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New Spectrophotometric Methods for Estimation of Ethacridine Lactate in Bulk and Pharmaceutical Formulations using 1, 10-Phenanthroline and Folin Ciocaltaeu Reagent

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Abstract: Ethacridine Lactate (EAL) is an antiseptic in 0.1% solutions. It is also used as an agent for inducing abortion. Two simple and sensitive spectrophotometric methods (Method A and Method B) were developed for the estimation of Ethacridine Lactate. Method A is based on the oxidative coupling of Ethacridine Lactate with 1, 10-phenanthroline in the presence of Fe(III) to form a chromophore with absorption maximum of 490nm. Method B is based on redox reaction of Ethacridine Lactate with Folin ciocaltaeu reagent to form a chromogen estimated at 600nm.

Methods A and B obey Beer's law in the concentration range of 2 to 18 and 5 to 35 µg/ml respectively.

Interference studies were conducted and it was found that the common excipients usually present in dosage forms do not interfere in the proposed methods.

The optical characteristics, regression analysis data and precision of the methods were calculated. The accuracy of the methods was evaluated by estimating the amount of Ethacridine Lactate in previously analyzed samples to which known amounts of Ethacridine Lactate was spiked. The accuracy of the methods was also conformed by comparison of the results obtained by proposed and reference methods. The methods were validated for use in routine quality control of Ethacridine Lactate in pharmaceutical formulations.

Key Words: Ethacridine Lactate, Spectrophotometry, Development, Validation, 1,10-Phenanthroline, Folin ciocaltaeu reagent.

Introduction:

Ethacridine lactate[1-6] is an antiseptic in solutions of 0.1%; it is also used as an agent for second trimester abortion. Upto 150ml of 0.1% solution is instilled extra amniotically using a foley catheter. Ethacredine as an abortificeant is found to be safer and better tolerated then 20% hypertonic saline. The chemical name of Ethacridine Lactate is 2-ethoxy-6,9-diamino acridine monolactate monohydrate.it is official in BP,USP and EP. For the estimation of Ethacridine Lactate few analytical methods by as HPLC[7-10] were reported. In the present investigation , we developed two simple and sensitive spectrophotometric methods (Method A and Method B) for the estimation of Ethacridine Lactate in pharmaceutical formulations. Method A is based on the oxidative coupling of Ethacridine Lactate with 1,10-phenanthroline[11] (PTL) in the presence of Fe (III) to form a orange coloured chromophore with absorption maximum of 490nm. Method B is based on redox reaction of Ethacridine Lactate with Folin ciocaltaeu reagent[12-19] to form a stable blue coloured chromogen, which can be estimated at 600nm.

Experimental :

Instrumentation:

Systronics double beam UV/Visible spectrophotometer 2201 with matched quartz cells were used for the present investigation.

Reagents Preparation :

Method-A:

1,10-phenanthroline solution (Qualigens, 0.198% w/v, 1.0 x 10^{-2} M): Prepared by dissolving 198 mg of 1,10-phenanthroline in 100 ml of 0.1N hydrochloric acid.

$FeCl_3$ stock solution (CDH, 0.162 % w/v, 1M) (3.3 x 10^{-3} M): About 162 mg of anhydrous ferric chloride was accurately weighed and dissolved in 100 ml of distilled water. 33.3 ml of above stock solution was further diluted to 100 ml with water.

Orthophosphoric acid solution (CDH, 2.0 x 10^{-1} M): 1.3 ml of orthophosphoric acid was diluted 100 ml with distilled water.

Method-B:

FC reagent solution (Loba, 0.67 N): Prepared by diluting three times of Folin-Ciocalteu reagent (2N) with distilled water.

Na_2CO_3 solution (Merck, 2% w/v, 1.89 x 10^{-1}M): Prepared by dissolving 2.0 gm of sodium carbonate in 100 ml of distilled water.

Standard Preparation:

About 100 mg of Ethacridine was accurately weighed and dissolved in 100 ml of water to get 1000 µg/ml stock solutions. This stock solution was further diluted with the same solvent to get working standard solution of 100 µg/ml.

Sample Preparation:

The content of five vials was taken and mixed thoroughly. From this an accurately measured portion of the liquid content equivalent to 50 mg of the drug was dissolved in 70 ml of water and filtered. The filtrate was diluted to 100 ml with methanol. Later this solution was further diluted to get absorbance values within the calibration curve range.

Procedure For Estimation:

Method A:

Aliquots of standard solution(100µg/ml) containing 2 to 18µg/ml were transferred into a series of 10 ml volumetric flasks and 1.0 ml of 0.003 M ferric chloride was added to each flask. Then 1.0 ml of PTL was added to all flasks and the volume was equalized with ethanol, boiled for 35 min and cooled to room temperature and 2.0 ml of OPA was added to all and final volume was made upto 10 ml with water. The absorbance was measured at 490 nm against corresponding reagent blanks. The amount of Ethacridine Lactate in sample was estimated from corresponding calibration graph.

Method B:

Into a series of 10 ml volumetric flasks, aliquots of standard solution (1000 µg/ml) of Ethacridine Lactate in the concentration range of 5-35 were transferred. Then 3.0 ml of Na_2CO_3 solution, 1.0 ml of FC reagent were successively added and kept aside for 5 min. The volume was made up to 10 ml with water. The absorbance was measured at 600 nm against reagent blank. The amount of Ethacridine Lactate was deduced from its Beer-Lambert's plot.

