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Development and Validation of RP-HPLC Method for Estimation of Etizolam in Bulk

Shubhangi L. Harde*, Ravindra B. Laware, Mahesh H. Kolhe

Department of Pharmaceutical Quality assurance, Pravara Rural College of Pharmacy, Pravaranagar, Tal – Rahata, Dist – Ahmednagar, India

ABSTRACT

The present research work describes a simple, accurate, precise, effective, stability indicating, RP-HPLC method for estimation of Etizolam in there tablet dosage form. A Reverse phase high performance chromatographic method was developed for estimation of Etizolam dosage. The separation was achieved by Cosmosil C18 (250mm×4.6ID, Particle size: 5 micron) and methanol: water in the proportion of 30:70v/v as mobile phase, at a flow rate 0.8ml/min. Detection was carried out at 242nm. For RP-HPLC method results of the validation indicate that the method was linear in the range of 100-600 µg/ml for Etizolam. The % recoveries for Etizolam obtained in the accuracy study were 99.77-99.51% respectively. The LOD for Etizolam were found to be 0.7877µg/ml. LOQ for Etizolam found to be 2.382µ/ml. Developed methods were found to be accurate, precise, rapid and stability indicating for estimation of Etizolam.

Keywords: Etizolam, RP-HPLC, tablet dosage form

1. INTRODUCTION

1.1 Drug Profile

Etizolam (ETI) 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]diazepines 1)[1,2]. ECS is official in IP'10 and ETI is official in JP XV [3,4].

Literature survey also reports few HPLC [5] methods for estimation of Etizolam (ETI) individually or in combination with other drugs. Present work describes rapid, simple, sensitive, accurate and reproducible stability indicating method.
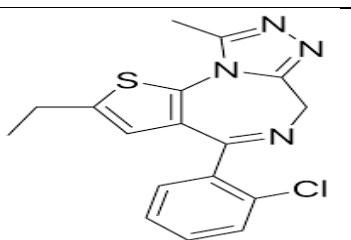
IUPAC Name :	4-(2-chlorophenyl)-2-ethyl-9-methyl-6H-thieno[3,2-f][1,2,4triazolo[4,3-a][1,4]diazepine.
Molecular Formula:	C17H15ClN4S [8]
Molecular weight:	342.854 g/mol
Appearance :	White powder
Melting Point :	145-148℃
Boiling Point :	545.3±60
Category :	Amnesic, anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant.[6]
Solubility :	No soluble in water. Soluble in acid water and in polyethylene glycol and ethanol.

Structure :	

Mechanism of Action

Etizolam is selectively a full agonist at GABA receptor to increase GABAergic transmission and enhance GABA-induced Cl-currents[11]. It is reported to bind to the benzodiazepine binding site which is located across the interface between the alpha and gamma subunits [12].

The present study aimed to develop a simple, sensitive, short retention time and accurate, validation of RP-HPLC method for estimation of Etizolam in bulk and tablet dosage form.

2.   MATERIALS AND METHOD :

Etizolam is gift sampled by Macleods Pharmaceutical Ltd., Daman, Gujarat.

All solvents and other reagents were of HPLC grade.

2.1. Instruments and Apparatus:

- System used for the research : HPLC Binary Gradient system

- Model no. : HPLC 3000 Series (Analytical Technologies Ltd.)

- Column : Cosmosil C18 (250mm×4.6ID, Particle size: 5 micron)

  2.2. Preparation of Standard Solution -

- ➢ Preparation of std stock solution of Etizolam – (1000ppm)

Weighed accurately 10mg of pure drug and dissolved in 10ml of solvent (Methanol: water (50:50); this gives 1000ppm solution of Etizolam. From this 0.1ml was transferred to 10 ml volumetric flask to prepare solution of 100 ppm.

2.3. Selection of Wavelength for Estimation:

Standard solution of Etizolam of concentration 10μg/ml was scanned between 200-400 nm using methanol as blank. The peak was obtained at 248nm.
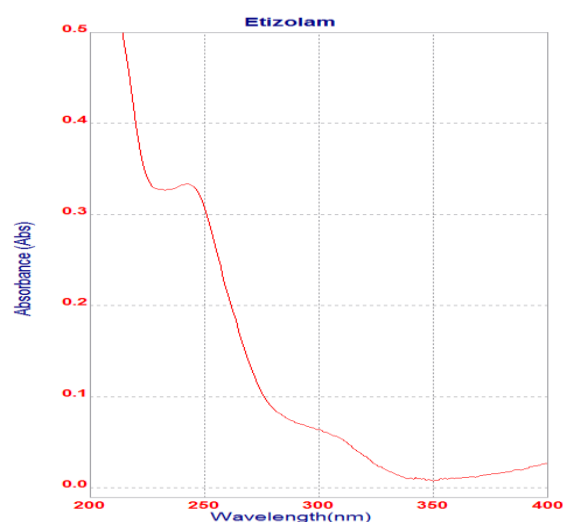
Fig : 1.Spectra of standard Etizolam for wavelength selection.

