Development of Validated Spectrophotometric Methods for Estimation of Ethacridine Lactate in Pharmaceutical Formulations

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Abstract: Ethacridine lactate (EAL) is an antiseptic in solutions of 0.1%. It is also used as an agent for second trimester abortion. Two simple and sensitive spectrophotometric methods (Method A and Method B) were developed for the estimation of EAL in pharmaceutical formulations. Method A is based on the condensation of the primary aromatic amino group of ethacridine lactate with an aromatic aldehyde (*p*-Dimethylaminocinnamaldehyde) to form a chromophore with absorption maximum of 600nm. Method B is based on redox reaction followed by complex formation of EAL with IO_3^- /Metol reagent to form a stable chromogen, which can be estimated at 520 nm. Method A obeys Beer's law in the concentration range of 2 to 12 $\mu\text{g/mL}$ and method B in the range of 20 to 100 $\mu\text{g/mL}$. Interference studies were conducted to see the influence of excipients with the proposed methods. The common excipients usually present in dosage forms do not interfere in the proposed method A and method B. The optical characteristics, regression analysis data and precision of the methods were also calculated. The methods were validated for use in routine quality control of EAL in pharmaceutical formulations.

Keywords: Ethacridine lactate, *p*-Dimethylaminocinnamaldehyde, Metol

Introduction

Ethacridine lactate¹⁻⁴ is an antiseptic in solutions of 0.1%; it is also used as an agent for second trimester abortion. Up to 150 mL of 0.1% solution is instilled extra amniotically using a foley catheter. Ethacredine as an abortifecant is found to be safer and better tolerated than 20% hypertonic saline. The chemical name of ethacridine lactate is 2-ethoxy-6,9-diamino acridine monolactate monohydrate. For the estimation of ethacridine lactate few HPLC

Methods⁵⁻⁸ were reported. The present investigation is to develop two simple and sensitive spectrophotometric methods for the estimation of EAL in pharmaceutical formulations based on; (a) The condensation of the primary aromatic amino group of EAL with *p*-dimethylaminocinnamaldehyde (PDAC)⁹ and (b) The redox reaction followed by complex formation of EAL with IO_3^- /metol reagent¹⁰⁻¹².

Experimental

A systronics double beam UV- visible spectrophotometer 2201 with 1 cm matched quartz cells was used for all spectral and absorbance measurements. A systronics digital pH meter was used for all pH measurements. Electronic dhona balance 200D was used for weighing the samples. Class 'A' volumetric glassware were used.

Reagents preparation

- PDAC solution (Loba, 0.2% w/v, 1.14×10^{-2} M): Prepared by dissolving 200 mg of *p*-dimethylaminocinnamaldehyde in 100 mL of methanol.
- Sulfuric acid solution (CDH, 10% v/v, 1.0×10^{-2} M): 10 mL of sulfuric acid was added to about 50 mL of ice cooled methanol solvent and finally volume was made up to 100 mL with methanol at room temperature.
- Metol solution (Qualigens, 0.2% w/v, 5.81×10^{-3} M): Prepared by dissolving 200 mg of *p*-*N*-methylaminophenol in 100 mL of distilled water.
- KIO_3 solution (Merck, 0.43%, 2.0×10^{-2} M): Prepared by dissolving 430 mg of potassium iodate in 100 mL of distilled water.
- pH 3.1 buffer solution: Prepared by diluting a mixture of 50 mL of 0.2 M potassium hydrogen phthalate and 19 mL of 0.2 N HCl to 200 mL with water and the pH was adjusted to 3.1.

Standard preparation

The stock solution (1 mg/mL) of EAL was prepared by dissolving 100 mg of the EAL in 100 mL of methanol. For Method A, this stock solution was suitably diluted with methanol to get working standard solution having a concentration of 100 $\mu\text{g/mL}$.

Sample preparation

The content of ten vials was taken and thoroughly mixed. From this an accurately measured portion of the liquid content equivalent to 100 mg of the drug was taken and diluted to 100 mL with methanol. Later this solution was further diluted to get absorbance values within the calibration curve range.

