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Development and Validation of Stability Indicating RP-HPLC Method for Estimation Etizolam in Tablet Dosage Form

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ABSTRACT:

Stability indicating RP-HPLC method for Etizolam in a tablet formulation was developed which is simple, precise, and sensitive and is applicable for quantification of Etizolam in tablet dosage form. An isocratic reversed phase HPLC method has been developed for the Etizolam on a Zorbax (250 x 4.6 mm) column using a mobile phase consisting of methanol: phosphate buffer pH-5 (42:58,v/v) at a flow rate of 1 mL/min and the detection was carried out at 238 nm. Retention times of Etizolam were found to be 2.28. The method is linear in range of 5-40 µg/ml. The method was validated with respect to specificity, linearity, accuracy, precision, ruggedness and robustness. This method can be used for quantification of Etizolam in tablet dosage form

KEYWORDS: Etizolam, Stability Indicating, RP- HPLC, validation, Zorbax.

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INTRODUCTION:

Etizolam is a thienodiazepine drug which is a benzodiazepine analog. It possesses amnesic, anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant properties.Etizolam is official in a japan pharmacopoeia . Literaturesurvey revealed that few analytical methods are availablefor determination of etizolam pharmaceutical formulations but no stability indicating analytical method has been reported for the simultaneous determination of etizolam.

Materials and methods:

INSTRUMENTATION:

High performance liquid chromatography including a thermo scientific product, UV detector, and zorbax, 5 µm column having dimensions 4.6mm x 250mm was used. Materials and reagents: Etizolam was obtained as a gift sample from astron research centre

Chromatographic condition:

A mobile phase consisted of methanol: phosphate buffer pH-5 (42:58, v/v) was pumped at a flow rate of 1 mL/min. The elution was monitored at 238 nm and the injection volume was 20 µL. The validation of the method was done following the ICH guidelines

Preparation of mobile phase and standard solutions:

Methanol and phosphate buffer used for mobile phase were filtered through 0.22

membrane filter (DuraporeMembrane, Miilipore GV 0.22µm) and degassed by ultrasonication for 15 min. The standard stock solution was prepared by dissolving etizolam in 100 ml of methanol to get a solution containing 100 µg /mL of etizolam and . Working standard solution was prepared by diluting 20ml of the stock solution with methanol to 100 ml to get a solution containing 20µg/mL of etizolam

Preparation of the sample solutions

Take equivalents weigth of 1 mg etizolam was dissolved in 10 mL of methanol and was sonicated for 20 min. The resulting mixture was filtered through 0.45µ membrane filter (SY25TG,mdi Membrane Technologies, California USA).The filtrate thus obtained was used for analysis.

STABILITY STUDY[7-28]

1. Preparation of hydrochloric acid solution (0.1 N):

Concentrated hydrochloric acid (0.85 ml) was transferred in 100 ml volumetric flask and diluted up to the mark with water.

2. Preparation of sodium hydroxide solution (0.1 N):

Accurately weighed 0.4 gm of sodium hydroxide was transferred in 100 ml volumetric flask and diluted up to mark with water.

3. Preparation of hydrogen peroxide solution (3% w/v):

Hydrogen peroxide (10 ml, 30%) was transferred in 100 ml volumetric flask and diluted up to the mark with water.

4. Acid hydrolysis:

Accurately measured 1 ml of Etizolam (100 µg/ml) and 1 ml 0.1N HCl was transferred in to 10 ml volumetric flask solution was heated for 2 hours at 80^{o} C for acid hydrolysis. neutralize with 0.1N NaOH Make up the volume with methanol up to mark to get 10 µg/ml . Filtered through 0.45 µm membrane filter paper and injected in to HPLC system.

5. Base hydrolysis:

Accurately measured 1 ml of Etizolam (100 µg/ml) and 1 ml 0.1N NaOH was transferred in to 10 ml volumetric flask , solution was heated for 2 hours at 80^{o} C for base hydrolysis. Make up the volume with methanol up to mark to get 10 µg/ml and Filtered through 0.45 µm membrane filter paper and injected in to HPLC system.

6. Oxidative hydrolysis:

Accurately measured 1 ml of Etizolam (100 µg/ml) and 1 ml of 3% H_2O_2 was transferred in to 10 ml volumetric flask solution was heated for 2 hours at 80^{o} C for oxidative hydrolysis.Than up the volume with methanol up to mark to get 10 µg/ml Filtered through0.45 µm membrane filter paper and injected into HPLC system.

7. Thermal Degradation:

For dry heat degradation study, Tablet powder equivalent to 5 mg Etizolam was spread over petri dish and exposed to dry heat (80˚C) for 12 hour in an oven then from that powder was transferred to 50 ml volumetric flask and dissolved in 30 ml of mobile phase. The flask was sonicated for 5 min and volume was made up to mark with mobile phase to get 100 µg/ml of Etizolam. Further dilution was carried out by diluting 1 ml of solution in 10 ml volumetric flask with mobile phase to get 10 µg/ml of Etizolam.

Calibration curve of standard of Etizolam

A calibration curve was plotted over a concentration range of 5-40 µg/ml for Etizolam. Accurately measured working standard stock solution of Etizolam (0.5,1, 1.5, 2, 3, and 4 ml) were transferred to a series of 10 ml volumetric flasks and the volume in each flask made up to the mark with methanol to get concentration range of 5-40 µg/ml for Etizolam. The resulting solution was injected into the column and the peak area obtained at 2.3 minute at flow rate 1 ml/min were measured at 238 nm for Etizolam. Calibration curve was constructed by plotting peak area versus concentration at 238 nm.

