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Development of New Spectrophotometric Methods for the Determination of Ethacridine Lactate in Bulk and Dosage forms

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Three simple and sensitive visible spectrophotometric methods (A-C) for the determination of Ethacridine Lactate in pure samples and pharmaceutical formulations are described in the present study. They are based on the formation of colored species by treating with P-Dimethylaminocinnamaldehyde (PDAC) reagent (Method A, λ_{max} 600 nm) or Methyl orange (MO) reagent (Method B, λ_{max} 420 nm) or Bathophenanthroline (BPL) reagent in presence of Ferric chloride (Method C, λ_{max} 600 nm). These methods were extended to the analysis of pharmaceutical preparations and results are compared with the reference method (USP).

Key words: Spectrophotometry, Ethacridine, Pharmaceutical preparations.

Ethacridine lactate[1, 2] (EAL) is chemically 2-ethoxy-6, 9-diaminoacridine monolactate monohydrate. It is used as an antiseptic and also as an agent for second trimester abortion[3]. A survey of literature revealed only a few reported methods which include titrimetric[4], selective FTIR[5], visible spectrophotometry[6] and HPLC[7] methods for estimation of EAL in urine. The analytically important groups of EAL were not exploited for designing suitable spectrophotometric methods. Hence an attempt has been to develop simple and sensitive visible spectrophotometric methods for EAL determination for routine quality control analysis of EAL in formulations.

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The developed methods are based on the formation of colored species by treating with PDAC reagent (Condensation reaction), Methyl orange (ion association complex formation) or Bathophenanthroline in presence of Ferric chloride (Oxidation followed by Complexation).

EXPERIMENTAL

Instrument

A Systronics Model 2201 UV-Vis Spectrophotometer with 1 cm matched quartz cells were used for absorbance measurements.

Reagents

All the chemicals used were of Analytical grade and prepared with double distilled water.

Method A

Methanolic solution of PDAC reagent (0.4% w/v) and Sulphuric acid (used as such).

Method B

Aqueous solutions of Methyl orange (0.2 % w/v) and 0.1 M Hydrochloric acid solutions were used .

Method C

Bathophenanthroline solution (0.332% w/v): Prepared by dissolving 332 mg of Bathophenanthroline in ethanol and made upto 100 ml with ethanol.

Ferric Chloride Solution (0.0033M)

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.

Standard Drug solution

Method A

Working standard solution of EAL was prepared by dissolving 10 ml of the drug solution (1 mg/ml) in methanol and made upto the mark of 100 ml with methanol (100 µg/ml).

Methods B & C

Working standard solution of EAL was prepared by dissolving 10 ml of the drug solution (1 mg/ml) in water and made upto the mark of 100 ml with water (100 µg/ml).

Procedures

Method A

Aliquots of standard Methanolic EAL solution (100 µg/ml) ranging from 0.2 to 1.2 ml were transferred to a series of 10 ml volumetric flasks. To each flask 1 ml of PDAC solution followed by 0.1 ml of 10% Sulphuric acid were added. The volume was made upto 10 ml with methanol. The absorbance was measured at 600 nm against reagent blank. The amount of EAL was deduced from its Beer-Lambert's plot.

Method B

Aliquots of standard EAL solution (100 µg/ml) ranging from 0.5 to 3.5 ml was transferred into a series of 125 ml separating funnels A volume of 1 ml of Dye solution was added. The total volume of aqueous phase in each separating funnel was adjusted to 10 ml with 0.1N Hydrochloric acid. To each separating funnel 10 ml of Chloroform was added and shaken for 3 minutes. The absorbance of the separated chloroform layer was measured at 420 nm against reagent blank. The amount of EAL was calculated from its Beer-Lambert's plot.

Method C

Aliquots of standard EAL solution (100 µg/ml) ranging from 0.2 to 1.0 ml were transferred to a series of 10 ml volumetric flasks. To each flask 1 ml of Ferric chloride solution and 1 ml of Bathophenanthroline reagent were added and the volumes in all the flasks were equalized with ethanol. The contents were gently boiled for 15 minutes, cooled to room temperature and 2ml of o-Phosphoric acid was added to all the flasks. The volume was made upto 10 ml with ethanol. The absorbance was measured at 600 nm against reagent blank. The amount of EAL was deduced from its Beer-Lambert's plot.

