ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Development and Validation of a Simple UV Spectrophotometric and Stress Studies Method for the Determination of Etomidate both in Bulk and Marketed Dosage Formulations

Dr.K. Bhavyas*, Sumaiya Tasneem, Dr.Mogili.Sumakanth .
Department of Pharmaceutical Analysis,
RBVRR Women's College of Pharmacy, Barkatpura, Hyd, India. Pincode:500027

Abstract:
A simple, accurate, precise UV spectrophotometric method was developed in pure and pharmaceutical formulations for etomidate. Etomidate exhibiting maximum absorbance at 243 nm using diluent as methanol and distilled water in the ratio (75:25). the method was validated for linearity, precision, sensitivity, and specificity. The drug obeyed linearity at concentration range of 0.1-34µg/ml. The proposed method was validated statistically with significant high value of correlation coefficient 0.9994. The percentage recovery value for etomidate for the proposed method for 50%, 100%,150% was in the range of 98-100.89%. The values of %RSD precision studies were found to be less than 2%. Forced degradation studies was performed on acid hydrolysis, alkaline hydrolysis, oxidation, photolytic and thermal and degraded absorbance was found. Therefore, the proposed method could be applied for the routine analysis of pharmaceutical dosage forms and injectables containing etomidate.

Key words: Etomidate, UV spectrometry, method development, validation.

1. INTRODUCTION:

Etomidate is currently approved for induction and maintenance 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. The structure is a carboxylated imidazole and the chemical compound is [R-1-(1-ethylphenyl) imidazole-5-ethyl ester] etomidate is the only anesthetic agent marketed as an active isomer, with a molecular weight of 342.36 kilodaltons. Since the introduction of etomidate into clinical practice in 1972.it initially gained widespread popularity due to its many beneficial properties with minimal side effects.

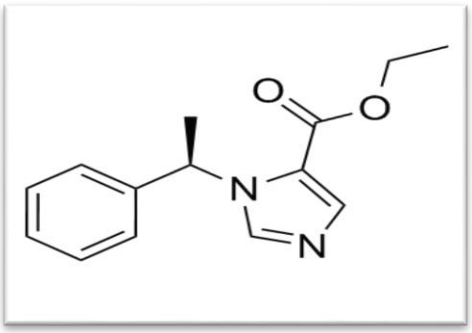

Figure 1: structure of etomidate

2. MATERIALS AND METHODS:

2.1. Apparatus:

Weighing balance, a double beam UV-Visible spectrophotometer "ELICO SL 210" and double beam UV-Visible spectrophotometer "SYSTRONIC 2203".

Chemicals and reagents: etomidate was gifted by pharma company,Hyderabad, Telangana, India. celodate (etomidate injection) 2mg/ml was purchased from local market. HPLC grade methanol procured from Rankem chemicals limited, New Delhi, India, and distilled water was used.

Instrumentation: Double beam UVspectrophotometer; Model: SL 210; Make: ELICO. The data was obtained using Spectra Treats the analysis was performed using UV SL120 using UV detector used for method development and validation. The output signal was checked and the acquisition and integrationof data was performed using spectral threats, Software on a computer.detection was monitored at 242nm.

Procedure:

Selection of wavelength: 10mg of etomidate drug was accurately weighed and transferred into 10 ml volumetric and makeup to the mark by using methanol and water in the ratio 75:25 as diluent. Then from this 0.1 ml was pipped out and transferred into another 10ml volumetric flask and make up to the mark using methanol and water to give 10ppm solution and this was scanned between 200 to 400nm and its absorbance was measured at 242nm (figure 2)