Results and Discussion:

Methods A: Ethacridine Lactate exhibits reducing property due to the presence of functional moieties (one or more) vulnerable to oxidation selectively with oxidizing agents such as Fe (III) under controlled experimental conditions. When treated with known excess of oxidant, Ethacridine Lactate undergoes oxidation, giving products of oxidation (inclusive of reduced form of oxidant, Fe (II) from Fe (III), besides unreacted oxidant. It is possible to estimate the drug content colorimetrically, which is equivalent to either the reacted oxidant or reduced form of oxidant formed. The reduced form of Fe III (Fe II) has a tendency to give colored complex on treatment with PTL.

The first step in the methods mentioned above is the oxidation of Ethacridine Lactate with the oxidant.

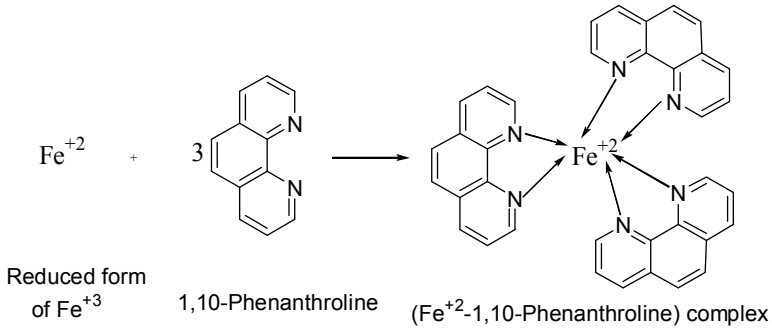
EAL + Fe (III) →
(Excess)

Oxidation products + Fe (II) + Fe (III)
(Reducedform ofOxidant) (Unreacted)

In this method, as Fe (III) interferes, even though to a little extent in the determination of Fe (II), the reactivity of the interfering entity has to be made insignificant by complexing it with o-phosphoric acid .

Fe (III) +o-phosphoric acid → Complex
(unreactive)

The second step concerns with the estimation of the reduced form of oxidant with appropriate chromogenic agent as described in method M_{A_1}. The complex formation for this method is shown in scheme no. 1.



Scheme - 1

Table-1: Optical characteristics, regression analysis,precision and accuracy of proposed methods for EAL

PARAMETER	M_A	M_B
λ_{max} (nm)	490	600
Beer's law limits (µg ml^{-1})	2 – 18	5-35
Molar absorptivity (l $mole^{-1}$ cm^{-1})	$1.64*10^4$	$7.517*10^3$
Detection limits (µg ml^{-1})	0.442	0.337
Sandell's sensitivity (µg cm^{-2} / 0.001 absorbance unit)	0.023	0.0483
Optimum photometric range (µg ml^{-1})	4-16	6-34
Regression equation (Y = a + bC)* Slope (b)	0.042	0.0198
Standard deviation of slope (S_b)	$4.62x\ 10^{-4}$	$1.343x\ 10^{-4}$
Intercept (a)	0.0098	0.0025
Standard deviation of intercept (S_a)	$5.60\ x\ 10^{-3}$	$2.023\ x\ 10^{-3}$
Standard error of estimation (S_e)	$7.73*10^{-5}$	$2.7*10^{-5}$
Correlation coefficient (r)	0.9999	0.998
Relative standard deviation (%)*	0.038	0.536
% Range of error (Confidence limits)**		
0.05 level	0.45	0.51
0.01 level	0.75	0.6

*y=a+bx, where 'x'is the concentration of Ethacridine Lactate in µg/ml and y is the absorbance value.
** average of six determinations.

Table-2: Assay and Recovery of Ethacridine Lactate in pharmaceutical formulations

sample	Labeled amount (mg/ml)	Amount found by proposed methods* (mg)±SD		Amount found by reference method (mg)±SD	% Recovery by roposed methods** ±SD	
		Method A	Method B		Method A	Method B
1	1	0.98±0.08	0.99±0.10	0.98±0.005	99.41±0.018	99.54±0.019
2	1	1.02±0.11	1.01±0.14	1.02±0.015	100.12±0.007	100.11±0.010

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

Method B:

The color formation by FC reagent with Ethacridine Lactate may be explained in the following manner based on the analogy with the reports of earlier workers.[25, 26] The mixed acids in FC reagent preparation involve the following chemical species.

$3H_2O.\ P_2O_5.\ 13\ WO_3.\ 5MoO_3.\ 10\ H_2O$ and

$3H_2O.\ P_2O_5.\ 14\ WO_3.\ 4MoO_3.\ 10\ H_2O$

Ethacridine Lactate probably effects a reduction of 1, 2 or 3 oxygen atoms from the tungstate and/or molybdate, thereby producing one or more of several possible reduced species, which have characteristic intense blue color.

The two developed methods follow Beer's law in the concentration range of 2-18 µg/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 are presented in table no 1. The accuracy of the methods was evaluated by estimating the amount of Ethacridine Lactate in previously analyzed samples to which known amounts of Ethacridine Lactate was spiked. The accuracy of the methods was also conformed by comparison of the results obtained by proposed and reference methods. The results of accuracy were given in table-2. Some of the commercially available formulations were procured from the local market and analyzed by the developed methods and the results comply with the labeled claim (table-2).

Conclusion:

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

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