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Spectrophotometric Determination of Esmolol Hydrochloride Using Analytical Reagents

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ABSTRACT

In the present study, four simple, rapid, and sensitive charge-transfer spectrophotometric methods were developed for the determination of esmolol hydrochloride (ESM). 2,3-Dichloro-5, 6-dicyano-1, 4-benzoquinone, 1-chloro 2,4-dinitro benzene, 2,5-dichloro-3,6-dihydroxycyclohexa-2,5-diene-1,4-dione (chloranilic acid), and sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate (Folin's reagent) were utilized as reagents in method A, B, C, and D, respectively. The developed charge- transfer complexes were measured at λ_{max} of 460, 430, 410, and 490 nm, respectively. The developed methods were applied successfully for the determination of ESM in its pure form as well as in its pharmaceutical formulations. The accuracy and reliability of the method were further ascertained by performing recovery test via standard addition technique.

Key words: *Spectrophotometric method, Esmolol hydrochloride, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone, Beer's law.*

1. INTRODUCTION

Esmolol hydrochloride (ESM) is a class II antiarrhythmic and is chemically known as methyl (RS)-3-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} propanoate hydrochloride (Figure 1). It is cardioselective beta$_1$ receptor blocker with rapid onset [1]. It is used in the treatment for the rapid control of heart rate [2]. 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 Pharmacopoeia [3] and United States Pharmacopoeia [4]. The methods which were reported in the literature for the determination of ESM includes reversed-phase high performance liquid chromatographic (RP-HPLC) with solid-phase extraction [5], stereoselective RP-HPLC [2], capillary electrophoresis [6], and liquid chromatography-mass spectrometry [7] and spectrophotometric [8] methods.

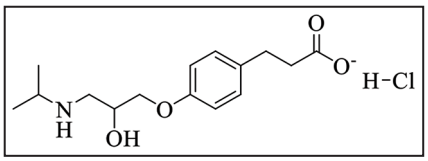

Figure 1: Structure of esmolol hydrochloride.

Since the above-mentioned methods are complex and expensive, there is a need to develop simple, less expensive, and more selective method for the determination of ESM. Hence, in the present investigation, an attempt was made to develop new spectrophotometric methods for determination of ESM in bulk and pharmaceutical formulations.

2. EXPERIMENTAL PROCEDURE

2.1. Instrumentation

A shimadzu ultraviolet (UV)-visible spectrophotometer (UV-160A) with a matched pair of 10 mm quartz cells are used in this analysis. Mettler Toledo analytical balance (accuracy 0.1 mg) was used for weighing all the samples.

2.2. Materials 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. Commercial dosage in the form of injections, Esocard, Neotech, and Miniblock were purchased from the local market, Tirupati. Double distilled water was used throughout the investigation. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 1-chloro 2,4-dinitro benzene (CDNB), chloranilic

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acid (CAA), and Folin's reagent were obtained from Merck.

2.2.1. Preparation of Standard Solutions

The standard stock solution of ESM was prepared by adding accurately weighed 100 mg of pure drug into 100 ml standard flask and dissolved in double distilled water, and the resulting solutions was equivalent to 1 mg/ml. The stock solution, thus prepared was further diluted to obtain the required concentrations for the current investigations.

2.3. Method Development

2.3.1. Method A (DDQ method)

Aliquots of standard ESM solution were transferred into a series of clean and dry volumetric flasks and made up to the mark with distilled water to obtain concentrations ranging from 10 to 50 µg/ml. 2 ml of 0.2% DDQ solution was added to each flask followed by mixing of the contents to obtain pinkish red chromogen. The maximum absorbance of the formed charge transfer complex was measured at 460 nm against the reagent blank.

2.3.2. Method B (CAA method)

Standard ESM solution was transferred into a series of clean and dry volumetric flasks and diluted to get the required concentration ranging from 12 to 60 µg/ml. A 2.2 ml of 2.5% CAA solution was added to each flask and kept the solution for about 5 min. The obtained yellowish green complex was measured at the maximum absorbance of 430 nm against the reagent blank.

2.3.3. Method C (CDNB method)

Different volumes of ESM standard solution were transferred into volumetric flasks and further diluted to obtain the concentrations in the range 4-28 µg/ml. To each flask, 3 ml of 3% CDNB solution was added and heated the contents up to 98°±2°C. The resultant yellow-colored solution was cooled at room temperature and measured the maximum absorbance at 410 nm against the reagent blank.

2.3.4. Method D (Folin's method)

Various volumes of standard ESM solution was diluted in standard flasks to acquire the concentrations in the range 15-50 µg/ml. 2.5 ml of Folin's reagent was added to each flask, and the formed pale yellow chromogen was measured at the maximum absorbance of 490 nm against the reagent blank. Calibration curve was constructed with the concentration of ESM versus absorbance for all methods, and respective regression equations were computed from the calibration graph using Beer's law and presented in Figure 2.

