Spectrophotometric Methods for the Estimation of Ethamsylate in Tablets

B. S. KUCHEKAR*, V. S. RANDIVE, A. P. CHAUDHARI AND S. B. BHISE
Government College of Pharmacy, Vidyanagar, Karad-415 124.

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Two simple, accurate, rapid and sensitive methods have been developed for the estimation of ethamsylate in pharmaceutical dosage forms. Method A is based on the reduction of Folin-Ciocalteau phenol reagent by ethamsylate in presence of 20% sodium carbonate solution giving blue chromogen, which shows maximum absorption at 740 nm while method B is based on the reduction of ferric ions (ferric nitrate) to ferrous ions by ethamsylate which then reacts with 1,10-phenanthroline to produce reddish coloured complex showing maximum absorption at 435 nm against reagent blank. In both the methods Beer's law was obeyed in the concentration range of 5-25 μg/ml.

Chemically ethamsylate is diethylammonium 2,5-dihydroxybenzenesulphonate. It is a haemostatic agent given for the prophylaxis and control of hemorrhages from small blood vessles[1]. The drug is official in British Pharmacopoeia and Martindale[1,2]. Literature survey reveals potentiometric[2], IR[2] and UV spectrophotometric[3] methods for the estimation of ethamsylate in tablets.

The present work describes two simple colourimetric methods for the estimation of ethamsylate in pharmaceutical dosage forms. Method A involves reduction of Folin-Ciocalteau (FC) phenol reagent by ethamsylate giving a blue chromogen in presence of 20% sodium carbonate solution, which shows maximum absorption at 740 nm against reagent blank. Method B is based on the reduction of ferric ions (ferric nitrate) to ferrous ions by ethamsylate, which then reacts with 1,10-phenanthroline producing a reddish coloured complex showing maximum absorption at 435 nm against reagent blank. A Systronics spectrophotometer 106 with 1 cm-matched couvettes was used for the measurement of absorbance. Folin-Ciocalteau phenol reagent (1:2 in distilled water), 20% sodium carbonate solution, 5% ferric nitrate in 5% nitric acid and 0.02 M 1,10-phenanthroline in 1% hydrochloric acid were freshly prepared.

*For correspondence
E-mail: bskuchekar2000@yahoo.com

Standard solution of ethamsylate was prepared by dissolving 100 mg in 100 ml and diluting 10 ml of this solution to 100 ml with distilled water (100 μg/ml). Twenty tablets of ethamsylate were weighed and powdered in a glass mortar. Powder equivalent to 100 mg of ethamsylate was accurately weighed and dissolved in distilled water to make 100 ml. The solution was then filtered and 10 ml of filtrate was diluted to 100 ml with distilled water.

In the method A, aliquots of 0.5 ml to 2.5 ml portion of standard solution were transferred to a series of 10 ml corning test tubes. To each test tube 3 ml of 20% sodium carbonate solution and 3 ml of FC reagent were added. The solution was kept for 2 min to complete the reaction and volume in each test tube was adjusted to 10 ml with distilled water. The absorbance of the solution in each test tube was measured at 740 nm against reagent blank prepared in the same manner without the addition of the drug and calibration curve was plotted. Similarly the absorbance of sample solution was measured and the amount of ethamsylate was determined by referring to the calibration curve.

In the method B, aliquots of 0.5 ml to 2.5 ml portion of standard solution were transferred to a series of 10 ml corn-

ing test tubes. To each test tube, 0.5 ml of ferric nitrate and 1.5 ml of 1,10-phenanthroline reagents were added. The solution was kept for 5 min to complete the reaction and volume in each test tube was adjusted to 10 ml with distilled water. The absorbance of the solution in each test tube was measured at 435 nm against reagent blank prepared in the same manner without the addition of the drug and the calibration curve was plotted. Similarly the absorbance of sample solution was measured and the amount of ethamsylate was determined by referring to the calibration curve.

To test the accuracy and reproducibility of the proposed method, recovery experiments were carried out by adding known amount of the drug to the preanalyzed formulation and reanalyzing the mixture by proposed method. The results are shown in Table 1. Both the proposed methods are based on the reducing behavior of ethamsylate. In method A, FC reagent is reduced by ethamsylate, the reduced FC gives blue colour in alkaline medium (20% sodium carbonate) showing maximum absorption at 740 nm. Method B involves reduction of ferric ions by ethamsylate to ferrous ions, which in turn reacts with 1,10-phenanthroline to give reddish coloured complex showing maximum absorption at 435 nm.

