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SIMPLE SPECTROPHOTOMETRIC ESTIMATION OF LABETALOL IN BULK AND MARKETED FORMULATION

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ABSTRACT

Two simple, sensitive and specific methods have been developed for the quantitative estimation of Labetalol in bulk and pharmaceutical dosage form. Method A is based on oxidation of labetalol with ferric chloride followed by complex formation of resulting ferrous ion(Fe^{2+})with 1,10- phenanthrolin to form orange red colored chromogen which showed at 509nm.MethodB forms blue color chromogen with Gibbs reagent in alkaline condition by electrophilic aromatic substitution giving maximum absorbance 655nm.The methods were validated as per ICH guidelines.

KEYWORDS: Labetalol, Gibbs reagent, 1, 10- phenanthrolin.

INTRODUCTION

Labetalol[1] chemically 2-hydroxy-5-{1-hydroxy-2-[(4-phenylbutan-2-yl) amino] ethyl} benzamide is an adrenergic receptor blocking agent[2] that has both selective alpha -1 and non selective beta adrenergic receptor blocking actions. Literature survey reveals that Labetalol was assayed by capillary electrophoresis [3], LC-MS [4] and spectroflorimetric [5], [6]methods in pharmaceutical preparation and biological fluids. Determinations of Labetalolby colorimetric method in pharmaceutical dosage form have not been reported in litrerature.Hence present work describes two colorimetric methods for estimation of Labetalol in tablet formulation. The methods are validated[7], [8] in terms of accuracy, precision, ruggedness and specificity as per ICH [9] guidelines.

MATERIALS AND METHODS

Instrument

The analysis was performed by Jasco V-630 series with 1cm matched glass cuvettes were used.

Chemicals

1. Labetalol (Sun Pharma Medication Pvt.Ltd)
2. Ferric chloride 2%
3. Nitric acid 1%
4.1,10 phenanthrolin 1%
5. Gibbs reagent 0.15%
6. Borax 0.25%

Standard Stock Solution for Method A and B

Stock solution A of 1mg/ml was prepared by dissolving 100mg of Labetalol in 100ml of distilled water. For working solution 10ml was pipette from standard stock solution into 100ml calibrated volumetric flask and made up the volume with distilled water to get concentration of 100mcg/ml (stock solution B).

Method A

From standard working solution B different concentrations were prepared ranging from 10-50 µg/ml were transferred into a series of 10ml volumetric flasks. To each flask 1ml 2% of ferric chloride was added, followed by 1% of 1ml of 1,10-phenanthroline and kept aside for 10mins for the completion of reaction and volume was made upto 10ml with distilled water. The absorbance was measured at 509nm against corresponding reagent blank.

Method B

Six different aliquots were taken from working standard stock solution B.1ml of 0.25%borax and 0.15%Gibbs reagent was added (kept for 10mins) to prepare series of concentration from 5-30mcg/ml.Then volume was made upto the mark with distilled water. The absorbance of the resulting solution was measured at 655nm.

Results of assay of formulation are given in Table no. 01 and 02.

Validation

Linearity

Linearity was determined over the range of 10-50mcg/ml for method A and 5-30mcg/ml for method B respectively. Calibration plots were constructed by plotting absorbance vs. concentration. This method obeys the Beer- Lambert's law**.** Table No.03 and 04.

Accuracy

Accuracy was established across the specified range of the analytical procedure. Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels taking into consideration percentage purity of added bulk drug samples. Table No.05

Ruggedness

To establish ruggedness of the proposed method, assays for two different concentrations of Labetalol were performed by two different analysts. The results of assays were represented as % Recovery and % RSD showing the ruggedness of the proposed method.Table no.06

RESULTS AND DISCUSSION

Method A

The proposed method involve the oxidation of Labetalol with ferric chloride and subsequent complexation of resulting ferrous ion(Fe^{2+}) with 1,10 phenanthrolin to form red orange colored chromogen. Fe^{3+} oxidizes drug and the produced Fe^{2+} forms orange red colored complex by reacting with 1,10 phenanthrolin which shows maximum absorbance at 509nm.The reaction is given in Fig 01.