2.3.5. Procedure for pharmaceutical formulations

Accurately weighed amount of ESM formulation was transferred into clean and dry volumetric flask, subsequently diluted with water to get the required concentration and analyzed by the above-mentioned procedure.

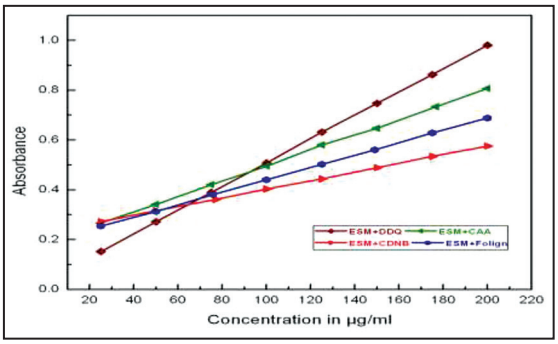

Figure 2: Calibration plot of esmolol hydrochloride with analytical reagents.

2.3.6. Procedure for excipients

Into a series of volumetric flasks, required quantity of ESM along with excipient was transferred, diluted, and sonicated for 30 min. Further, the volume was made up to the mark, filtered through 0.45 µ filter paper and subjected to analysis using the described procedure.

3. RESULTS AND DISCUSSION

The molecular interaction between electron donors and acceptors is generally associated with the formation of intensely colored charge transfer complexes, which absorb in the visible region [9]. The photometric methods based on molecular interactions are simple and appropriate since they result in the rapid formation of the complexes. ESM is n-electron donor and will form charge-transfer complexes with selected reagents which act as π-acceptors.

3.1. Absorption Spectrum

As described, ESM was reacted with DDQ, CDNB, CAA, and Folin's reagent and measured the maximum absorbance of the colored complex at the wavelength of 460, 430, 410, and 490 nm, respectively, against the reagent blank.

3.2. Effect of the Reagent Concentration

ESM was allowed to react with different volumes of DDQ, CDNB, CAA, and Folign reagent solution to obtain the optimum concentration of reagent and it was found that 2.0 ml of 0.2%, 2.2 ml of 2.5%, 3 ml of 3%, and 2.5 ml DDQ, CDNB, CAA, and Folign reagent, respectively, produced maximum intensity of the chromogen.

3.3. Effect of the Concentration Drug

A fixed volume of all reagent solutions was added orderly into the different volumes of drug solution, for color development. The maximum absorbance was measured at 460, 430, 410, and 490 nm, respectively, and the same was obeyed the Beer's law which can be considered as the optimum concentration.

3.4. Analytical Method Validation

Validation is one of the important steps in analytical method evaluation. The validation parameters, i.e., linearity, accuracy, precision, recovery, specificity, limit of detection, limit of quantification, and robustness were evaluated to assess the method suitability.

3.4.1. Linearity

The linearity of calibration graphs was proved by the high values of the correlation coefficient and the small values of the Y-intercept of the regression equation. The apparent molar absorptivities of the resulting colored complexes and relative standard deviation (RSD) of response factors for proposed spectrophotometric method were also calculated and presented in Table 1.

Table 1: Spectral characteristics of the drug with reagent.

Parameter	Method A	Method B	Method C	Method D
λ_{max} (nm)	460	430	410	490
Beer's law limit (µg/ml)	10-50	12-60	4-28	15-50
Molar absorbance ($L.mol^{-1}/cm^{-1}$)	1.5961	1.3051	0.9441	1.1401
Sandell's sensitivity ($\mu g.cm^{-2}$/0.001 A.U)	0.0010	0.001271	0.00175	0.0014
Correlation coefficient (r^2)	0.9985	0.9975	0.9941	0.9988
Slope (m)	0.0046	0.0030	0.0017	0.0024
Intercept (c)	0.04435	0.1914	0.2324	0.1963
% RSD	0.2849	0.9591	0.5546	0.8267
Color	Pinkish red	Yellowish green	Yellow	Pale yellow
LOD	1.1468	5.525	4.5239	5.237
LOQ	3.4752	16.743	13.709	15.871

RSD=Relative standard deviation, LOD=Limit of detection, LOQ=Limit of quantification

3.4.2. Robustness and ruggedness

In the evaluation of the robustness, some parameters such as drug concentration, reagent concentration, wavelength range, and shaking time were interchanged. The capacity remains unaffected by small changes in the above parameters. Method ruggedness was expressed as RSD% of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical difference between different analysts and instruments suggesting that the developed methods were robust and rugged.

3.4.3. Accuracy and precision

The accuracy of the analytical method is the nearness in conformity between the established exact value or reference value and the experimental value. Accurate results proved that the recovery values in drug and pharmaceutical forms are within the acceptance criteria.

Precision of the method is a measure of the ability to create reproducible results. It is evaluated using six replicate determinations. The intra- and inter-day precision was evaluated and found % RSD is <1.0, which indicates that there are no significant variations for intra- and inter-day analysis. These results are presented in Tables 2 and 3.

3.4.4. Recovery

Recovery studies were carried out by standard addition method. The results are summarized in Table 4 which indicate that the proposed method can be successfully applied for the determination of ESM in pure form and pharmaceutical formulations.

