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[Research article]

METHOD DEVELOPMENT AND VALIDATION ON ETOMIDATE INJECTION BY RP-HPLC

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ABSTRACT

A new simple, accurate, rapid and precise isocratic High performance liquid chromatographic (HPLC) method was developed and validated for the determination of Etomidate (ETO) injection. The Method employs Waters HPLC system on Develosil –ods-UG column (300 x 3.9 mm x 5µm) and flow rate of 1.5 mL/min with a load of 20 µL. Acetonitrile and Phosphate buffer was used as mobile phase in the composition of 40:60. The Detection was carried out at 254 nm. Linearity ranges for Etomidate was 40-240 µg/ml respectively. Retention Time of Etomidate was found to be 12.061 minutes respectively. Percent recovery study values of Etomidate were found to be within 98-102 %. This newly developed method was successfully utilized for the Quantitative estimation of Etomidate in injectables. This method was validated for accuracy, precision, linearity and Robustness as per ICH guidelines.

Keywords: Etomidate, RP-HPLC, Validation.

INTRODUCTION

Etomidate [R-1-(1-ethylphenyl) imidazole-5-ethyl ester] is a unique drug used for induction of general anesthesia and sedation. The first report on etomidate was published in 1965 as one of several dozen aryl alkyl imidazole-5-carboxylate esters synthesized by Janssen pharmaceuticals (a division of ortho-Mcneil-Jansen pharmaceuticals, titussville, New Jersey, USA) initially developed as anti-fungal agents the potent hypnotic activity of several compounds, including etomidate appeared significantly barbiturates.

MATERIALS AND METHODS

Chemicals

Etomidate was obtained HUZHOU ZHAN WANG pharmaceutical co. ltd ., Hyderabad, India as a gift sample respectively. Monobasic sodium dihydrogen phosphate (AR Grade), ortho-phosphoric acid (AR Grade), Acetonitrile (HPLC Grade), was purchased from Merck (India) Ltd., Worli, Mumbai, India. Formulation Etomidate injection was obtained from Aurobindo containing ETO (2mg/ml). Double distilled water was used throughout the experiment.

Instrumentation

Analysis was performed on the chromatographic system of Equipment. High–performance liquid chromatography ALLIANCE e2695, PDA DETECTOR 2998 equipped with auto sampler .Hplc is controlled by Empower 2 software .Column Develosil –ods-UG (300 x 3.9mm x 5µm), Mobile phase having monobasic sodium

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dihydrogen phosphate Buffer and Acetonitrile (60 : 40) where flow rate is 1. 5mL / min ,Wavelength is 254 nm, Injection volume 20 (L, Column oven at Ambient and Run time 20 minutes. Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed.

Preparation of Standard Solution

Accurately weighed about 50mg of Etomidate working standard are transferred separately into 25mL clean dry volumetric flask, added about 5mL of mobile phase and sonicated to dissolve it completely and make the volume up to the mark with diluent. Further pipette out 4mL of the Etomidate of above stock solution into a 50mL volumetric flask and diluted up to the mark with diluent to get the concentration of 160µg/mL respectively.

Preparation of Sample Solution (Marketed formulation)

Pipette out 4mL of sample solution from a vial and transferred to a 50 mL volumetric flask finally make the volume up to the mark with the diluent.

Validation of Method

The HPLC method was validated in accordance with ICH guidelines.

Precision

The precision of the method was performed in the intra-day studies five repeated injections of standard solution were made and the response factor of drug peak and % RSD was calculated. .From the data obtained the developed method was found to be precise.

Accuracy

The accuracy of measurement is defined as the closeness of the measured value to the true value. Typically, accuracy is represented and determined by recovery studies. This study was performed by spiking analyte matrices. For assay methods, spiked placebo samples are prepared at three concentrations of 50%, 100% & 150%.

Robustness

Robustness was evaluated by making deliberate variations in few method parameters such as variation of the temperature; flow rate, change in mobile phase composition, wavelength. The robustness of the method was studied by using 160 µg mL-1 of ETO respectively.

Limit of detection and Limit of quantitation

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ).

LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S,

Where 'ASD' is the average standard deviation and 'S' is the slope of the line.

RESULTS AND DISCUSSION

Selection of Chromatographic Conditions and Optimization of Mobile Phase preparation of Phosphate buffer

Weighed 0.7 grams of NaH_2PO_4 and transferred into a 1000mL beaker dissolved and diluted to 1000mL with HPLC grade water. Adjust the P^H 4.8 with ortho phosphoric acid.

Preparation of mobile phase

A mixture of above buffer (PH 4.8) 600 ml (60%) and 400 mL of Acetonitrile
(40%) were mixed and degassed in ultrasonic water bath for 5 minutes, finally filtered through 0.45 µm membrane filter. Diluent Preparation Acetonitrile was used as Diluent and chromatogram shown in figure 1.