2.4   Trial batches for selecting optimum solvent system

Trial batches were run as per following chromatographic conditions by using methanol and water in the ratio of 90:10, 80:20 and 70:30 respectively.

Table 1: optimized chromatographic condition

Parameters	Condition
Stationary Phase	Cosmosil C18 (250mm×4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol : water
Pump Mode	Reciprocating
Flow Rate (ml/min)	0.8ml/min
Run Rate (min)	9.79
Volume of Injection (microliter)	20
Detection Wavelength	248
Retention time (min)	7.003

Table 2: System suitability parameters for final optimized chromatographic conditions

Parameters	Data Obtained	
	70 : 30	80 : 20
Retention Time (Rt)	7.003 min	4.345 min
Resolution	0.00	0.00
Theoretical Plates (N)	3827	8695
Tailing Factor (Tf)	1.44	1.15

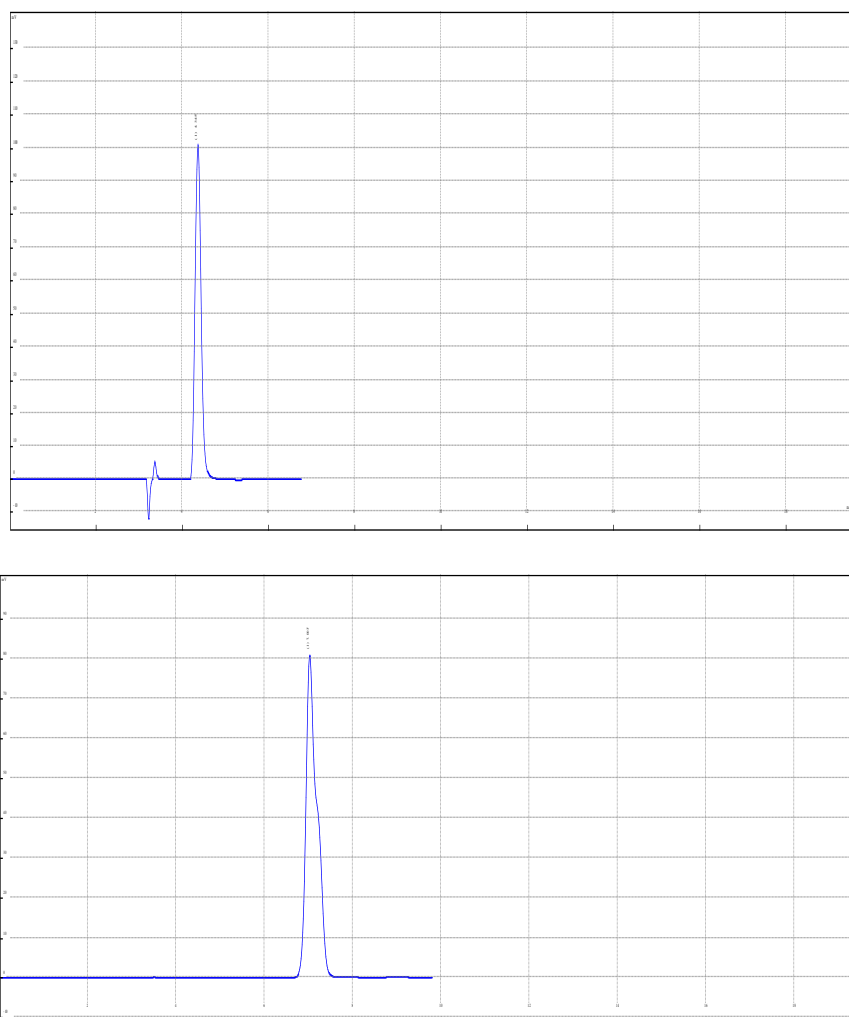Fig2. Chromatogram of standard showing separated peak of Etizolam.

