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A simple method for the spectrophotometric determination of cephalosporins in pharmaceuticals using variamine blue

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Abstract: A simple spectrophotometric method for the determination of cefotaxime, ceftriaxone, cefadroxil and cephalexin with variamine blue is presented. The determination is based on the hydrolysis of β-lactam ring of cephalosporins with sodium hydroxide which subsequently reacts with iodate to liberate iodine in acidic medium. The liberated iodine oxidizes variamine blue to violet colored species of maximum absorption at 556 nm. The absorbance is measured within the pH range of 4.0-4.2. Beer's law is obeyed in the range of 0.5-5.8 μg mL^{-1}, 0.2-7.0 μg mL^{-1}, 0.2-5.0 μg mL^{-1} and 0.5-8.5 μg mL^{-1} for cefotaxime, ceftriaxone, cefadroxil and cephalexin respectively. The analytical parameters were optimized and the method is successfully applied for the determination of cefotaxime, ceftriaxone, cefadroxil and cephalexin in pharmaceuticals.

Keywords: cephalosporins determination; spectrophotometry; variamine blue.

Introduction

Cephalosporins structurally differ from pencillins by the heterocyclic ring system. Cephalosporins are pencillinase-resistant antibiotics with significant activity against both gram-positive and gram-negative bacteria. The key intermediate for semisynthetic production of a large number of cephalosporins is 7-aminocephalosporanic acid, which is formed by hydrolysis of cephalosporin C produced by fermentation [1]. A few thousand semisynthetic cephalosporins have been described in the scientific literature, but only a small number of these has shown clinical importance.

Cefotaxime, ceftriaxone, cefradine and cephalexin are β-lactam antibiotics possessing a broad spectrum of antibacterial properties [2,3]. Several methods have been reported for the quantitative determination of cephalosporins. These include fluorimetric [4], polarographic [5] and isotachophoretic methods [6]. Some reported spectrophotometric methods for the determination of these analytes are chloranilic acid [3], paramolybdate anion [7], molybdophosphoric acid [8] formation of a complex with Cu(II) [9], a reaction with potassium iodate in acidic medium [10]. Cefotaxime, ceftriaxone and cefradine were also determined in pharmaceuticals preparations [11-15], Urine [14,16-19] and human serum [20]. Recently a rapid development of chromatographic determination methods of pharmaceuticals has been observed too [21,22].

The hydrolysis of ß-lactum ring, which is the common feature for cephalosporins and pencillins, has been achieved by the sodium hydroxide addition. Major difficulties in the determination of cephalosporin have been encountered at the β-lactum ring hydrolysis step [23]. A β-lactum enzyme

[24] has been used for the hydrolysed product of the analyte reacts with iodate in acid medium and liberates iodine. The liberated iodine oxidizes variamine blue to the violet colored species is the basis for the spectrophotometric determination of the analytes. The reaction mechanism followed the course similar to the one described for pencillins [25]. The proposed method has been successfully applied for the determination of cefotaxime, ceftriaxone, cefadroxil and cephalexin in pharmaceuticals.

Experimental

Apparatus

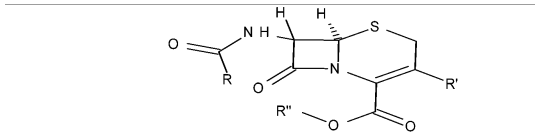
A Systronics 2201 UV-VIS Double Beam Spectrophotometer with 1 cm quartz cell was used for the absorbance measurements and a WTW pH 330, pH meter was used.

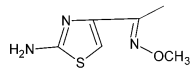
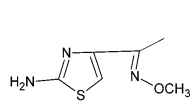
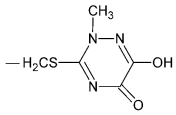
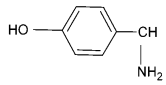
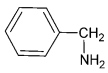
Reagents and solutions

All chemicals used were of analytical grade and doubly distilled water was used for dilution of the reagents and samples. Cefotaxime, ceftriaxone, cefadroxil, and cephalexin stock solutions (1000 μg mL^{-1}) were prepared by dissolving standard sodium cefotaxime (Alkem Lab. Ltd. Mumbai) or sodium ceftriaxone (Aristo Pharmaceuticals Ltd. Mumbai) or standard cefadroxil (Alkem Lab. Ltd. Mumbai) or standard cephalexin (Ranbaxy, India) in water. These compounds chosen to represent cephalosporins. They were prepared freshly, as required, by dissolving an appropriate amount of each antibiotic in water to provide a 1 μg mL^{-1} solution. The standard solution must be protected from contact with light. The structures of the cephalosporins studied are listed in table 1. Sodium hydroxide 0.1 mol L^{-1} aqueous solution, hydrochloric acid (Merck Limited, Mumbai) 1 mol L^{-1} aqueous solution, potassium iodate (S.D. fine – Chem Limited, Mumbai) 0.1 mol L^{-1} aqueous solution were used through out.

Taxim (Alkem Lab. Ltd. Mumbai), Monocef (Aristo Pharmaceuticals Ltd. Mumbai), Cefadrox (Aristo Pharmaceuticals Ltd. Mumbai) and Sporidex (Ranbaxy, India) were examined. A 0.05% solution of Variamine Blue (E-Merck Limited, Mumbai) in (75: 25) water-ethanol mixture was used and stored in an amber bottle.

