Simultaneous Estimation of Escitalopram oxalate and Etizolam in Bulk and Pharmaceutical Dosage Form by UV

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Abstract

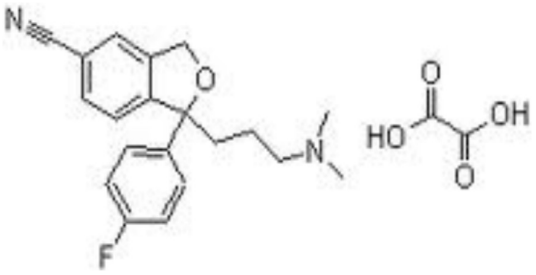
Three simple, fast, accurate, precise, and cost-effective UV spectrophotometric techniques have been devised and validated for the simultaneous quantification of Escitalopram oxalate and Etizolam in tablet dose. The UV methods were developed using the usual addition idea with Methanol: Water (70:30, v/v) as the solvent. The simultaneous equation approach is used to estimate the λmax of escitalopram oxalate at 236 nm and etizolam at 250 nm, respectively. Linearity was reported between 10-30 µg/ml and 2-10 µg/ml for Escitalopram oxalate and Etizolam, respectively. The correlation coefficient values were determined to be 0.990 and 0.997. The methods demonstrate high precision, with % RSD values within the range of less than 2, and recovery rates near to 100% for both medicines. The methodologies were statistically validated in accordance with ICH principles and can be used in subsequent analyses for tablet formulation.

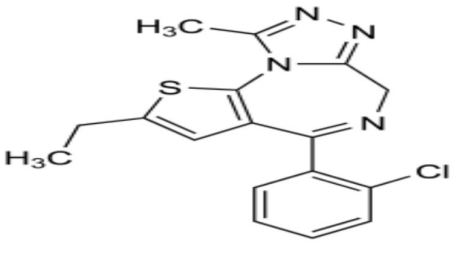
Keywords - Escitalopram oxalate, Etizolam, Simultaneous equation method.

Introduction:

Escitalopram oxalate (ESC) is the (Figure 1) selective serotonin reuptake inhibitor, antidepressant medication; 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 having antianxiety function, and its chemical name is 4-(2-Chlorophenyl)- 2-ethyl 9-methyl-6H-thieno [3,2-f] [1,2,4] Triazolo-[4,3-a] [1,4] diazepines 1)[1,2]. ESC is official in IP'10, while ETI is official in Japan XV.[3,4].





Escitalopram oxalate ($C_{20}H_{21}FN_2O.\ C_2H_2O_4$) Etizolam ($C_{17}H_{15}ClN_4S$)

Figure 1: Structural formulas of Escitalopram oxalate and Etizolam.

Material and Methods :

The UV spectrophotometric approach was carried out using a Shimadzu 1800 double beam UV-visible spectrophotometer with UV probe 2.33 software, spectral band width of 2 nm, and 1 cm matched pair quartz cells.Cadila Healthcare in Ahmedabad provided the ESC standard, and Intas Pharmaceuticals in Ahmedabad supplied the ETI standard. The UV technique was carried out using AR grade methanol and double distilled water. Macleods Pharmaceutical Ltd.'s ETIZOLA PLUS tablet dosage form, with the label claim of Escitalopram oxalate 5 mg and Etizolam 0.50 mg, was obtained from a local pharmacy.

Method Parameters:

a. Diluent: Methanol: Water (70: 30%, v/v)

b. Wavelength: **Escitalopram oxalate** λ1 = 236 nm; **Etizolam** λ2 = 250 nm

Drug	Concentration	Drug	Concentration
Escitalopram oxalate	10	Etizolam	2
	15		4
	20		6
	25		8
	30		10

Preparation of standard stock solution

To achieve a final concentration of 200 µg/ml of Escitalopram oxalate and 100 µg/ml of Etizolam, a standard powder of 10 mg of Escitalopram oxalate and 10 mg of Etizolam was accurately weighed, dissolved, and diluted with diluent. Then, 2 ml of Escitalopram oxalate and 1 ml of Etizolam from the above stock solution were added to the 10 ml volumetric flask. Different aliquots of this standard stock solution were placed into a 10 ml volumetric flask and filled with diluent to the desired volume. This answer was utilised as the working standard solution (WSS).

Selection of analytical wavelength

To make a solution with 20 µg/ml of Escitalopram oxalate and 10 µg/ml of Etizolam, dilute 1 ml of each above standard stock solution to 10 ml of methanol. These diluted solutions were scanned in the 200-400 nm range independently. Escitalopram oxalate exhibits λmax at 236 nm, while Etizolam has three λmax at 250 nm.The overlay spectra of Escitalopram oxalate and Etizolam revealed an isoabsorptive point at 247 nm.