Table 2: Evaluation of accuracy and precision results of the proposed method in bulk form.

Method	Taken mg/ml	Intra-day				Inter-day			
		Found*	Recovery %	±SD	% RSD	Found*	Recovery %	±SD	% RSD
A	8	7.90	98.85	0.0306	0.3869	7.87	98.45	0.0559	0.7097
	6	5.91	98.89	0.0523	0.8864	5.89	98.14	0.0386	0.6570
	4	3.93	98.13	0.0187	0.4766	3.92	98.08	0.0206	0.5264
B	4	3.94	98.50	0.0167	0.4247	3.95	98.79	0.0231	0.5862
	6	5.89	98.08	0.0446	0.7580	5.90	98.28	0.0512	0.8691
	8	7.89	98.63	0.0513	0.6512	7.92	99.00	0.0672	0.8488
C	2	1.96	98.08	0.0147	0.7503	1.97	98.58	0.0147	0.7465
	4	3.93	98.25	0.0357	0.9103	3.94	98.50	0.0228	0.5787
	6	5.93	98.89	0.0436	0.7359	5.93	98.78	0.0568	0.9584
D	6	5.94	99.02	0.0407	0.6850	5.92	98.66	0.0424	0.7166
	8	7.91	98.85	0.0719	0.9097	7.92	99.04	0.0683	0.8621
	10	9.81	98.10	0.0723	0.7378	9.83	98.25	0.0524	0.5337

*Average of six determinations, SD=Standard deviation, RSD=Relative standard deviation

Table 3: Evaluation of accuracy and precision results of the proposed method in pharmaceutical dosage form.

Method	Pharmaceutical formulation	Taken mg/ml	Intra-day				Inter-day			
			Found*	Recovery %	±SD	% RSD	Found*	Recovery %	±SD	% RSD
A	Esocard	2	1.96	98.08	0.0147	0.7503	1.97	98.50	0.0063	0.3210
	Neotech	6	5.89	98.16	0.0464	0.7890	5.93	98.75	0.0446	0.7529
	Miniblock	10	9.83	98.33	0.0816	0.8303	9.89	98.93	0.0637	0.6445
B	Esocard	2	1.97	98.58	0.0075	0.3817	1.96	98.16	0.0121	0.6168
	Neotech	6	5.91	98.58	0.0403	0.6825	5.92	98.75	0.0459	0.7752
	Miniblock	10	9.89	98.91	0.0581	0.5874	9.83	98.33	0.0816	0.8303
C	Esocard	2	1.96	98.16	0.0136	0.6958	1.96	98.25	0.0151	0.7717
	Neotech	6	5.92	98.72	0.0287	0.4853	5.88	98.13	0.0530	0.9013
	Miniblock	10	9.91	99.10	0.0303	0.3060	9.87	98.70	0.0600	0.6079
D	Esocard	2	1.97	98.50	0.0109	0.5560	1.96	98.16	0.0121	0.6168
	Neotech	6	5.92	98.77	0.0307	0.5191	5.90	98.47	0.0453	0.7675
	Miniblock	10	9.83	98.38	0.0541	0.5508	9.89	98.91	0.0397	0.4014

*Average of six determinations, SD=Standard deviation, RSD=Relative standard deviation

Table 4: Determination of recovery of esmolol hydrochloride in pharmaceutical formulation.

Name of the drug	Pharmaceutical formulation	Labeled amount (mg/ml)	Found (mg/ml)*	Recovery %	±SD	% RSD
Esmolol hydrochlo-ride	Esocard	5	4.947	98.92	0.023	0.472
	Neotech	5	4.951	98.98	0.022	0.457
	Miniblock	5	4.958	99.14	0.018	0.379

*Average of six determinations, SD=Standard deviation, RSD=Relative standard deviation

Table 5: Determination of esmolol hydrochloride in the presence of excipients.

Excipients	Amount taken mg/ml	Found mg/ml*	Recovery %	±SD	% RSD
Glucose	20	19.93	99.63	0.022	0.108
Sucrose	25	24.94	99.74	0.033	0.131
Lactose	15	14.92	99.43	0.039	0.261
Dextrose	20	19.94	99.68	0.024	0.122
Talc	25	24.95	99.78	0.022	0.091
Starch	25	24.94	99.74	0.023	0.094

*Average of six determinations, SD=Standard deviation, RSD=Relative standard deviation

3.4.5. Specificity and selectivity

The specificity and selectivity of the current method were evaluated by estimating ESM in presence of excipients. The results are summarized in Table 5 which indicate that there is no interference from excipients present in the formulation.

4. CONCLUSIONS

In the present method, the determination of the drug ESM in both bulk and pharmaceutical formulations has been carried using four simple, specific, and low-cost spectrophotometric methods. From the correlation coefficient values and recovery studies, it can be concluded that the present developed methods can be successfully utilized for determination ESM in regular quality control processes.

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****Bibliographical Sketch***

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