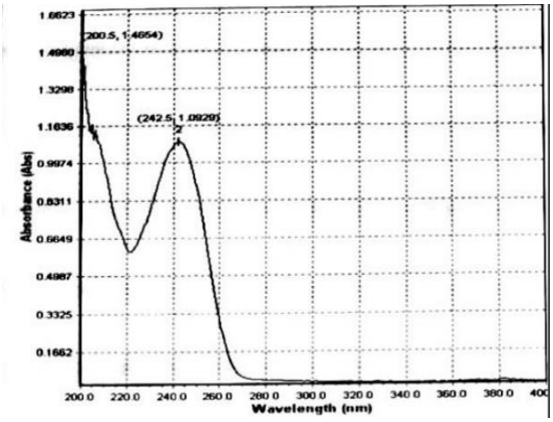

Figure 2: UV spectrum of etomidate

3. ASSAY:

Preparation of standard solution: 10mg of etomidate drug was accurately weighed and transferred into 10 ml volumetric and makeup to the mark by using methanol and water in the ratio 75:25 as diluent. Then from this 0.1 ml was pipped out and transferred into another 10ml volumetric flask and make up to the mark using methanol and water to give 10ppm solution and this was absorbance was measured at 242nm.

Preparation of test solution:0.2ml of drug was pipped out from celodate (etomidate injection) and transferred into another 10ml volumetric flask and make up to the mark using methanol and water Then from this 0.1 ml was pipped out and transferred into another 10ml volumetric flask and make up to the mark using methanol and water to give 10ppm solution and this was absorbance was measured at 242nm.

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

4. METHOD VALIDATION:

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

4.1. Specificity:

Definition: The ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components.

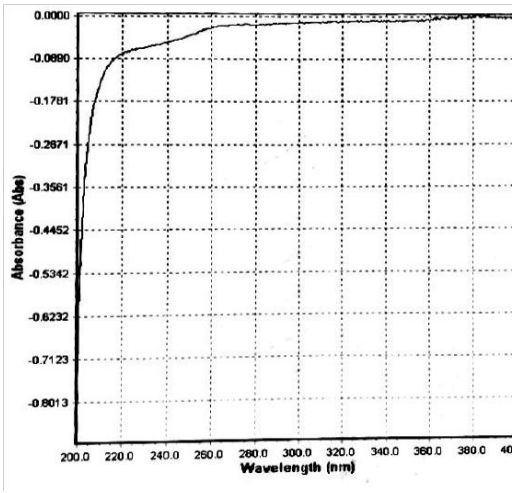

Figure 3 Blank spectrum

4.2. Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample

The proposed spectroscopic method was found to be linear in the range of 0-36 µg/ml with correlation coefficient was 0.9994 slope 0.1055 and intercept 0.0751 as shown in the table 1.

Table 1: Linearity study

S. No.	Parameters	Results
1.	Absorbance maximum(nm)	242
2.	Linearity and range(µg/ml)	0.1-34µg/ml
3.	Slope	0.1051
4.	Correlation coefficient	0.9997
5.	Y-intercept	0.0751

4.3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility,It was examined by evaluation of 6 replicates of etomidate standard solution under the same experimental conditions.

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

=0.000736/1.7784 x100

=0.0413

Limit=%RSD was found to be in limits less then 2 as per USP.

4.4. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy was determined by spiking standard solution to sample solution at three concentration i.e. 12.5ppm 15ppm 17.5ppm. Three replicates of each concentration level were prepared and absorbance was measured at wavelength. % recovery was determined.

4.5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, and deliberate variations in method parameters and provides an indication of its reliability during normal usage. 1- Change in pH of mobile phase. 2- Change of temperature 3- A little bit change of column.

Six aliqutes of 10ppm was prepared as standard solution and scanned under wavelenghth (+/-)1nm and the absorbance was noted down.

4.6. Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different days, etc.

10ppm standard solution was prepared and scanned for 6 times by different analyst and different instruments.

4.7. Limit of Detection (LOD):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.Limit of detection was calculated for etomidate by using the standard formula 3.3 x sd/slope as consistent With ich guidelines Where σ = the standard deviation of the response S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

4.8. Limit of quantification (LOQ):

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.Limit of quantification was calculated for etomidate by using the standard formula 10 x sd/slope as consistent With ich guidelines

Where σ = the standard deviation of the response S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

5. Stability studies

Stability evaluation should be carried out to ensure that every step taken during sample preparation, processing and analysis as well as the storage conditions used do not affect the concentration of the analyte.