Linearity

The linearity of the method was demonstrated over the concentration ranges of 40 to 240 µg/mL for ETO. Preparation of standard stock solution 50mg working standard drug is transferred into 25mL volumetric flask, which contains 2000 µg/mL further pipette out Aliquots of 1mL, 2mL, 3ml, 4mL, 5mL, 6mL and for Etomidate was prepared from above prepared standard stock solution. Different concentrations of the pure drugs were injected into the chromatographic system. Calibration curve of Etomidate were constructed by plotting peak area vs applied concentrations and shown in Fig. 2. The obtained results have shown an excellent correlation between peak area and concentration of pure drug within the concentration range. The correlation coefficient for the average area at each level vs concentration of analyte was calculated and presented in Table 1.

Precision

The precision of the analytical method was studied

by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or relative standard deviation. In the above Precision, study was assessed by injection repeatability tests. For injection, repeatability mixed standard solution of ETO was injected in replicate. In this method, precision was confirmed by low % RSD values of peak area for all components and reported in Table 2. The % RSD values was 1.172 value should be within 2, and the method was found to be precise.

Accuracy

Preparation of Standard Solution

Accurately weighed about, 50 mg of Etomidate working standards are transferred separately into 25 ml clean dry volumetric flask, added about 5ml of diluent and sonicated to dissolve it completely and make the volume up to the mark with diluent. Further pipette out 4 ml of the Etomidate above stock solutions into a 50mL volumetric flask and diluted up to the mark with diluent to get the concentrations of 160µg/ml respectively. These stock solutions were filtered through 0.45µm membrane filter paper by using the vacuum filters.

Preparation of Sample solution

Preparation of 50% solution

Accurately weigh and transfer 25 mg of Etomidate working standard was separately dissolved into a 25 ml clean dry volumetric flask. Add 4mL of placebo and sonicate to dissolve it completely and make volume up to the mark with the diluent. Further pipette 4 ml of etomidate of the above stock solution into a 50 ml volumetric flask and dilute up to the mark with diluent.

Preparation of 100% solution

Accurately weigh and transfer 50 mg of Etomidate working standards was dissolved separately into a 25mL clean dry volumetric flask. Add 4ml of placebo and sonicate to dissolve it completely and make volume up to the mark with the diluent. Further pipette 4ml of etomidate of the above stock solution into a 50mL volumetric flask and dilute up to the mark with diluent.

Preparation of 150% solution

Accurately weigh and transfer 75 mg of Etomidate working standards was dissolved separately into a 25mL clean dry volumetric flask. Add 4ml of placebo and sonicate to dissolve it completely and make volume up to the mark with diluent. Further pipette 4mL of etomidate of the above stock solution into a 50mL volumetric flask and dilute up to the mark with diluents.

The RP-HPLC method developed in the present study has been used to quantify ETO. The average area was taken and % accuracy was calculated. The mean recoveries were found in the range of 98 - 100%. The results are presented in Table 3. No interfering peaks were found in the chromatogram indicating that Excipients usually present in the Etomidate injection did not interfere with the estimation of the drug by the proposed RP-HPLC method.

Sensitivity

The LOD ETO was found to be 1.088 µg/ml respectively. The LOQ for ETO was found to be 3.29 µg/ml respectively. The low values of LOD and LOQ indicates high sensitivity of the method.

Robustness

Robustness of the method was studied by making deliberate changes in the chromatographic conditions, and the effects on the results were examined. the low values of % RSD (less than 2 %) indicating robustness of the method.

Analysis of marketed tablet formulation

Six replicates of the samples solutions (20 µL) were injected for quantitative analysis. The amount of ETO estimated was found to respectively. Drug indicates that there was no interference from the excipients commonly present in pharmaceutical formulations. The results are shown in Table 4.