Method B

Labetalol forms a blue colored chromogen with gibbs reagent in alkaline condition by electrophillic aromatic substitution.Gibbs reagent couples at para position to the ring structure.The decomposition of Gibbs reagent in a solution shown to occur first under the formation of quinineimine.The solution was analysed at 655nm.The reaction is given in Fig 02.

Tables No. 01 Assay Results of Marketed Formulation of Method A

Formulation	Actual concentration of Labetalol (µg/ml)	Amount obtained of Labetalol(µg/ml)	% Labetalol
Tablet	20	19.49	97.45

Tables No. 02 Assay Results of Marketed Formulation of Method B

Formulation	Actual concentration of Labetalol (µg/ml)	Amount obtained of Labetalol (µg/ml)	% Labetalol
Tablet	10	9.89	98.9

Table No. 03 Absorbance of different concentration of Labetalol obeying beer's law(MethodA)

SL.No	Volume of drug taken(ml)	Concentration in µg/ml	Absorbance At 509 nm
1	1.0	10	0.085
2	2.0	20	0.165
3	3.0	30	0.241
4	4.0	40	0.325
5	5.0	50	0.411

Table No. 04 Absorbance of different concentration of Labetalol obeying beer's law(MethodB)

SL. No	Volume of drug taken(ml)	Concentration in µg/ml	Absorbance At 655 nm
1	1.0	10	0.0987
2	2.0	20	0.1798
3	3.0	30	0.2287
4	4.0	40	0.3665
5	5.0	50	0.4578

Table No. 05 Determination of Accuracy

Methods	Amt of sample	Amt. of drug added	Amt. of drug recoveredµg/ml	% Recovery
A	10	8	7.92	99.75
	10	10	10.01	100.01
	10	12	11.95	99.58
B	20	16	15.92	99.55
	20	20	20.10	100.5
	20	24	23.86	99.41

Table no. 06 Ruggedness results for Labetalol at 509 nm and 655nm.

Methods	Concentration (µg/ml)	Analyst I		Analyst II	
		Amount found (µg)	(%) Recovery	Amount found (µg)	(%) Recovery
A	10	10.05	100.5%	9.98	99.08%
	20	19.91	99.55%	20.01	100.05%
B	10	9.98	99.8%	10.11	101.1%
	20	20.21	101.05%	20.16	100.8%

Table No: 07 Summary of Parameters of Spectrophotometry

Parameter	Method A	Method B
λmax(nm)	509 nm	655nm
Beer's law limits (µg/ml)	10-50 µg/ml	10-50 µg/ml
Regression equation (y=a+bc) Slope (b) Intercept (a)	b=0.008 a=0.001	b=0.009 a=0.002
Correlation coefficient (r2)	0.999	0.999
% Recovery	1) At Level-1 (80%)=99.05 2) At Level-2 (100%)=100.5 3) At Level-3 (120%)=99.41	1) At Level-1 (80%)=99.05 2) At Level-2 (100%)=100.5 3) At Level-3 (120%)=99.41
Repeatability (%RSD)	0.0876-0.4146	0.0985-0.4476
Limit of Detection (µg/ml)	0.08058	0.03804
Limit of Quantitation (µg/ml)	0.2441	0.1152

