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Research Article

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Development and Validation of a UV-Visible Spectrophotometer Method for Estimation of Etomidate Spiked in Human Plasma

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Abstract UV-visible spectrophotometer method for determination of etomidate in human plasma was developed and validated. Acetonitrile and water (75:25) as diluent. The absorption maximum for the drug was observed at 243nm in methanol. The calibration curve range from 5-60µg/ml. the % recovery was found to be 98-100%, the applicability of the method was examined by analysing the human plasma. The method was successfully applied for estimation of etomidate in pharmaceutical formulations.

Keywords Etomidate, validation, plasma, pharmaceutical formulations

1. Introduction

Etomidate is a general anesthetic that acts as a level of reticular- activating system to produce anesthesia.it is an imidazole compound that depresses CNS functions via GABA.it does not induce analgesia like barbiburates and propofol. Etomidate is currently approved for induction and maintenance of general anesthesia and sedation. The structure is a carboxylated imidazole and the chemical compound is [R-1-(1-ethylphenyl) imidazole-5-ethyl ester] with a molecular weight of 342.36 kilodaltons. Since the introduction of etomidate into clinical practice in 1972.it has gained popularity due to its minimal side effects. Etomidate does not inhibit myocardial function, and anesthetic induction tends to produce minimal blood pressure and heart rate changes in patients. Etomidate is used to increase seizure duration potential when compare to propofol and thiopental, As it is most favorable profile for anesthetic induction in many critical cases.propofol is also used as replacement for etomidate it also acts as sedative drug. In the present study an attempt was made to develop a simple, precise and accurate method for the estimation of drug in pharmaceutical dosage form and validate as per International Conference on Harmonization (ICH) guidelines.

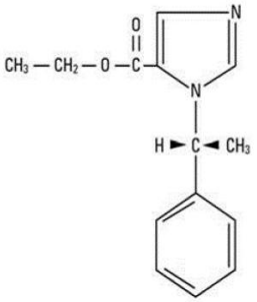

Figure 1: Structure of Etomidate

2. Material and Methods

2.1. Instruments

A double beam UV-Visible spectrophotometer "ELICO SL 210" and double beam UV-Visible spectrophotometer "SYSTRONIC 2203"

2.2. Chemicals

Etomidate was obtained as gift sample from pharmaceutical industry, Hyderabad, Telangana, India. Celodate injection (2mg/ml) was purchased from local market. Human plasma was procured from the healthy volunteers. Analytical reagents such as acetonitrile, methanol was used.

3. Extraction of Etomidate from Plasma

Previously frozen blood plasma were thawed from which 400µl was placed into the glass tube to which 1ml of ACN and methanol (75:25) was added. The tube was vortex mixed at low speed for 10 sec to mix the solutions. After which the tubes were covered with paraffin and placed in refrigerator for 10mins. The tube was then again vortex mixed for 10 sec and centrifuged at 2000 rpm for 15mins. The supernatant was removed into a clean glass test tube.

4. Method Development

4.1. Preparation of Standard Solution

Standard solution of Etomidate drug was prepared by taking 10mg in 10ml volumetric flask and was diluted using methanol and the volume was made up to the mark with methanol. From the above solution 0.1ml was pipetted out into 10ml volumetric flask and made up to the mark with methanol to get 10µg/ml.

4.2. Selection of Wavelength

Solutions of 10µg/ml of Etomidate were prepared and the solution was scanned in the spectrum mode from 200nm to 400nm. The maximum absorbance of Etomidate was observed at 243nm

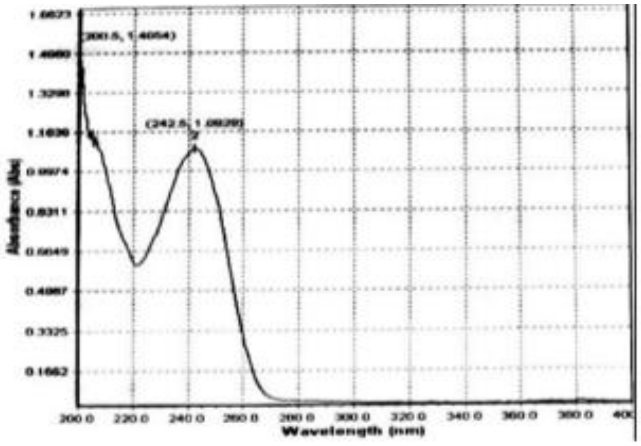

Figure 2: UV spectrum of standard solution

4.3. Optimization of Parameters

All the optimization parameters were performed at room temperature. Etomidate was found to yield clear colorless solution with methanol, showing maximum absorbance at 243nm. Various concentrations and various volumes were tried for all the solvents. The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance.

4.4. Preparation of Calibration Curve

Standard stock solution of Etomidate was further diluted to get concentration in the range of 5-60µg/ml. The resultant absorbance of the solution was measured at 243nm using methanol as blank.

5. Method Validation

The developed method was validated as per ICH guidelines. The proposed method was validated in terms of specificity, linearity, precision, accuracy, robustness, LOD and LOQ.

5.1. Specificity

It is the ability to assess unequivocally the analyte in the presence of components which are expected to be present typically this may include degradants, impurities etc.