From above findings, it was concluded that the most efficient resolution and peak symmetry for Etizolam achieved with the above chromatographic conditions and with a mobile phase composed of Methanol: water (70:30%v/v)

3. METHOD VALIDATION

The described method has been validated for linearity, accuracy, limit of detection, limit of quantification, and robustness, as per ICH guidelines.

3.1 Linearity

Linear relation was obtained between mean peak area and concentration of the drug in the range of 10-50μg/ml. The data of peak areas obtained with the respective concentration in microgram/ml are shown in Table 3 for Etizolam. The linearity curves for Etizolam shown in Fig 3.

Table 3: Data of peak area of Etizolam (10-50μg/ml)

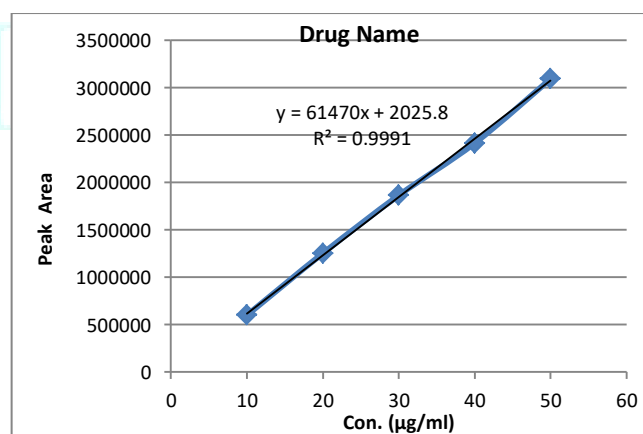| Sr. No.	Concentration (μg/ml)	Peak area of Etizolam
1	10	601683
2	20	1253654
3	30	1865239
4	40	2415986
5	50	3093992

Fig 3: Calibration Curve for Etizolam.

3.2 Accuracy

Accuracy refers to closeness of the test results obtained by the method to the true value. Accuracy was performed by the standard addition methods. To a fixed amount of the pre-analysed mixture add a 50%, 100%, and 150% of the standard solution and % recovery was calculated. The results are shown in table 4.

Table 4: Recovery data for Etizolam

Sr. No	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	1865239	1861110	99.77863427
2	100% Recovery	2415986	2399110	99.30148602
3	150% Recovery	3093992	3078990	99.5151248

3.3. Precision

3.3.1 Repeatability –

It was performed by 100% test concentration level and % RSD was calculated. The data for repeatability for Etizolam is presented in table no. 5

Table 5 : Repeatability data for Etizolam

Sr. No	Conc.	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	%SD	%RSD
	10	601683				
1	10	605015	603668.6667	1755.59572	0.2908211	0.290821077
	10	604308				
	30	1865239				
2	30	1861683	1865816	449.647065	0.2384826	0.2848263
	30	1870526				
	50	3093992				
3	50	3080256	3090886.667	9467.967329	0.3063188	0.306318812
	50	3098412				

3.3.2 Reproducibility –

In that the data for Interday and Intraday precision for Etizolam was performed. It was performed by 100% test concentration level and % RSD was calculated.

A: Interday Precision of Etizolam -

The data for Interday precision for Etizolam are present in table 6. For Interday sample solution containing 100 µg/ml were prepared six times and measured on the different days and % RSD was calculated. The % RSD for Interday precision for Etizolam was found to be 0.33%.

Table 6: Interday Precision -

Sr. No	Day 1	Day 2	Mean	% RSD
1	1865239	1855353		
2	1861683	1860249	1871110	0.33%
3	1870526	1871110		

B: Intraday Precision of Etizolam -

The data for Intraday precision for Etizolam shown in table 7. For Intraday sample solution of 100 µg/ml of Etizolam were prepared six times and measured on the same day and % RSD was calculated. The % RSD for Intraday precision was found to be 0.23%.

Table 7: Intraday Precision -

Sr. No	Morning	Evening	Mean	% RSD
1	1865239	1870976		
2	1861683	1867709	1868304	0.23%
3	1870526	1873693		

3.4 Limit of Detection (LOD) and Limit of Quantification (LOQ) :

3.4.1: Limit of detection (LOD) –

Limit of detection is calculated by using formula,

LOD = 3.3× (SD/Slope)

LOD = 3.3× (0.2384/0.999)

LOD = 0.7875µg/ml.

