl –9 –methyl –6H- thieno [3,2-f] [1,2,4] triazolo -[4,3-a] [1,4] diazepine 1)[1,2]. ESC is official in IP'10 and ETI is official in JP XV [3,4]. Literature survey indicate some spectrophotometric[5-11], HPLC [12-15], HPTLC [16-18], fluorimetry [19-20], LC-MS [21-24], LC-MS/MS [25], enantiomeric separation [26-27], CE [28] and TLC [29] methods for estimation of ESC either individually or in combination with other drugs . Literature survey also reports few HPLC [30], HPTLC [31], LC-MS [32,33] and GC-MS [34,35] methods for estimation of ETI individually or in combination with other drugs. However there is no analytical method reported for simultaneous estimation of both drugs in their combined tablet dosage form. Present work describes rapid, simple, sensitive, accurate and reproducible spectrophotometric methods.
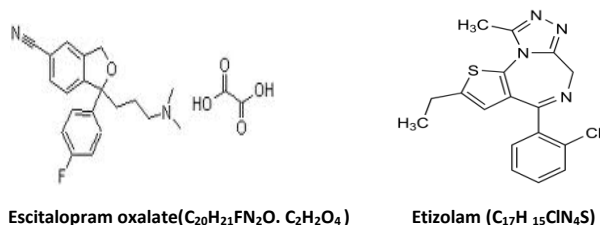
Escitalopram oxalate($C_{20}H_{21}FN_2O.\ C_2H_2O_4$)   **Etizolam ($C_{17}H_{15}ClN_4S$)**

Figure 1: Structural formulas of ESC and ETI

MATERIAL AND METHODS

UV spectrophotometric method was carried out using Shimadzu 1800 double beam UV - Visible spectrophotometer with UV probe 2.33 software, spectral band width of 2 nm, wavelength accuracy ±0.5 nm and 1 cm matched pair quartz cells .Standard of ESC was obtained from Cadila Healthcare, Ahmedabad and ETI was obtained from Intas Pharmaceuticals, Ahmedabad. AR grade methanol used for UV method and 0.1N HCl was prepared in double distilled water. Tablet dosage form having brand name ETIZOLA PLUS 5 with label claim of ESC 5 mg and ETI 0.5 mg of Macleods Pharmaceutical Ltd. was purchased from local pharmacy.

Preparation of standard stock solution

An accurately weighed standard powder of 5 mg of ESC and 2.5 mg of ETI were transferred in 25 ml volumetric flask separately, dissolved and diluted up to the mark with methanol AR grade, to get final concentration 200 µg/ml of ESC and 100 µg/ml of ETI. From this standard stock solution, different aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with 0.1N HCl. This solution was used as a working standard solution (WSS).

Selection of analytical wavelength

By appropriate dilution of standard stock solution, solution containing 20 µg/ml of ESC and 10 µg/ml of ETI was prepared in 0.1N HCl. These diluted solutions were scanned in range 200-400 nm separately. ESC shows λmax at 238.2 nm and ETI showed three λmax at 251.6, 292.8, 363.8 nm .The overlay spectra of ESC and ETI showed isoabsorptive point at 248.8 nm **(figure 2)**
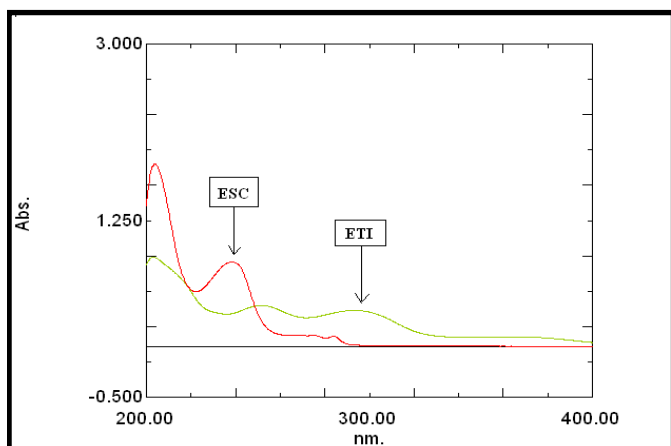

