ORIGINAL RESEARCH PAPER

Pharmacy

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ESMOLOL HYDROCHLORIDE IN BULK AND OPHTHALMIC FORMULATION BY UV VISIBLE SPECTROPHOTOMETER .

KEY WORDS: Esmolol hydrochloride, UV spectroscopic method, validation.

Dr. K. Bhavyasri* Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy Barkatpura, Hyderabad- 500027, India *Corresponding Author

K V Manisha Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy Barkatpura, Hyderabad- 500027, India

Dr. M. Sumakanth Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy Barkatpura, Hyderabad- 500027, India

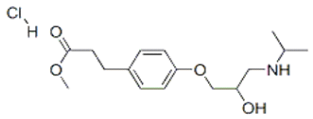
R. Swethasri Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy Barkatpura, Hyderabad- 500027, India

ABSTRACT

Aim: In the present study, simple, rapid and sensitive spectrophotometric method was developed for the determination of Esmolol hydrochloride (ESM). Esmolol hydrochloride showed absorption maxima at 223nm. There was linear response in the concentration range 1 - 6 µg/ml with correlation coefficient 0.998 .The method was validated by determining its accuracy, linearity and precision .The proposed method is simple, economical and hence can be applied for routine quality control of esmolol hydrochloride in bulk and injections.

INTRODUCTION:

Esmolol Hydrochloride (ESM) is a class 2 Antiarrhythmic and is chemically known as methyl (RS)-3-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} proponate hydrochloride. It is cardio selective beta$_1$ receptor blocker with rapid onset. It is used in the treatment for the rapid control of heart rate. ESM decreases the force and rate of heart contractions by blocking beta- adrenergic receptors of the sympathetic nervous system .It was officially published in Indian pharmacopeia and United States pharmacopeia. The method which is reported in the literature for the determination of ESM includes UV spectrophotometric method. The method was validated by parameters such as linearity, accuracy, precision and robustness.



Structure of esmolol hydrochloride.

Experimental procedure:

Instrumentation:

UV Spectrophotometer:

ELICO SLV 210 UV spectrophotometer with a matched pair of 10mm quartz cells are used in this analysis.

Sonicator:

Lab man, Digital ultrasonic Cleaner, Model no: LMUC-2, Serial: L8486.

Micro pipette:

P'fact A (100-1000µL).

Chemicals and reagents:

All the chemicals used were of analytical reagent grade. Double distilled water was used for all the experimental studies. ESM was procured from sigma Aldrich Pvt limited. Commercial dosage is in the form of injections, Esocard, Neotach and Miniblock were purchased from local market, Hyderabad. Double distilled water was procured from (SDFCL).

Preparation of standard stock solution:

The standard stock solution was prepared by dissolving 10.0mg of esmolol hydrochloride in 10.0ml of water to acquire a concentration of 1000µg/ml. The working standard solution of 10µg/ml was prepared by appropriate dilution of the stock solution with distilled water.

Selection of analytical wavelength and absorption maxima:

Appropriate dilutions were prepared for drug from standard stock solution and the solutions were scanned in the wavelength range of 200-400nm. The absorption maximum was found at 223nm.

Selection of analytical concentration range:

Aliquots of 2, 4, 6, 8, 10µg/ml of 100µg/ml of esmolol hydrochloride were pipette into six 10ml volumetric flask. The volume was made up to 10ml with water was measured at 223nm against water as blank. For standard solution analytical concentration range was found to be and overlatin spectra were observed.

Calibration curve of esmolol hydrochloride:

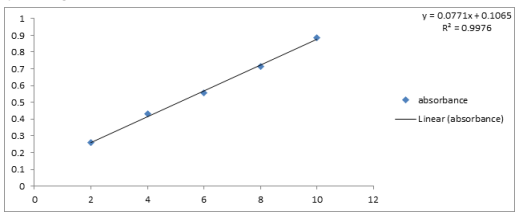
Aliquots of 2, 4, 6, 8, 10µg/ml of 100µg/ml solution of esmolol hydrochloride were pipette from stock solution into each of five 10ml volumetric flask. The volume was made up to 10.0ml with water. The absorbance of the solution was measured at 223nm against water as blank.

Analytical method validation:

Validation is one of the most important steps in analytical method evalution. The validation parameters, i.e.; linearity, accuracy, precision, robustness and ruggedness were evaluated to assess the method suitability.

Linearity:

Linearity is a measure of how well a calibration plot of response vs. concentration approximates a straight line. From 100ppm solution, appropriate dilutions were made to get final concentration of 2, 4, 6, 8, 10µg/ml and absorbance was taken at λmax-223nm.



Linearity curve of esmolol hydrochloride.