Procedure for assay

Method A

To a series of 10 mL volumetric flasks, methanolic EAL standard solution (100 $\mu\text{g/mL}$) ranging from 0.2 to 1.2 mL was transferred and 1.0 mL of PDAC followed by 1 mL of 10% sulfuric acid. The final volume was adjusted to 10 mL with methanol. The absorbance was measured at 530 nm against the reagent blank. The amount of EAL present in the given sample solution was computed from its calibration curve.

Method B

Into a series of 25 mL volumetric flasks, 15 mL of pH 3.1 buffer, 1.0 mL of 0.02 M KIO_3 solution and 2.0 mL of 0.2% metol solution were successively placed. To this standard solution (1000 $\mu\text{g/mL}$) of EAL in the range of 0.5-2.5 mL were added. The final volume in

each flask was made up to the mark with distilled water. The absorbance of the solutions was measured at 520 nm after 20 min against the reagent blank. The amount of EAL in test was computed from the corresponding Beer-Lambert's plot.

Results and Discussion

Method A

This method is based on the formation of Schiff's base of EAL with *p*-dimethylamino-cinnamaldehyde as shown in Scheme 1.

Method B

The color development is due to the formation of a CT complex as represented in Scheme 2. The composition of colored species formed between PMBQMI and EAL can be explained as per the analogy of earlier workers.

Scheme 1

Scheme 2

Interference studies were conducted to see the influence of excipients with the proposed methods. The common excipients usually present in dosage forms do not interfere in the proposed method A and method B. The optical characteristics, regression analysis data and precision of the methods are presented in Table 1. The results of accuracy were given in Table 2.

Table 1. Optical characteristics and regression analysis parameters

Parameter	Method A	Method B
$\lambda_{\max}$, nm	600	520
Beer's law limits, $\mu\text{g mL}^{-1}$	2-12	20-100
Molar absorptivity, $\text{l mole}^{-1} \text{cm}^{-1}$	2.39×10^4	2.82×10^3
Detection limits, $\mu\text{g mL}^{-1}$	0.857	1.683
Sandell's sensitivity, $\mu\text{g cm}^{-2} / 0.001$ absorbance unit	0.015	0.127
Optimum photometric range, $\mu\text{g mL}^{-1}$	4-10	20-80
Regression equation ($Y = a + bc$) * Slope (b)	0.066	0.008
Standard deviation of slope (S_b)	1.53×10^{-4}	6.65E-05
Intercept (a)	0.001	-0.0018
Standard deviation of intercept (S_a)	1.10×10^{-3}	4.03×10^{-3}
Standard error of estimation (S_e)	1.62×10^{-3}	5.57×10^{-3}
Correlation coefficient (r)	0.9999	0.9999
Relative standard deviation, % *	0.39	0.656
% Range of error (Confidence limits) **		
0.05 level	0.0008	0.75
0.01 level	0.001	0.95
% Error in bulk samples ***	0.06	0.71

* $y = a + bx$, where 'x' is the concentration of EAL in $\mu\text{g/mL}$ and y is the absorbance value. ** Average of six determinations. *** Average of three determinations

Table 2. Estimation of ethacridine lactate in pharmaceutical formulations

Method	Pharmaceutical Formulation	Labelled Amount, mg/mL	Proposed method			Found by reference method $\pm$ S.D	% Recovery by proposed methods ** $\pm$ S.D
			Amount found *, mg $\pm$ S.D	t (Value)	F (Value)		
A	Injection	1	0.97 $\pm$ 0.013	0.271	1.401	1 $\pm$ 0.009	99.88 $\pm$ 0.25
B	Injection	1	1.03 $\pm$ 0.011	0.328	1.258	0.96 $\pm$ 0.012	100.1 $\pm$ 0.81

* Average of six determinations. ** Average of three determinations

Conclusion

The proposed methods are economic, simple, sensitive, reproducible and accurate and can be used for the routine analysis of EAL in bulk as well as in its pharmaceutical preparations.

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