Figure 2: Overlay spectra of ESC (20 µg/ml) and ETI (10 µg/ml) in 0.1N HCl

Preparation of calibration curve

For construction of calibration curve, two series of different concentration in range of 10-60 µg/ml for ESC and 5-30 µg/ml for ETI were prepared in 0.1N HCl from stock solution. These solutions were scanned in range of 200-400 nm and absorbance were measured at selective wavelength and calibration curve were plotted for absorbance vs. concentration.

Method I (Simultaneous equation method)

Two wavelengths selected for the method are 238.2 nm(λ_1) and 251.6 nm(λ_2) that are absorbance maxima of ESC and ETI respectively in 0.1N HCl . The stock solutions of both the drugs were further diluted separately with 0.1N HCl to get a series of standard solutions of 10-60 µg /ml of ESC and 5-30 µg /ml of ETI. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations:

$$\mathbf{Cx} = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \qquad \mathbf{Cy} = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2}$$

Cx and Cy = Concentration of ESC and ETI respectively (gm/100 ml)
ax_1 and ax_2 = Absorptivity of ESC at λ_1 and λ_2 respectively
ay_1 and ay_2 = Absorptivity of ETI at λ_1 and λ_2 respectively
A_1 and A_2 = Absorbance of test at λ_1 and λ_2 respectively

Method II (Q ratio (absorbance ratio) method)

From the overlay spectrum of ESC and ETI, two wavelengths were selected, one at 248.8 nm (λ_2) which is the isoabsorptive point for both the drugs and the other at 238.2 nm (λ_1) which is λ max of ESC. The absorbances of the sample solutions , prepared in a similar manner as in the previous method, were measured and the absorbance ratio values for both the drugs at selected wavelengths were also calculated. The method employs Q-values and the concentrations of drugs in sample solution were determined by using the following formula**:**

$$\mathbf{Cx} = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{A}{a_1} \qquad\qquad \mathbf{Cy} = \frac{Q_0 - Q_1}{Q_2 - Q_1} \times \frac{A}{a_2}$$

$Q_0 = \dfrac{\text{Absorbance of test at } \lambda_1}{\text{Absorbance of test at } \lambda_2}$

$Q_1 = \dfrac{\text{Absorptivity of ESC at } \lambda_1}{\text{Absorptivity of ESC at } \lambda_2}$

$Q_2 = \dfrac{\text{Absorptivity of ETI at } \lambda_1}{\text{Absorptivity of ETI at } \lambda_2}$

A = Absorbance of test at λ_2 , a_1 and a_2 = Absorptivity of ESC and ETI at λ_2 respectively

Cx and Cy = Concentration of ESC and ETI respectively (gm/100 ml)

Method III (Absorbance correction method)

From the overlay spectra of ESC and ETI, two wavelengths were selected, one at 238.2 nm (λ_1) for ESC and the other at 292.8 nm (λ_2) for ETI at which ESC shows zero absorbance. The absorbances of the sample solutions, prepared in a similar manner as in the previous method, were measured for both the drugs at selected wavelengths. The concentrations of drugs in sample solution were determined by using the following formula:

$Cy = A_{292.8\,nm} / A(1\%, 1cm)_{292.8nm}$ of ETI
$Cx = C_{Ax238.2\,nm} / A(1\%, 1cm)_{238.2\,nm}$ of ESC
$C_{Ax238.2\,nm} = A_{238.2\,nm} - Ay_{238.2\,nm}$
$Ay_{238.2\,nm} = Cy \times A(1\%, 1cm)_{238.2\,nm}$ of ETI
Cx and Cy = Concentration of ESC and ETI respectively (gm/100 ml)

Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed and powdered. An accurately weighed tablet powder equivalent to 5 mg of ESC and 0.5 mg of ETI was transferred in to 25 ml volumetric flask. To this 2 mg of standard ETI powder was added to achieve ratio of ESC to ETI 2: 1. To this 20 ml of methanol was added and sonicated for 15 min. Volume was made up to the mark with methanol then solution was filtered through whatman filter paper no. 41. From this stock solution, necessary dilutions were made with 0.1N HCl to get final concentration of 20 µg/ml and 10 µg/ml of ESC and ETI respectively. The above solution was then analyzed for the content of ESC and ETI using the methods described above.