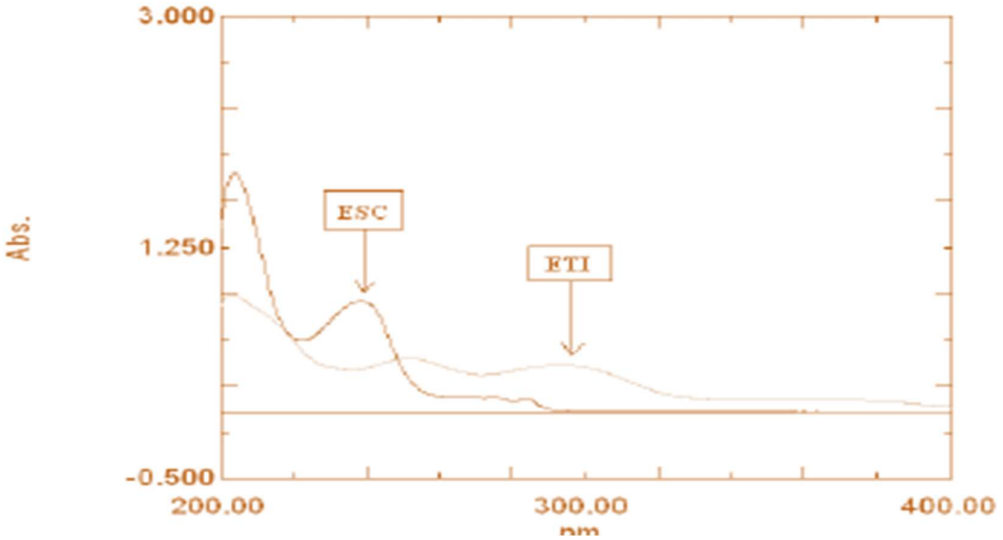


Figure 1: Overlay spectra of Escitalopram oxalate (20 µg/ml) and Etizolam (10 µg/ml) in diluent.

Procedure for Analysis of Tablet Formulation:

Twenty tablets were weighed and pulverised. An carefully weighed tablet powder containing 5 mg of Escitalopram oxalate and 0.5 mg of Etizolam was put to a 25 mL volumetric flask. To this, 2 mg of regular Etizolam powder was added, yielding a 2:1 ratio of Escitalopram oxalate to Etizolam. To this, 20 mL of diluent was added and sonicated for 15 minutes. The volume was filled to the mark with diluent, and the solution was filtered through Whatman filter paper No. 41. The stock solution was diluted with diluent to achieve final concentrations of 20 µg/ml of Escitalopram oxalate and 10 µg/ml of Etizolam. The aforementioned solution was then analysed for the presence of Escitalopram oxalate and Etizolam, using.

The absorbance at λ1 and λ2 was measured and the concentration was calculated using following formula;

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

Where,

Cx and Cy are the concentrations of Escitalopram oxalate and Etizolam, respectively,A1 and A2 are the absorbances of sample at λ1 and λ2, respectively,

ax1 and ax2 are the absorptivity of Escitalopram oxalate at λ1 and λ2, respectively,ay1 and ay2 are the absorptivity of Etizolam at λ1 and λ2, respectively.[5,6]

Analysis of marketed formulation by proposed methods:

Drug	**ETIZOLA PLUS**	**Assay* ± SD**
Escitalopram oxalate	5 mg.	99.97 ± 0.44
Etizolam	0.5 mg	99.09 ± 0.37

Validation:-

The proposed methods were validated accordance to ICH Q2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification.[7,8]

1) Linearity & Range:

The linearity of the proposed approaches was assessed using linear regression analysis, which was obtained using the least squares method. Calibration standards were generated by spiking the needed amount of working standard solution.Pour 100 µg/ml of Escitalopram oxalate and Etizolam into 10ml volumetric flasks and dilute with diluent to achieve concentrations of 10, 15, 20, 25, and 30 µg/ml of Escitalopram oxalate and 2, 4, 6, 8, and 10 µg/ml of Etizolam. The absorbance of the medicines was measured. A calibration curve was created by plotting the drug absorbance versus its concentration. The results showed that there was a strong association between absorbance and analyte concentration. The medications were linear in the concentration range of Escitalopram oxalate (10-60 µg/ml) and Etizolam (5-30 µg/mL).
(Figure 2 & 3).

Five concentrations of Escitalopram oxalate and Etizolam ranging from 10 to 30 µg/ml and 2 to 10 µg/ml were prepared. The sample preparations are as follows: X ml of Escitalopram oxalate and Y ml of Etizolam were diluted to 10 mL.