3.4.2: Limit of Quantification (LOQ) –

Limit of Quantification is calculated by using formula,

LOQ = 10× (SD/Slope)

LOQ = 10× (0.2384/0.999)

LOQ = 2.3863 µg/ml.

3.5: Robustness

Robustness was calculated by using two methods, i.e; change in flow rate and change in wavelength.

3.5.1: The data for Robustness of Etizolam for change in flow rate is shown in table no.8.

Table 8: Robustness data for change in flow rate –

Sr. No	Conc.	Area	Mean	SD	%SD
	20	1250874			
1.	20	1253654	1251021	2562.18	0.20480698
	20	1248536			

3.5.2: The data for Robustness of Etizolam for change in wavelength is shown in table no.9.

Table 9: Robustness data for change in wavelength –

Sr. No	Conc.	Area	Mean	SD	%SD
	20	1253654			
1.	20	1251683	1253657	1975	0.15753925
	20	1255633			

3.6: Ruggedness

The data for Ruggedness of Etizolam is shown in table no. 10

Table 10: Data for Ruggedness of Etizolam –

Sr. No	Concentration	Area
1	10	605246
2	20	1255624
3	30	1868954
4	40	2420158
5	50	3095487

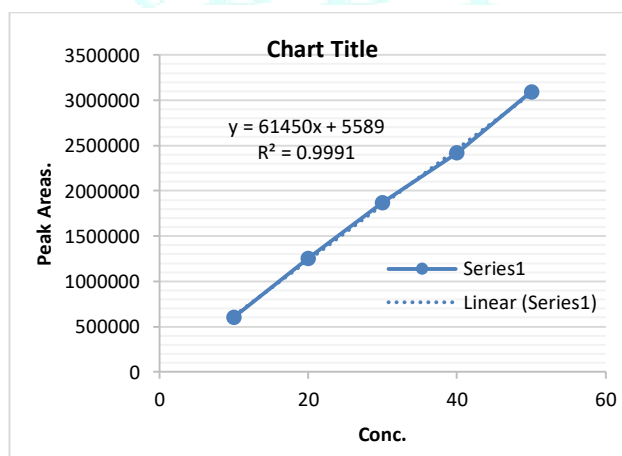The Ruggedness curve for Etizolam is shown in Fig. 4.

Fig 4. Ruggedness curve for Etizolam.

3.7: Assay

By using the standard areas from linearity of reported concentration the % assay was calculated.

The data for assay of Etizolam is shown in table no. 11

Table 11: Data for assay of Etizolam –

Sr. No	% Composition	Area of Standard	Area of Sample	% Assay
1.	% Assay	1865239	1865239	100

4. RESULTS AND DISCUSSION

4.1. Optimized chromatographic conditions

The optimized chromatographic conditions fig 1.The best peak shape and maximum separation was achieved with mobile phase Methanol : Water in the proportion of 70:30 %v/v, peak symmetry and reproducibility obtained on Cosmosil C18 (250mm*4.6ID, Particle size : 5 micron), optimum wavelength. For detecting analyte, λmax was found to be 248nm, A flow rate of 0.8ml/min yielded optimum separation and peak symmetry. Chromatogram of Etizolam fig 2 and optimized chromatographic condition is shown in table 1.

4.2. Linearity

Linear relation was obtained between mean peak area and concentration of the drug in the range of 10-50μg/ml for Etizolam. Shown in fig.3

4.3. Accuracy

The percentage recovery for Etizolam were 99.77–99.51%, shown in table 4 and 5

4.4. Precision

4.4.1. Interday –

The data for Interday precision for Etizolam is present in table.6. The % RSD for Interday was found to be 0.33%.

4.4.2. Intraday –

The data for Intraday precision for Etizolam is present in table.7. The % RSD for Intraday was found to be 0.23%.

4.5. LOD and LOQ

The LOD for Etizolam were found to be 0.7875μg/ml.

LOQ for Etizolam were found to be 2.3863μg/ml.

4.6. Robustness

Result of the Robustness table no. 8. The %SD for Robustness for change in flow rate was found to be 0.2048%. Shown in table no.8.

The % SD for Robustness for change in wavelength was found to be 0.1575%. Shown in table no. 9.

4.7. Ruggedness

The result for Ruggedness curve for Etizolam is shown in fig. 4.

5. CONCLUSION

A simple, fast, accurate and precise RP-HPLC analytical method has been developed and validated for the Quantitative analysis of Etizolam in Bulk form. The results of stress testing undertake according to the ICH guidelines reveal that the method is specific and accurate.

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