The following stability tests should be evaluated:

a) Freeze-thaw stability

b) Bench-top/short-term stability

Freeze-thaw stability:

It will be performed for 3 cycles. The solution should be stored in the freezer for at least 24 hours, after that the same samples are withdrawn from the freezer and allow them to thaw unassisted at room temperature and then refreeze the samples for minimum of 12 hours. Repeat the same exercise for two or more times. While thawing at each cycles, the samples should be uncapped. After the required number of cycles the absorbance was measured at 242nm.

Bench-top stability:

It will be performed to evaluate the stability of the samples, which are kept on bench during the process. The anticipated time for the bench top stability(usually 4 to 24 hours) should cover the duration of the time and then the absorbance was measured at 242nm.

6. Force degradation studies

Acid hydrolysis: From the standard stock solution take 1ml separately in two 10ml volumetric flask. To that add 1ml of 0.1N HCl in one volumetric flask and 1N HCl in another volumetric flask and kept it aside for 24hrs. After completion of time neutralize it with 1ml of 0.1N NaOH to 0.1N HCl flask and 1ml of 1N NaOH to 1N HCl flask. Then the absorbance was measured at 242nm.

Alkali hydrolysis: From the standard stock solution take 1ml separately in two 10ml volumetric flask. To that add 1ml of 0.1N NaOH in one volumetric flask and 1N NaOH in another volumetric flask and kept it aside for 24hrs. After completion of time neutralize it with 1ml of 0.1N HCl to 0.1N NaOH flask and 1ml of 1N HCl to 1N NaOH flask. Then the absorbance was measured at 242nm.

Photolytic degradation: The minimum quantity of drug was exposed to UV light in UV chamber for over night by placing the drug in petri dish. After completion of time the sample was diluted with water to obtain required concentration and the absorbance was measured at 242nm.

Thermal degradation: The minimum quantity of drug was exposed to dry heat at 30-40 Oc in hot air oven for 3hrs by placing the drug in petri dish. After completion of the time weigh the required quantity and dissolve in distilled water and measure the absorbance at 242nm

Peroxide degradation: From the standard stock solution take 1ml in 10ml volumetric flask to that add 1ml 0f 3% hydrogen peroxide solution and keep it aside for 12hrs. After completion of time measure the absorbance at 242nm.

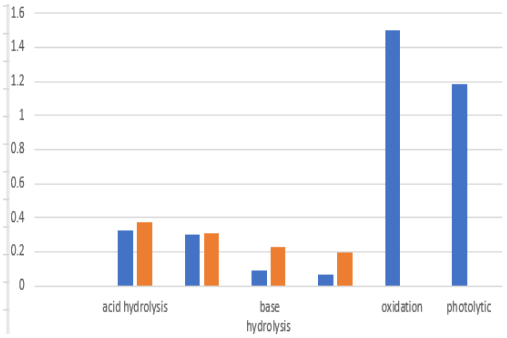

Figure 4: Degraded absorbance

7. RESULTS AND DISCUSSION

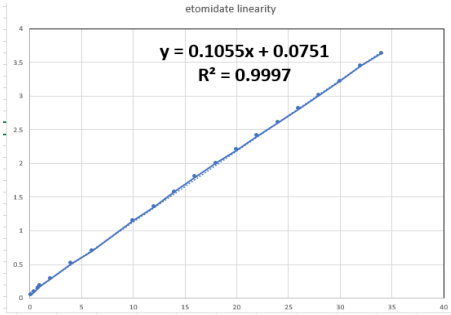

Figure 5: linearity curve of etomidate

Table 2: Conc vs abs table for linearity study.