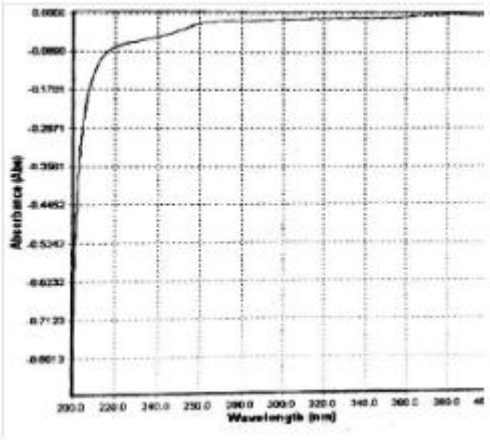

Figure 3: Blank Spectrum

5.2. Linearity

The linearity of analytical procedure is its ability to obtain test result within the range is directly proportional to the concentration of the analyte of the sample.

The proposed method was found to be linear in the range of 5-60µg/ml with correlation coefficient was 0.9995 (figure 4), slope and intercept was shown in table 1.

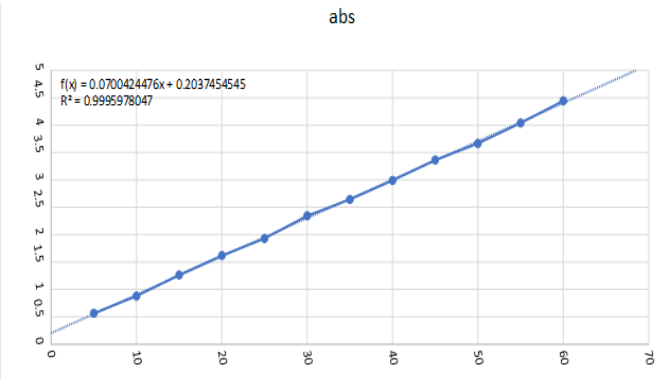

Figure 4: Linearity Chart

Table 1: Results of quantitative determination of etomidate

S. No.	Parameters	Results
1.	Absorbance maximum(nm)	243
2.	Linearity and range(µg/ml)	5-60 µg/ml
3.	Slope	0.070042
4.	Correlation coefficient	0.9995
5.	Y-intercept	0.203745

5.3. Precision

The precision of analytical procedures the closeness of agreement between a series of measurement obtain from multiple sampling fro same homogenous sample under the prescribed conditions.

The precision of the proposed method was estimated in terms of inter-day and intra-day precision wherein the method was repeated for 6 times respectively. The results shown in table 2 indicating %RSD of less than 2% each level clearly indicate that the proposed method was precise enough for the analysis of drug.

%RSD = (SD of measurement/mean value of measurement) x 100.

Table 2: Results of Precision

Concentration	Intraday precision (%RSD)	Inter-day precision (%RSD)
10µg/ml	0.02126	Day 1:0.4252 day 2: 0.8951

5.4. Accuracy

Accuracy is the closeness of the test results obtained by that of true value.

The accuracy of the method was determined by performing recovery studies by spiking standard solution to that of the human plasma at three different levels i.e., 50%, 100%, 150%. Values of %recovery greater than 90-125% indicate that the proposed method was accurate for the analysis of drug and the results were reported in table 3.

% Recovery = (Amount found/ Amount added) x 100

Table 3: Results of accuracy studies

Level	Amount of standard added (µg/ml)	Pre-analysed sample (µg/ml)	% Recovery
50%	5	10	98.33%
100%	10	10	99.95%
150%	15	10	100.89%

5.5. Robustness

The capacity of remaining unaffected when Deliberately changes are made in the method such as wavelength. + or- nm from the fixed wavelength.

The robustness of the proposed method was evaluated by changing wavelength. The %RSD was calculated. The low values of %RSD obtained after small deliberate changes in method indicates that the method was robust and the results were presented in table 4.

Table 4: Results of Robustness

S. No.	Concentration	Wavelength	% RSD
1	10µg/ml	241	0.0251
2		242	0.0498
3		242	0.0214
4		245	0.0151

5.6. Ruggedness

The degree of reproducibility of the results obtained by the analysis of the sample under a variety of conditions such as different instrument and different analyst.

The ruggedness of the proposed method was evaluated by varying conditions different analyst and different instrument (“ELICO SL 210" and "SYSTRONIC 2203"). The %RSD was calculated. The low values of %RSD obtained by changing the conditions indicates that the method was rugged and the results were presented in table 5.

Table 5: Results of Ruggedness

S. No.	Concentration	Analyst	% RSD	Instrument	% RSD
1	10µg/ml	Analyst1	0.035%	Instrument 1	0.0248
2		analyst2	0.035538%	(SYSTRONIC)	
				instrument 2	0.0418
				(ELICO)	

5.7. LOD

The detection limit of analytical procedure were lowest amount of analyte can be detected but not quantitated as exact value.

LOD = 3.3 x SD/ slope.

The LOD of the proposed method was found to be 0.00432µg/ml.

5.8. LOQ

The quantitation limit of an analytical procedure was lowest amount of analyte can be detected and quantitatively determined with suitable precision and accuracy.

LOQ = 10 x SD/ slope.

The LOQ of the proposed method was found to be 0.02669 µg/ml

5.9. Analysis of Injectable Formulation Studies (Assay)

Celodate injection of etomidate was prepared by by taking 0.1ml from the vial in 10ml volumetric flask and diluted with methanol to obtain 10µg/ml. The absorbance of the dilution was observed at selected wavelength and the concentration was obtained from calibration curve method.

The % assay is calculated by using the following formula

% Assay = [(Absorbance of the sample/ Absorbance of the standard)*(Concentration of the standard/ Concentration of the sample)]*100.

=99%

6. Conclusion

A simple and selective UV Spectrophotometric method was developed for the estimation of Etomidate in injectable formulations. The developed method was validated as per ICH guidelines.

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