Table 1: Different concentration of Escitalopram oxalate and Etizolam.

X ml of ESCO	Y ml of ETIZ	Diluted to	Conc. of ESCO (µg/ml)	Conc. of ETIZ (µg/ml)
0.1 ml	0.2 ml	10 ml	10	2
1.5 ml	0.4 ml	10 ml	15	4
2 ml	0.6 ml	10 ml	20	6
2.5 ml	0.8 ml	10 ml	25	8
3 ml	1 ml	10 ml	30	10

ESCO: Escitalopram oxalate, ETIZ: Etizolam

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

LOD is the smallest amount of analyte in a sample that can be identified but not quantified under the specified experimental circumstances. The limit of quantification (LOQ) is the lowest concentration of analyte in a sample that can be quantified with acceptable precision and accuracy under specified experimental conditions.[9,10]

The LOD and LOQ for Escitalopram oxalate and Etizolam 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

3) Precision:

Precision was investigated to determine intra- and inter-day changes in the test technique for Escitalopram oxalate and Etizolam. To establish intra-day precision, three replicate measurements of 100% concentrations within a drug's linearity range were taken at separate times on the same day. The

system's inter-day precision was measured over three consecutive days of ordinary operation. The precision of an analytical method is stated as the percentage RSD of a set of measurements, which should be less than 2%.**(Table 6, 7, 8, 9)**

4) Accuracy:

The methods' accuracy was determined at three distinct concentration levels for each medication, 50%, 100%, and 150%, in triplicate, in accordance with ICH recommendations. The percentage recovery from the total amount of medication discovered was between 98.42 and 100.69%.

(Table 10). [11,12]

Results and Discussion :

The proposed methods for simultaneous estimation of Escitalopram oxalate and Etizolamin tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines.

Table 2: Analysis of Tablet Formulation by proposed methods:

Drug	ETIZOLA PLUS	Assay* ± SD
Escitalopram oxalate	5 mg.	99.97 ± 0.44
Etizolam	0.5 mg	99.09 ± 0.37

1) Linearity:

Table 3: Summary of Linear regression analysis of Escitalopram oxalate

Sr. No	con	abs
1	10	0.385
2	15	0.565
3	20	0.705
4	25	0.815
5	30	0.94

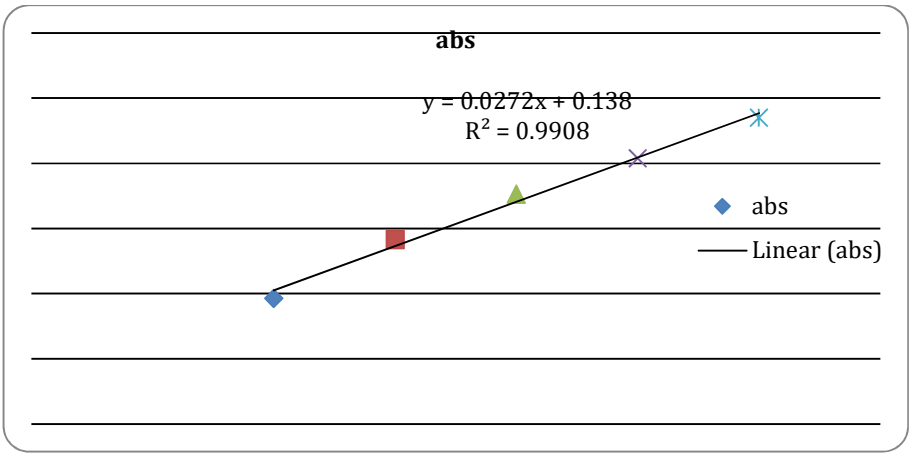


Figure 2: Linearity for Escitalopram oxalate

Table 4: Summary of Linear regression analysis of Etizolam.

Sr no.	Concentration	Absorbance
1	2	0.218
2	4	0.387
3	6	0.570
4	8	0.770
5	10	0.987

