Material Science Research India Vol. **7**(2), 487-490 (2010)

Development and validation of novel spectrophotometric methods for the determination of ethacridine lactate in bulk

E. VENUMADHAV[1], P. SAI PRAVEEN[2], T. NEHA[2], P. BHARGAVI[2] and G. DEVELA RAO[2]*

[1]Cheif Operating Officer, Veeda CR, Ahmedabad, Gujarat (India).
[2]KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada - 520 010 (India).
Email: drgdr1964@gmail.com

(Received: June 27, 2010; Accepted: July 19, 2010)

ABSTRACT

Three simple and sensitive visible spectrophotometric methods (A-C) for the determination of Ethacridine Lactate in pure samples and pharmaceutical formulations are described. They are based on the formation of colored species by treating with Folin-Ciocalteau reagent (Method A, λ_{max} 600nm) or 3-methyl 2-benzathiazolinone hydrazone in the presence of Ferric chloride (Method B, λ_{max} 580nm), or 1, 10 Phenathroline in presence of Ferric chloride (Method C, λ_{max} 490nm). These methods were extended to the analysis of pharmaceutical preparations and results are compound with the refernce method (USP).

Key words: Spectrophotometry, ethacridine, pharmaceutical preparation.

INTRODUCTION

Ethacridine lactate[1,2] (EAL) is chemically 2-ethoxy-6, 9-diamino acridine monolactate monohydrate. It is used as an antispetic and also as an agent for second trimester tabortion[3]. A survey of literature revealed only a few reported methods which include titrimetric[4], selective FTIR[5], visible spectrophotometry[6] and HPLC methods for estimation of EAL in urine[7]. 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.

The developed methods are based on the formation of colored species by treating with Folin-Ciocalteau reagent (Redox reaction)[8],3-methyl 2-benzathiazolinone hydrazone in the presence of Ferric chloride (Oxidative coupling)[9] or 1, 10 Phenanthroline in presence of Ferric chloride (Oxidation followed by complexation)[10].

EXPERIMENTAL

Instrument

A systronics model 220 UV-Vis spectrophotometer with 1cm matched quartz cells were used for absorbance measurements

Reagents

All the chemicals used were of Analytical grade and prepared with double distilled water Aqueous solutions of FC reagent (2N) and 20% w/v Sodium carbonate solutions were used for Method A

Aqueous solutions of MBTH (0.2% w/v) and 0.01 % w/v Ferric chloride solutions were used for Method B.

1, 10 Phenanthroline solution (0.198% w/v)

Prepared by dissolving 198 mg of 1, 10 Phenathroline in 0.1 N HCl and made upo the mark with 0.1 N HCl.

Ferric chloride solutions (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

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

Method A

Aliquots of standard EAL solution (100 µg/ml) ranging from 0.5 to 2.5 ml were transferred to a series of 10 ml volumetric flasks. To each flask 3 ml of Sodium carbonate solutions and 1ml of FC reagent were added and kept aside for 5 min. The volume was made upto 10 ml with 0.1 N HCl. The absorbance was measured at 600 nm against reagent blank. The amount of EAL was deduced from its Beer-Lamberts plot.

Method B

Aliquots of standard EAL solution (100 µg/ml) ranging from 0.15 to 0.9 ml were transferred to a series of 10 ml volumetric flasks. To each flask 2 ml of Feeric chloride solutions and 1ml of MBTH reagent were and allowed to stand at room temperature for 15 min. The volume was made upto 10 ml with 0.1 N HCl. The absorbance was measured at 580 nm against reagent blank. The amount of EAL was deduced from its Beer-Lamberts 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 Feeric chloride solutions and 1ml of1, 10 Phenanthroline reagent were and the volumes in all the flasks were equalized with ethanol. The contents were gently boiled for 35 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 490 nm against reagent blank. The amount of EAL was deduced from its Beer-Lamberts plot.

For Pharmaceutical formulations

For injection formulations, 10 vials were broken and the constent 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 1mg/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 inrercept 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 keeping the other 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 & 2. shows that the values of % recovery are between 98 to 102% and the value of coefficient of variation are sufficiently low indicating that the proposed methods are free of interferences from any excipients like strach, 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 preprations.

Table 1: Optical Regression characteristics of proposed methods

Parameters	Methods		
	A	B	C
λmax (nm)	600	540	490
Beers law limits (µg/ml)	5-25	1.5-9	2-10
Molar Absorptivity (L. mole^{-1} cm^{-1})	7.5117×10^{3}	3.552×10^{4}	1.64×10^{4}
Sandell's sensitivity (µg/cm²/0.001 abs.unit)	0.0483	0.0121	0.023
Regression equation (Y)*			
Slope (b)	0.0198	0.078	0.042
Intercept (a)	0.0025	0.012	0.0098
Correlation coefficient	0.998	0.9998	0.9999
% Relative standard deviation **	0.536	0.503	0.038
% Range of error**			
0.05 level	0.51	0.78	0.45
0.01 level	0.6	0.95	0.75

*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 Amount found±SD	T (value)	F (value)	% Recovery**
A (FC)	Injection	1	0.97±0.12	0.676	1.406	99.41±0.69
B (MBTC)	Injection	1	1.03±0.15	0.422	1.832	100.1±0.85
C (1, 10PTL)	Injection	1	0.89±0.125	0.648	2.324	99.42±0.92

*Average ±SD of 6 determinations

The t and F-values refer to comparision of proposed method with 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", Advance in contraception., **4**(2): 154-157 (1987).
4. T. Sakai, N. Miyatake and N. Ohno, *Appl. Spectrosc.*, **50**: 1203 (1996).
5. Bratu, I. Chiosa V, Stanculescu, *Annal of West University of Timisoara, Series of Chemistry, An International Journal.* **46**: 951

(1997).

6. R.K. Gilpin, L.A., Pachla, *Anal. Chem*. **67**(12): 295-313 (1995).
7. Zhi-Yong Guo, Dan-Yi Wei, Yuan-Yuan Wang, Kun-Fei Xuan, Xu-Fei Yu, Qui-Luan Yu, Yun Chu "An HPLC method for the determination of ethacridine lactate in human urine "*Biomedical chromatography*, **21**(5): 480-483 (2007).
8. CSP Sastry and BS Sastry "Application of MBTH chemistry in the determination of pharmaceuticals", *The Easter Pharmacist* 30-33 (1986).
9. K.P.R. Chowdary and G. Devala Rao, "Spectrophotometric determination of Salmeterol xinafoate with Folin-Ciocalteau Reagent, *"Indian Drug*". **34**(10): 606-607 (1997).
10. S. Vijaya Saradhi, V. Hima Bindu and G. Devala Rao, "New Spectrophotometric methods for the determination of Simvastatin". *Acta Ciencia Indica*, **XXXIIIC,** No **2**: 205 (2007).