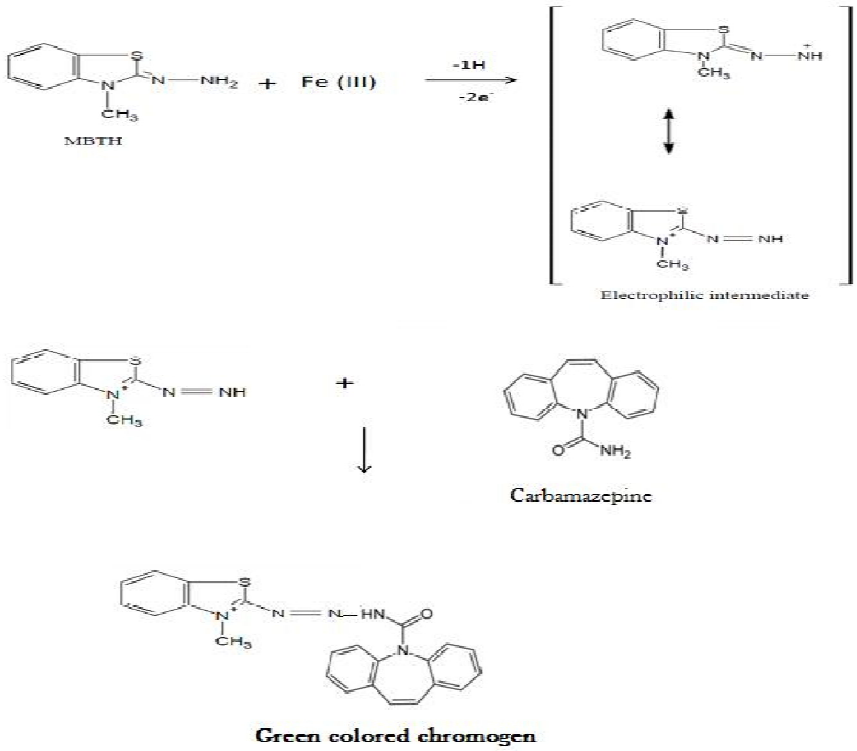

Figure 01

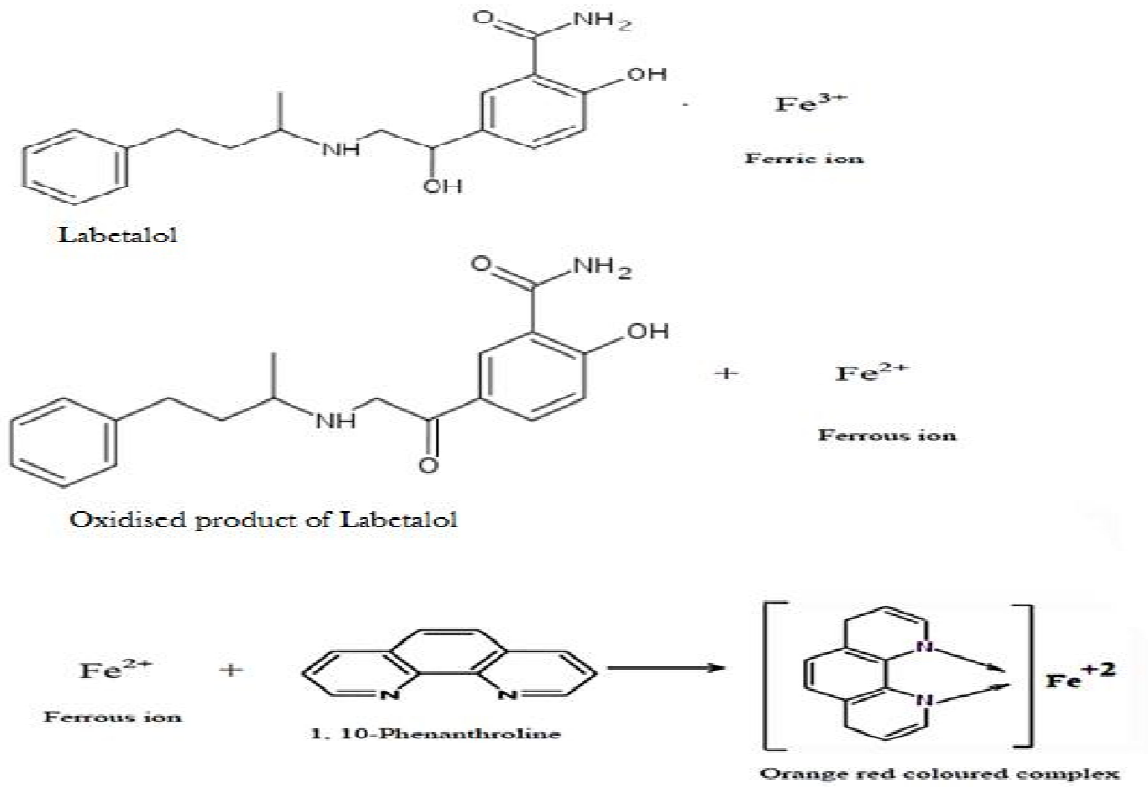

Figure 02

CONCLUSION

Two simple colorimetric methods A and B were developed for the determination of Labetalol in pure as well as in its dosage form. The proposed method is highly reproducible and reliable and is in good agreement with label claim of the drugs. The methods was validated in accordance with ICH guidelines. The method can be used for the routine analysis of Labetalol.

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