For Pharmaceutical Formulations

For injection formulations, 10 vials were broken and the contents were transferred to a clean and dried beaker. From this a volume of the drug solution equivalent to 100 mg of EAL was taken and a standard stock solution of 1 mg/ml was prepared. This was appropriately diluted and the amount of EAL was found out as described in the procedure.

Recovery study

To study the accuracy, reproducibility and precision of the proposed methods, recovery experiments were carried out. The recovery of the added standard was studied at 3 different levels. Each level was repeated 6 times. A plot of amount of drug found by proposed method (Y-axis) against standard added (X-axis) was drawn. The intercept on Y-axis indicates the amount of drug present per formulation.

RESULTS AND DISCUSSION

The optimum conditions for each method were established by varying one parameter at a time and keeping the others fixed and observing the effect of product on the absorbance of colored species and incorporated in the procedure. The optical characteristics are given in Table-1, together with regression equation for calibration plots. The precision and accuracy were found by analyzing six replicate samples containing known amount of drugs and the results were summarized in Table-1. Table-2 shows that the values of % recovery are between 98 to 102 % and the values of coefficient of variation are sufficiently low indicating that the proposed methods are free of interferences from any excipients like starch; talc etc and the results are reproducible.

Thus these developed methods can be employed for the routine determination of EAL in pure and in pharmaceutical preparations.

Table 1. Optical and Regression Characteristics of Proposed Methods

Parameters	Methods		
	A	B	C
λ_{max} (nm)	600	420	600
Beers law limits(µg/ml)	2-12	5-35	4-24
Molar Absorptivity(L.mole^{-1} cm^{-1})	2.3851×10^4	7.98×10^3	1.07×10^4
Sandell's sensitivity(µg/cm^2/0.001 abs. unit)	0.0151	0.045	0.0337
Regression equation(Y)*			
Slope (b)	0.0659	0.227	0.029
Intercept (a)	0.00053	-0.007	0.0076
Correlation coefficient	0.9999	0.9998	0.9996
% Relative standard deviation**	0.390	0.664	0.821
% Range of error **			
0.05 level	0.29	0.37	0.6
0.01 level	1.23	0.33	0.42

*Y= a+bc where c is the concentration and Y is the absorbance unit
** Average of six determinations

Table 2. Results of Assay and Recovery Experiments

Method	Formulation	Labelled amount (mg)	Proposed method			% Recovery**
			Amount found± SD	T (value)	F (value)	
A(PDAC)	Injection	1	0.87± 0.125	0.648	2.324	99.42± 0.92
B (MO)	Injection	1	1.03± 0.15	0.422	2.844	100.42± 0.62
C(BPL)	Injection	1	0.98 ± 0.32	0.584	3.25	100.14 ±0.56

*Average ±SD of 6 determinations
The t and F-values refer to comparision of proposed method with the reference method.
Theoretical values at 95 % confidence limits, T = 2.571 and F = 5.05
** Average of 6 determinations

REFERENCES

1. *United States Pharmacopoeia,* 24, National Formulary, 19, section 1225 "Validation of compendial methods, US Pharmacopoeial convention, Rockville, 2000
2. *European Pharmacopoeia*, 3rd edition, 1997 and Supplement, Council of Europe, Strasburg, 1999
3. A.Malpani and U. Krishna, "Second trimester abortion with ethacridine lactate plus Carboprost*", Advances in contraception*, 1987; **4**(2): 154-157.
4. T.sakai, N.Miyatake and N.Ohno, *Appl. Spectrosc*., 1996; **50**: 1203.
5. Bratu I, Chiosa V, Stanculescu., *Annals of West University of Timisoara, Series of Chemistry, An International Journal* ,46, 951, 1997
6. RK Gilpin, LA Pachla, *Anal. Chem*., 1995; **67**(12): 295-313.
7. Zhi-Yong Guo, Dan-Yi Wei , Yuan-Yuan Wang, Kun-Fei Xuan, Xu-Fei Yu, Qiu-Luan Yu, Yun Chu "An HPLC method for the determination of ethacridine lactate in human urine" *Biomedical Chromatography*, 2007; 21(5): 480-483.