Concentration	Absorbance
0.1	0.0414
0.4	0.0912
0.8	0.1501
1	0.1816
2	0.2842
4	0.5121
6	0.7011
10	1.1511
12	1.3512
14	1.5755
16	1.8012
18	1.9999
20	2.2051
22	2.4111
24	2.6022
26	2.8111
28	3.0121
30	3.2212
32	3.4512
34	3.6298

Table 3: Evaluation data of precision study

Concentration	Absorbance
15µg/ml	1.7755
15µg/ml	1.7745
15µg/ml	1.7735
15µg/ml	1.7754
15µg/ml	1.7745
15µg/ml	1.7755
Mean	1.7784
SD	0.000736
% RSD	0.0467

Table 4 : Accuracy data

%Level	Absorbance(nm)	%Recovery	Mean% recover
50% (10+2.5ppm)	1.8453 1.8412 1.8399	98.49% 98.28% 98.22%	98.33%
100% (10+5ppm)	2.0031 2.0013 2.0071	99.8% 99.9% 100.1%	99.93%
150% (10+7.5ppm)	2.2999 2.3421 2.3231	99.94 101.78% 100.95%	100.89%

Table 5: Robustness data

S.no	Concentration (ppm)	Absorbance(nm)	
1	15ppm	241	243
2	15ppm	1.7665	1.7655
3	15ppm	1.7755	1.7756
4	15ppm	1.7845	1.7840
5	Mean	1.7750	1.7755
6	SD	0.2883	0.2802

Table 6: Ruggedness data

S.no	Concentration	Absorbance	
		Analyst 1	Analyst 2
1	15µg/ml	1.7745	1.7542
2	15µg/ml	1.7742	1.7545
3	15µg/ml	1.7755	1.7556
4	15µg/ml	1.7756	1.7543
5	15µg/ml	1.7743	1.7556
6	15µg/ml	1.7756	1.7548
7	15µg/ml	1.7745	1.7542
8	Mean	1.7748	1.75474
9	SD	0.000577	0.000621
10	%RSD	0.0325	0.03538

Assay

%Assay

= (absrobance of sample/ absorbance of standard) x (concentration of standard/concentration of sample) x 100.

=17/15x100

=99%

7. CONCLUSION:

A simple and selective Spectrophotometric method was developed for the analysis of Etomidate in bulk and injectables formulation. The developed method was validated as per ICH guidelines, stability studies and a force degradation study was also conducted for Etomidate and the amount of drug degraded was determined.

Acknowledgement:

I want to acknowledge our beloved principal Prof. M. Sumakanth RBVRR women's college of pharmacy for giving me the opportunity for performing the research work.

REFERENCES:

[1] Michael J.Avram, Robert J.Fragen, Harry W.Lind performedon High performance liquid chromatographic assay for etomidate in human plasma:Results of preliminary clinical studies using etomidate for hypnosis in total

[2] Intravenous anesthesia. Journal of Pharmaceutical Sciences Volume 72, Issue 12, December 1983, pages 1424– 1426 .

[3] M.P McIntosh R,A Rajewski performed on A simple and efficient high- performance liquid chromate graphic assay for etomidate in plasma

[4] Journal of pharmaceutical and biomedical analysis volume 24, issue 4, Febrauary 2001, pgno:689 -694 .

[5] Xin-sheng Deng, Victoria J Simpson performed on Gas chromatographic–mass spectrometric determination of etomidate in Mousebrain. Journal of Pharmacological and Journal methods volume 43 , Issue 1, Febrauary 2000, pgno:73 -77

[6] Esener Z, Sarihasan.B, Guven H Br J performed on Thiopentone and etomidate Concentrations in maternal and umbilical plasma and in colostrum. Anaesth volume 69, issue 6, December 1992, pg no :586-588.

[7] Kugler A, Vallma.N, Suttman H, Taeger K performed on Etomidate using a new solubilizer.Experimental clinical studies on venous tolerance and bioavailability Anaesthesist volume 39,issue10, pg no:475-80.

[8] Shirozu K, Akata T, Yoshino J, Setoguchi H,Morikawa K, Hoka S performed onThe mechanisms of the direct action of etomidate on vascular reactivity in rat mesenteric resistance arteries.Pub med.gov Anesth analg ,volume 108 issue 2, Feb 2009 ,pg no:496-507