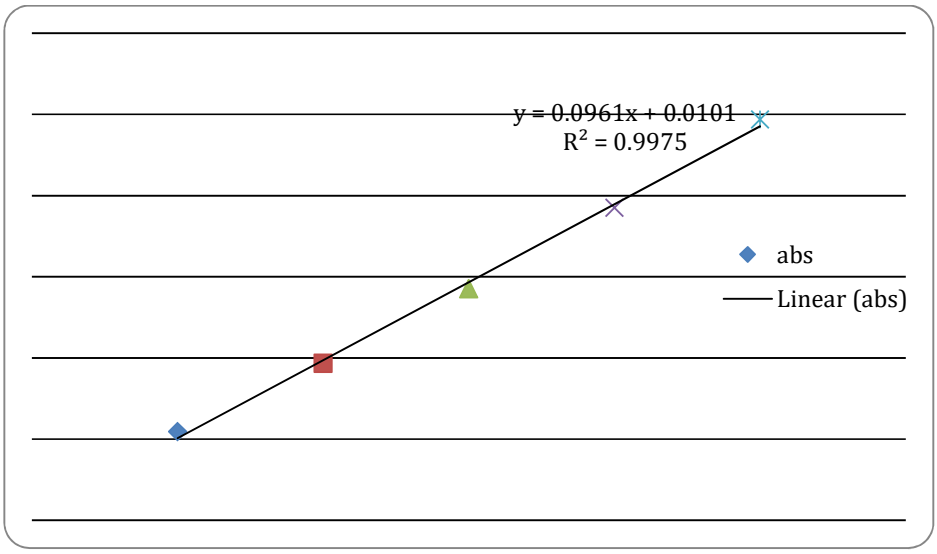


Figure 3: Linearity for Etizolam

2) Range: 10 to 30 µg/ml for Escitalopram oxalate and 2 to 10 µg/ml for Etizolam.

3) LOD and LOQ: Limit of Detection and Limit of Quantitation was calculated on the basis of Slope and Standard deviation of response.

Table 5: LOD and LOQ of Escitalopram oxalate and Etizolam.

Drug	**LOD (µg/ml)**	**LOQ (µg/ml)**
Escitalopram oxalate	2.926535	8.868289
Etizolam	0.599709945	1.817302864

4) Precision

a) Intraday precision:

Intraday precision was performed by analyzing three different concentrations within linearity range, three times in a day.

Table 6: Intraday precision For Escitalopram oxalate.

Con	Trial 1	Trial 2	Trial 3	Mean	SD	% RSD
10	0.382	0.376	0.386	0.381333	0.005033	1.319901
15	0.568	0.562	0.572	0.567333	0.005033	0.887172
20	0.768	0.775	0.762	0.768333	0.006506	0.846821
Mean					0.005524	1.017965

Table 7:Intraday precision For Etizolam.

Con	Trial 1	Trial 2	Trial 3	Mean	SD	% RSD
4	0.387	0.388	0.39	0.388333	0.001528	0.393354
6	0.57	0.572	0.568	0.57	0.002	0.350877
8	0.77	0.771	0.772	0.771	0.001	0.129702
Mean					0.001509	0.291311

B) Interday precision:

Table 8: Interday precision For Escitalopram oxalate.

Con	Trial 1	Trial 2	Trial 3	Mean	SD	% RSD
10	0.394	0.386	0.39	0.39	0.004	1.025641
15	0.578	0.582	0.588	0.582667	0.005033	0.863825
20	0.784	0.79	0.792	0.788667	0.004163	0.527895
Mean					0.004399	0.805787

Table 9:Interday precision For Etizolam.

Con	Trial 1	Trial 2	Trial 3	Mean	SD	% RSD
4	0.387	0.381	0.391	0.386333	0.005033	1.302819
6	0.571	0.569	0.571	0.570333	0.001155	0.202461
8	0.77	0.771	0.772	0.771	0.001	0.129702
Mean					0.002396	0.544994

5)Accuracy: (% Recovery) :

Table 10: Accuracy of Escitalopram oxalate and Etizolam.

Drug Name	Level of Recovery	Amount Added (µg/ml)		Theoretical Conc. (TC)	% Recovery
		Test	Standard		
Escitalopram oxalate	50%	20	10	30	99.88 %
	100%	20	20	40	98.42 %
	150%	20	25	45	100.58 %
Etizolam.	50%	10	5	15	99.33 %
	100%	10	10	20	100.69 %
	150%	10	15	25	100.10 %

The proposed methods for simultaneous estimation of Escitalopram oxalate and Etizolam in tablet dosage forms were found to be simple, accurate, cost-effective, and fast. The approach was validated using the ICH Q2 (R1) criteria. Standard calibration curves for ESC and ETI were linear, with correlation coefficients (r2) ranging from 0.9908 to 0.9975 at all wavelengths tested. The methods demonstrate high precision, with % RSD values within the range of less than 2, and recovery rates near to 100% for both medicines.

Conclusion :

The developed UV methods were discovered to be more accurate, precise, and repeatable. The study of tablets containing two medicines produced satisfactory results. The statistical parameters of these strategies produced satisfactory results. Recovery studies demonstrated that the approach was extremely accurate and precise. The procedures were found to be easy and time-saving. All three recommended approaches are suitable for routine analysis in quality control laboratories.

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