METHOD VALIDATION [36,37]

The proposed methods were validated accordance to ICH Q2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification. The results are shown in Table 1, 2, 3 & 4.

Linearity & Range:

The linearity of proposed methods was evaluated by linear regression analysis, which was calculated by least square method. Calibration standards were prepared by spiking required volume of working standard solution200 µg/ml of ESC and 100 µg/ml of ETI into different 10ml volumetric flasks and volume made with 0.1N HCl to yield concentrations of 10, 20, 30, 40, 50 and 60 µg/ml of ESC and 5, 10, 15, 20, 25 and 30µg/ml for ETI. The absorbances of the drugs were measured. Calibration curve was plotted between absorbance of drug against concentration of the drug. These results shown there was an excellent correlation between absorbance and analyte concentration (Table 1). The drugs were linear in the concentration range of 10-60 µg/ml for ESC and 5-30 µg/ml for ETI (Figure 3 & 4).

Accuracy:

Accuracy of the methods was determined at three different concentration levels i.e.80%, 100% and 120% in triplicate for

each drug as per ICH guidelines. From the total amount of drug found, the percentage recovery was fond in range of 99.92 – 101.35 %.(Table 2).

Precision:

Precision was studied to find out intra and inter-day variations in the test method of ESC and ETI. Intra-day precision was determined by analyzing six replicate measurements of 100% concentrations within linearity range of drugs on three different times in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. The precision of an analytical method is expressed as %RSD of a series of measurements which should be less than 2 %. (Table 3)

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

LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions.

The LOD and LOQ for ESC and ETI were determined according to ICH guideline

LOD = 3.3 σ / S   LOQ = 10 σ / S

Where,
σ = Standard deviation of the y intercept of calibration curves
S = Slope of the calibration curve

The results of LOD and LOQ were shown in table 1.

RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of ESC and ETI in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration curves for ESC and ETI were linear with correlation coefficients (r^2)values in the range of 0.9970- 0.9998 at all the selected wavelengths and the values were average of three readings with standard deviation in the range of 0.11 –1.94. The methods show high precision with % RSD value within range of 0.34-0.95 and recovery was close to 100% for both the drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of ESC and ETI in marketed formulations.
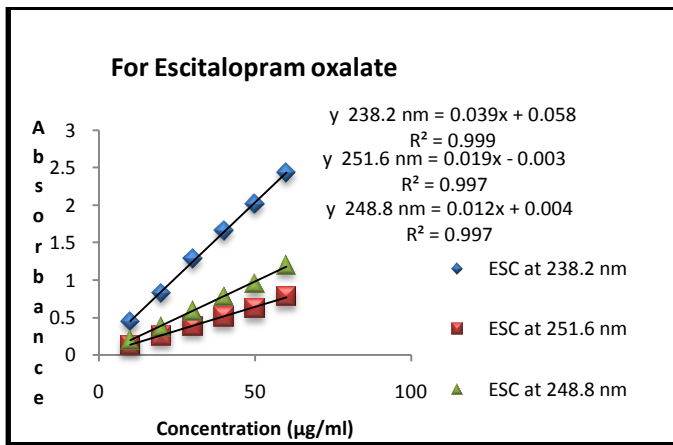
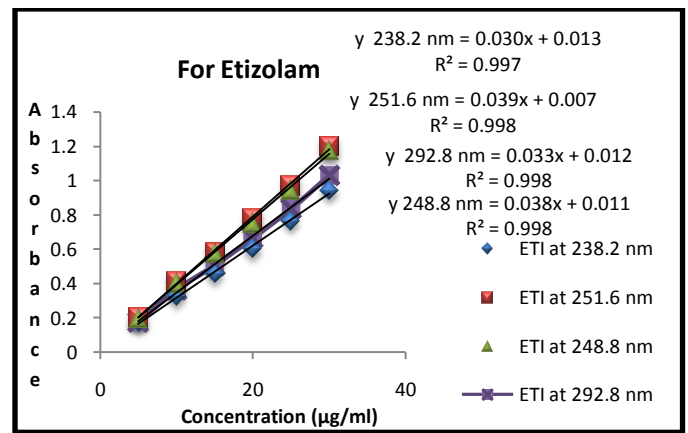**Figure 3:** Calibration Graph for Escitalopram oxalate