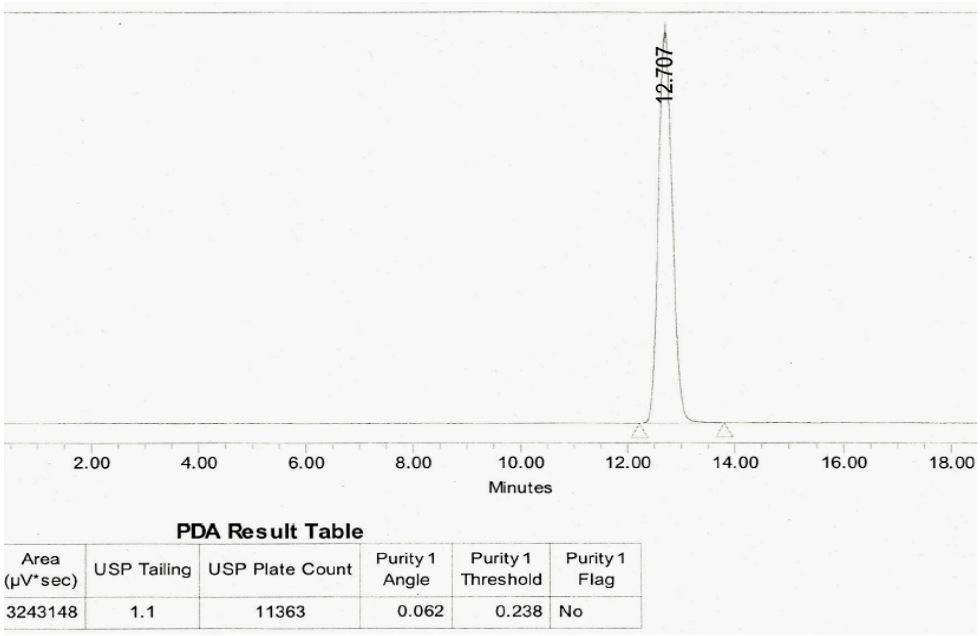
System Suitability Test

According to USP, system suitability tests are integral part of liquid chromatographic methods. The resolution, number of theoretical plates, peak asymmetry and capacity factor were calculated for standard solutions. The results obtained from validation of the methods and system suitability studies are summarized in Table 5.

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method is adequately rugged and robust and can be used for simultaneous determination of thiocolchicoside and etodolac in tablet formulation. The method was validated as per ICH guidelines.

Figure 1 Optimized chromatographic condition



PDA Result Table

Area (µV*sec)	USP Tailing	USP Plate Count	Purity 1 Angle	Purity 1 Threshold	Purity 1 Flag
3243148	1.1	11363	0.062	0.238	No

Figure 2 calibration curve of Etomidate

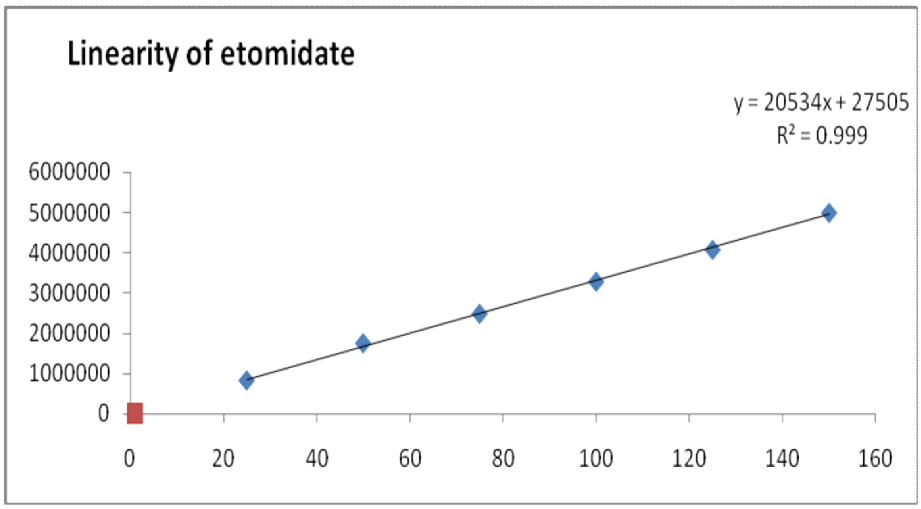


Table 1.Linearity data

SNO	INJECTIONS	AREA	AVERAGE
1	INJECTION1	814671	814690.5
	INJECTION2	814710	
2	INJECTION 1	1745440	1746445
	INJECTION2	1747449	
3	INJECTION1	2480971	2480433
	INJECTION2	2479894	
4	INJECTION1	3282186	3282767
	INJECTION2	3283348	
5	INJECTION1	4076661	4078178
	INJECTION2	4079695	
6	INJECTION1	4987923	5001493
	INJECTION2	5015062	

Table 2. Precision

SNO	INJECTIONS	AREA
1.	INJECTION 1	3177238
2.	INJECTION 2	3280549
3.	INJECTION 3	3224041
4.	INJECTION 4	3199547
5.	INJECTION 5	3225432
6.	INJECTION 6	3183621

Table 3. Accuracy

Drug	% Level	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean % recovery
ETO	50	0.08064	0.08028	99.59	99.48
	100	0.161209	0.162476	100.78	
	150	0.241813	0.237152	98.07	

Table 4. Assay Results

S.No.	DRUG NAME	LABLE CLAIM (mg/mL)	AMOUNT FOUND (mg/mL)	% ASSAY FOUND
1	ETOMIDATE	2	1.919461	98.80

Table 5. System suitability parameters

S.No.	Parameters	ETOMIDATE
1	% R.S.D	0.2
2	Tailing factor (T)	1.1
3	No. of theoretical plates (N)	11336

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