Precision:
The precision of an analytical procedure represents the nearness of agreement b/w a series of measurements got from multiple sampling of same sample under similar conditions. Precision was carried out by measuring response for a single concentration 10ppm for 6times at 223nm.

Accuracy:
It is defined as the closeness of measures value to the true value. Accuracy was determined by performing recovery experiments in which determination of % mean recovery of sample by percentage method at three different levels (80%-120%)

Ruggedness:
It is the degree of reproducibility of test results obtained by the analysis of the same samples under various conditions. It was carried out by two different analysts on different days to check the reproducibility which indicates the method developed is rugged.

Robustness:
It is the reliability of an analysis with respect to deliberate variations in method parameters such as change in wave length. It was determined by performing the same proposed method on different wavelengths, which indicates that the method developed is robust.

Limit of detection (LOD) and limit of Quantitation (LOQ):
LOD & LOQ: Calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the ten replicate determinations of same conc. (10µg/mL), standard deviation (SD) of the responses was calculated. From these values, the LOD & LOQ were determined on the basis of standard deviation and slope of the regression equation.

Assay:
Measured accurately 10ml of drug (injection) and transferred to 10ml volumetric flask. Dissolved it by using water as diluent and made up to mark (1000ppm). Then 0.1ml of above solution is transferred into 10ml volumetric flask, made up to mark to get concentration 10ppm using micropipette. The results indicates that the amount of drug in (injection) sample was in good agreement with label claim of formulation as indicated by % recovery(98.56%)

RESULTS AND DISCUSSION:
The method discussed in the present work provides convenient and accurate way for analysis of esmolol hydrochloride.

Table: 1 linearity of esmolol hydrochloride by UV-Visible Spectrophotometer.

Concentration	Absorbance
2	0.25
4	0.42
6	0.55
8	0.71
10	0.88

Table: 2 Accuracy of esmolol hydrochloride by UV-Visible spectrophotometer.

%recovery	Sample(Bulk + formulation)	Absorbance	%recovery	%RSD
80	2+10	1.4230	99.75	0.18
80	2+10	1.4231	99.76	
80	2+10	1.4232	99.78	
100	4+10	1.5960	100.02	0.19
100	4+10	1.5961	100.03	
100	4+10	1.5962	100.04	
120	6+10	1.7332	101.06	0.20
120	6+10	1.7333	101.07	
120	6+10	1.7334	101.08	

Table: Inter day and Intraday Precision of Assay UV-Visible spectrophotometer.

Concentration (µg/ml)	Intraday precision	Inter day precision
10	0.8874	0.8872
10	0.8871	0.8871
10	0.8874	0.8872
10	0.8867	0.8869
10	0.8878	0.8875
10	0.8882	0.8880
%RSD	0.19%	0.18%

Table: Ruggedness values of esmolol hydrochloride.

Instrument -1			Instrument – 2	
Day – 1			Day – 2	
Analyst – 1		Analyst – 2	Analyst -1	Analyst – 2
Concentration	Absorbance	Absorbance	Absorbance	Absorbance
10µg/ml	0.8544	0.8564	0.8545	0.8562
10µg/ml	0.8594	0.8597	0.8592	0.8596
10µg/ml	0.8654	0.8597	0.8653	0.8596
10µg/ml	0.8632	0.8597	0.8630	0.8595
10µg/ml	0.8542	0.8611	0.8541	0.8612
10µg/ml	0.8632	0.8611	0.8532	0.8612
%RSD	0.19%	0.16%	0.18%	0.17%

Table: Robustness values of esmolol hydrochloride.

Instrument – 1	Wavelength (nm)	
Concentration	222nm	224nm
10µg/ml	0.8511	0.8828
10µg/ml	0.8490	0.8835
10µg/ml	0.8482	0.8809
10µg/ml	0.8482	0.8816
10µg/ml	0.8515	0.8835
10µg/ml	0.8515	0.8842
%RSD	0.17%	0.14%

Table:Validation parameters of Esmolol Hydrochloride.

Validation Parameters	Results
Wavelength	223nm
Linearity range	2-10µg/ml
Correlation coefficient	0.997
Precision %RSD	0.19%
Accuracy (% recovery)	97-102
Robustness %RSD	222nm-0.17% 224nm-0.14%
Ruggedness %RSD	Analyst 1-0.19%,0.16% Analyst 2-0.18%,0.17%
LOD	0.054
LOQ	0.018
Assay	98.56%

CONCLUSION:
The UV – Spectrophotometric method that was developed for the determination of esmolol hydrochloride are based on calibration curve. The methods are validated and found to be simple, sensitive, accurate and precise. Hence, it can be used successfully for routine analysis of esmolol hydrochloride from injection.

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