The colour intensity of the chromogen in method A was intensified with 3 ml of FC reagent and in method B with 0.5 ml of 5% ferric nitrate and 1.5 ml of 0.02 M 1,10-phenanthroline. Stability study of the chromogen was carried out by measuring the absorbance values at time intervals of 10 min for 1.5 h and it was found to be stable for 1 h for both methods. The optical characteristics such as absorption maxima, Beer's law limits, correlation coefficient (r), slope (m), y-intercept (c), molar absorptivity, Sandell's

TABLE 1: ANALYSIS DATA OF TABLET FORMULATION.

Formulation	Label claim (mg/tab)	Method	% of label claim* ± S.D.	% COV	S.E. of Mean	% Recovery	Reported method[3]
Tablet 1	250	M_1	98.8±1.59	1.6177	1.9844	98.88	98.70
		M_2	99.9±1.42	1.4219	0.7099	99.90	
Tablet 2	250	M_1	99.1±1.50	1.5142	1.8630	98.52	98.90
		M_2	99.7±1.67	1.6707	0.8331	99.67	
Tablet 3	250	M_1	99.1±1.27	1.2853	1.5818	99.85	99.56
		M_2	100.1±0.93	0.9299	0.4658	99.81	

*Mean of five determinations. M_1 – Method A, M_2 – Method B. The commercial preparations used were: tablet 1-Hemsyl, Indoco Remedies Ltd., tablet 2-Cosklot, CFL Pharmaceuticals Ltd., and 3- Dicynene, Dr. Reddy's Laboratories Ltd.

TABLE 2: OPTICAL CHARACTERISTICS AND PRECISION.

Observation	Method A	Method B
Absorption maxima (nm)	740	435
Beer's law limit (μg/ml)	5-25	5-25
Correlation coefficient	0.9998	0.9998
Molar absorptivity ($lit^{-1}\ mol^{-1}cm^{-1}$)	$1.279x10^4$	$1.1248x10^4$
Sandell's sensitivity ($\mu g/cm^2/0.001$)	$2.058x10^{-2}$	$2.3408x10^{-2}$
Regression equation (y=mx+c)		
Slope (m)	0.04858	0.04272
Intercept (c)	-0.1129	0.0382
% Range of error (confidence 95%)	0.0037	0.00104

sensitivity and the percent range of error (95% level confidence limit) calculated from 5 replicate readings containing ¾ of upper Beer's limit are incorporated in Table 2. The molar absorptivity and sandell's sensitivity values show the sensitivity of both the methods, while the precision is confirmed by % COV (coefficient of variance) values included in Table 1, which are less than 2%. The analysis results of marketed formulations are in good agreement with the reported method[3], which is also a spectrophotometric method for estimation of ethamsylate. The reproducibility, repeatability and accuracy of these methods were found to be good, which is evidenced by low standard deviation. The percent recovery obtained (98.5-98.9 for method A and 99.7–99.9 for method B) indicates non-interference from the common excipients including lactose used in the formulation. Thus these methods developed in the present investigation are simple, sensitive, accurate and precise and can be successfully applied for the routine estimation of ethamsylate in pharmaceutical dosage forms.

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Antibacterial Activity of Aerial Part Extracts of *Achyranthes bidentata* Blume

B. R. BALAKRISHNAN*, B. SANGAMESWARAN, B. ARUL AND V. H. BHASKAR
Vinayaka Mission's College of Pharmacy, Salem-636 008.

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***Achyranthes bidentata* Blume belonging to the family Amaranthaceae was investigated for antibacterial activity against *Bacillus subtilis* (NCM-2439), *Staphylococcus aureus* (NCIM-2492), *Pseudomonas aeruginosa* (NCIM-2053) and *Escherichia coli* (NCIM-2068) organisms, using agar diffusion method. The petroleum ether, chloroform, methanol and aqueous extracts showed significant antibacterial activity. Our findings offer experimental support to the therapeutic claims on this herb as useful against bacterial infections.**

India is one among the countries in the world today where ancient systems of medicine such as Ayurveda, Siddha and Unani have been in practice for many years. In common, all the above-mentioned systems of medicine are directly or indirectly depend upon the natural resources such as plants, animals and minerals. There is massive wealth available in these medical systems, but what comes in the way of making these systems of medicines globally acceptable is the lack of standardized products, lack of reliable production techniques and the absence of pharmacological proof of concept for these drugs[1]. The plant *Achyranthes bidentata* Blume (Family: Amaranthaceae) is a small herb widely found in western ghat areas of Emerald, Edakkadu, about 30 Km away from Ooty, the capital town of Nilgris District. This plant is claimed to have good medicinal value and is widely used as an antitumour, antispasmodic and cytotoxic[2,3] in the native systems of medicine.

The Ariel parts of the plant were collected in the month of November and cleaned to remove the debris. The plant

*For correspondence