Figure 4: Calibration Graph for Etizolam

Table 1: Summary of Linear regression analysis and optical characteristics of ESC and ETI

Parameter	Method I		Method II		Method III	
	ESC	ETI	ESC	ETI	ESC	ETI
Analytical wavelength(nm)	238.2	251.6	238.2	248.8	238.2	292.8
Beer's law limit (µg/ml)	10-60	5-30	10-60	5-30	10-60	5-30
Coefficient of Correlation(r^2)	0.9998	0.9989	0.9998	0.9989	0.9998	0.9989
Slope	0.039	0.039	0.039	0.038	0.039	0.033
y intercept	0.058	0.007	0.058	0.011	0.058	0.013
Molar absorptivity (lit/mole/cm)	18476	14148.48	18476	14424.86	18476	12411.155
Sandell's sensitivity (mcg/cm^2/0.001AU)	0.011614	0.020833	0.011614	0.024312	0.011614	0.028198
LOD(µg/ml)	1.13	0.60	1.13	0.57	1.13	0.57
LOQ(µg/ml)	3.42	1.83	3.42	1.73	3.42	1.72

Table 2: Results of Recovery Study

Amount taken			% Recovery*					
ESC (µg/ml)	ETI (µg/ml)	% Added	Method I		Method II		Method III	
			ESC	ETI	ESC	ETI	ESC	ETI
		80	100.62	101.22	100.97	100.31	100.42	101.75
20	10	100	100.3	101.42	101.07	99.52	100.56	100.76
		120	100.93	101.31	101.46	99.92	100.84	101.54
	Mean		100.62	101.32	101.17	99.92	100.61	101.35
	SD		0.32	0.10	0.26	0.40	0.21	0.52
	% RSD		0.3130	0.0988	0.2559	0.3953	0.2125	0.5146

**Average value of three determinations,* RSD *–Relative standard deviation, SD – Standard Deviation*

Table 3: Results of Precision Study

	ESC (µg/ml)	ETI (µg/ml)	Method I		Method II		Method III	
			ESC	ETI	ESC	ETI	ESC	ETI
Intraday *(% RSD)	20	10	0.424585	0.612011	0.424585	0.574889	0.424585	0.950605
Interday *(% RSD)			0.417387	0.582713	0.417387	0.348955	0.417387	0.72336

**Average value of six determinations,* RSD *–Relative standard deviation*

Table 4: Analysis of marketed formulation by proposed methods

Drug	ETIZOLA PLUS 5	Assay* ± SD		
		Method I	Method II	Method III
ESC	5 mg	101.36 ±0.57	101.53 ±0.98	101.19 ±0.57
ETI	0.5 mg	101.55 ±0.59	99.83 ±0.81	102.02 ±1.38

***Average value of six determinations, SD –standard deviation*

CONCLUSION

The developed UV methods were found to be more accurate, precise and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. Thestatistical parameter of these methods showed good results. The recovery studies revealed excellent accuracy and high precision of the method. The methods were found to be simple & time saving. All three proposed methods could be applied for routine analysis in quality control laboratories.

ACKNOWLEDGEMENTS

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