4.7 Method validation for ETIZOLAM

A. Linearity

The linear response of Etizolam was determined by analyzing six independent levels of the calibration curve in the range of 5-40 µg/ml for Etizolam

B. Precision volumetric flask and dissolved in 30 ml of mobile phase. The flask was sonicated for 5 min and volume was made up to mark with mobile phase to get 100 µg/ml of Etizolam. Further dilution was carried out by diluting 1 ml of solution in 10 ml volumetric flask with mobile phase to get 10 µg/ml of Etizolam.

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4.7 Method validation for ETIZOLAM

A. Linearity

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It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variance of a series of measurements.

1) Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Repeatability of method was performed by preparing the test solution of etizolam for six times from tablet dosage form and analyzed as per the proposed method. Percentage relative standard deviation (RSD) should be less than 2% (Table 5.4).

2) Intermediate Precision

It expresses within laboratory variations as on different days analysis or equipment within the laboratory

a. Intra-day precision

Variation of results within same day is called Intra-day precision. The Intra-day precision for HPLC method was determined for three concentration of Etizolam solution for the three times on the same day

b. Inter-day precision

Variation of results amongst days called Inter-day precision. The Inter-day precision for HPLC method was determined for Etizolam for three days

D. Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions.

Limit of detection can be calculated using following equation as per ICH guidelines

$$\mathrm{LOD} = \frac{3.3\ \sigma}{\mathrm{S}}$$

Where , σ = Standard deviation of the y intercept

S = Slope of the calibration curve

E. Limit of Quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guideline.

$$\mathrm{LOQ} = \frac{10\ \sigma}{\mathrm{S}}$$

Where , σ = Standard deviation of the y intercept

S = Slope of the calibration curve

F. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness of the method was studied by changing the flow rate; change in wavelength, change in pH, and composition of mobile phase and change in response of drugs were studied.

G. System Suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used.

The parameters used in these were asymmetry of chromatographic peak, Theoretical plates, resolution, retention time and repeatability as RSD of peak area for replicate injections.

RESULT AND DISCUSSIONS:

Several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained by using the mobile phase containing methanol: phosphate buffer pH-5 (42:58, v/v). Quantification was achieved with UV detection at 238 nm based on peak area.

A representative chromatogram is shown in Chromatogram of etizolam System suitability tests were carried out on freshly prepared standard solutions (n = 6) containing etizolam. System suitability parameters obtained with 1 µL injection volume are summarized

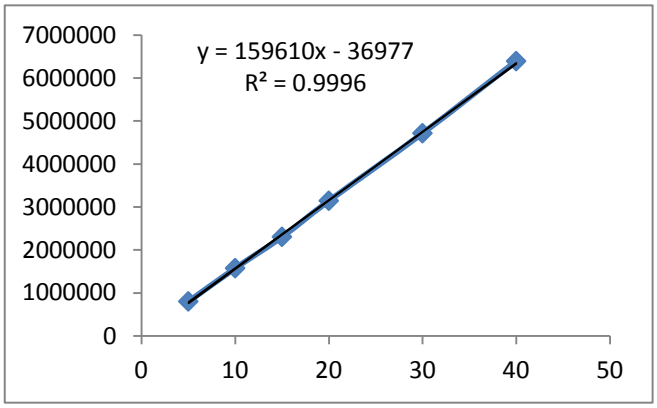

Figure 1 linearity graph

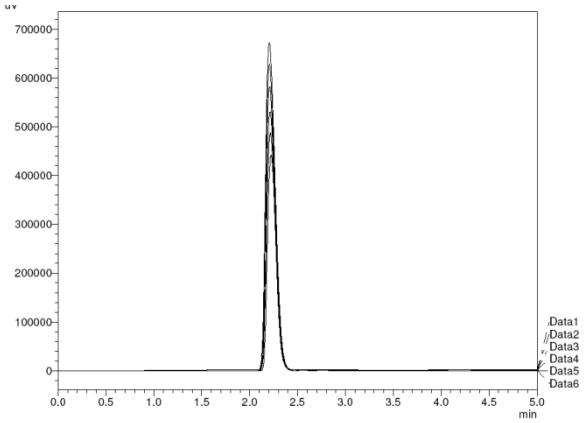

figure 2 linearity curve of etizolam

Table 1 linearity range

Sr. No.	Conc. (µg/ml)	Area (Mean ± S.D, n=3)	%RSD
1	5	805379±6360.96	0.78981
2	10	1572946±5850.253	0.37193
3	15	2307884±2315.83	0.100344
4	20	3147074±3210.073	0.102002
5	30	4649755±55925.75	1.202759
6	40	6427349±62471.4	0.971962

PRECISION

Repeatability:

Relative standard deviation of all the parameters is less than 2% (Table 5.3), which indicates that the proposed method is repeatable.

Intermediate Precision

The low % RSD values of intra-day and inter-day precision reveals that the proposed method is precise

LOD and LOQ

Based on calibration curve LOD and LOQ was calculated for

Table 3 LOD & LOQ

DRUG	LOD(µg/ml)	LOQ(µg/ml)
Etizolam	0.391855	1.187439

System Suitability Parameters:

Statistical analysis of parameters required for system suitability testing of the HPLC method

Table 4 System Suitability Parameters for HPLC method

System Suitability Parameters	Etizolam
Tailing Factor	1.47
Theoretical Plates	20024
Retention Time (min.)	2.28
Area ± SD	1572946
% RSD	0.37193

5.5 Application of Proposed Method for analysis of Marketed formulation

The proposed method was applied successfully for analysis of marketed formulation and results obtained are shown in following table.

CONCLUSION :

Parameter	Etizolam
Linearity	5-40(µg/ml)
Precision(% RSD, NMT 2)	
Repetability	1.32
Intermediate pricision	0.66
Accuracy (% recovery)	100.8
LOD	0.8
LOQ	0.15
Assay ±%RSD	99.97±1.0

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