Table 1. Structures of the cephalosporins studied.



Cephalosporin	R	R'	R"
1. Cefotaxime		$-CH_2OCOCH_3$	Na
2. Ceftriaxone			Na
3. Cefadroxil		$-CH_3$	H
4. Cephalexin		$-CH_3$	H

Procedure

An aliquot of a sample solution containing 0.5 – 5.8 μg mL^{-1} of cefotaxime, 0.2-7.0 μg mL^{-1} of ceftriaxone, 0.2-5.0 μg mL^{-1} of cefadroxil and 0.5-8.5 μg mL^{-1} of cephalexin was transferred into a series of 25 mL calibrated flasks, 1 mL of 0.1 mol L^{-1} sodium hydroxide were added and the mixture was kept on a water bath (80°C) for 10 min. after being cooled to room temperature (27 ± 2°C), 1.5 mL of 0.1 mol L^{-1} potassium iodate and 2 mL of 1 mol L^{-1} hydrochloric acid were added The mixture was gently shaken until the appearance of yellow color, indicating the liberation of iodine, 1 mL of 0.05 % of variamine blue was then added to it followed by the addition 2 mL of 1 mol L^{-1} of acetate buffer of pH 4 and the reaction mixture was shaken for 2 min. The contents were diluted up to 25 mL with distilled water and mixed well. The absorbance of the oxidized species variamine blue formed was then measured at 556 nm against the reagent blank prepared in the same manner, without the analyte. The amount of the cefotaxime, ceftriaxone, cefadroxil and cephalexin present in the volume taken was computed from the calibration graph.

Analysis of injection solution

An appropriate amount of each antibiotic was dissolved in water so as to prepare 1mg mL^{-1} solution and then the recommended procedure was followed without modification. The presence of other substances caused no significant interference with the determination of antibiotics.

Analysis of formulations

Weighed an amount of the sample equivalent to about 250 mg cephalosporin and was dissolve in a sufficient amount of distilled water. The solution was shaken and filtered off through Whatman No. 1 filter paper and washed with water. The filtrate was diluted up to the mark with distilled water and made upto 100 mL. The general procedure was applied with no modification and the presence of excipients in the sample such as glucose, fructose, lactose, sucrose, calcium or starch caused no interference in the determination and process of separation was not required.

Results and discussion

This method is based on the hydrolysis of ß-lactum ring of the analytes on heating with sodium hydroxide and the reaction of the hydrolysed product with potassium iodate in acidic medium to which liberates iodine. The liberated iodine oxidizes variamine blue to violet colored species of maximum absorption at 556 nm. The reagent blank had negligible absorbance at this wavelength. Beer's law is obeyed in the range of 0.5-5.8 μg mL^{-1}, 0.2-7.0 μg mL^{-1}, 0.2-5.0 μg mL^{-1} and 0.5-8.5 μg mL^{-1} for cefotaxime, ceftriaxone, cefadroxil and cephalexin respectively. Determination of cefotaxime, ceftriaxone, cefadroxil, and cephalexin are represented in scheme 1. The absorption spectra of the oxidized form of variamine blue are presented in figure 1, the absorption spectra of colored species of variamine blue with cefotaxime, ceftriaxone, cefadroxil and cephalexin against reagent blank in the range 300 – 800 nm are illustrated in figure 2. The maximum absorption is at 556 nm and reaction systems are presented in scheme 2.

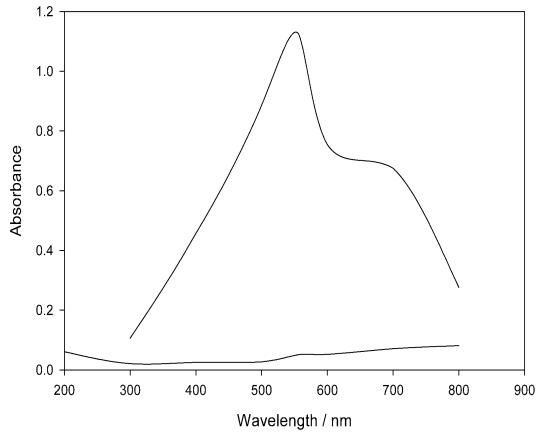


Figure 1. Absorption spectra of the oxidized form of variamine blue with reagent blank.

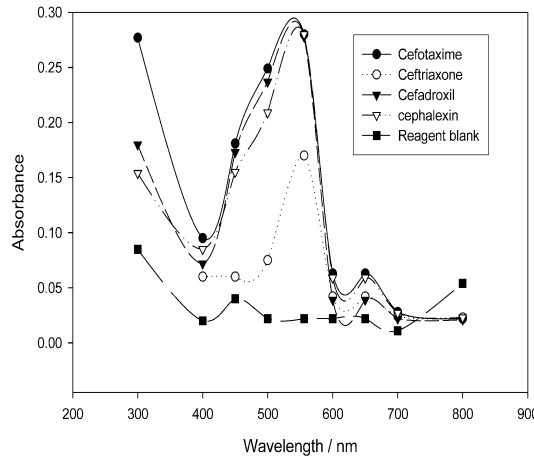
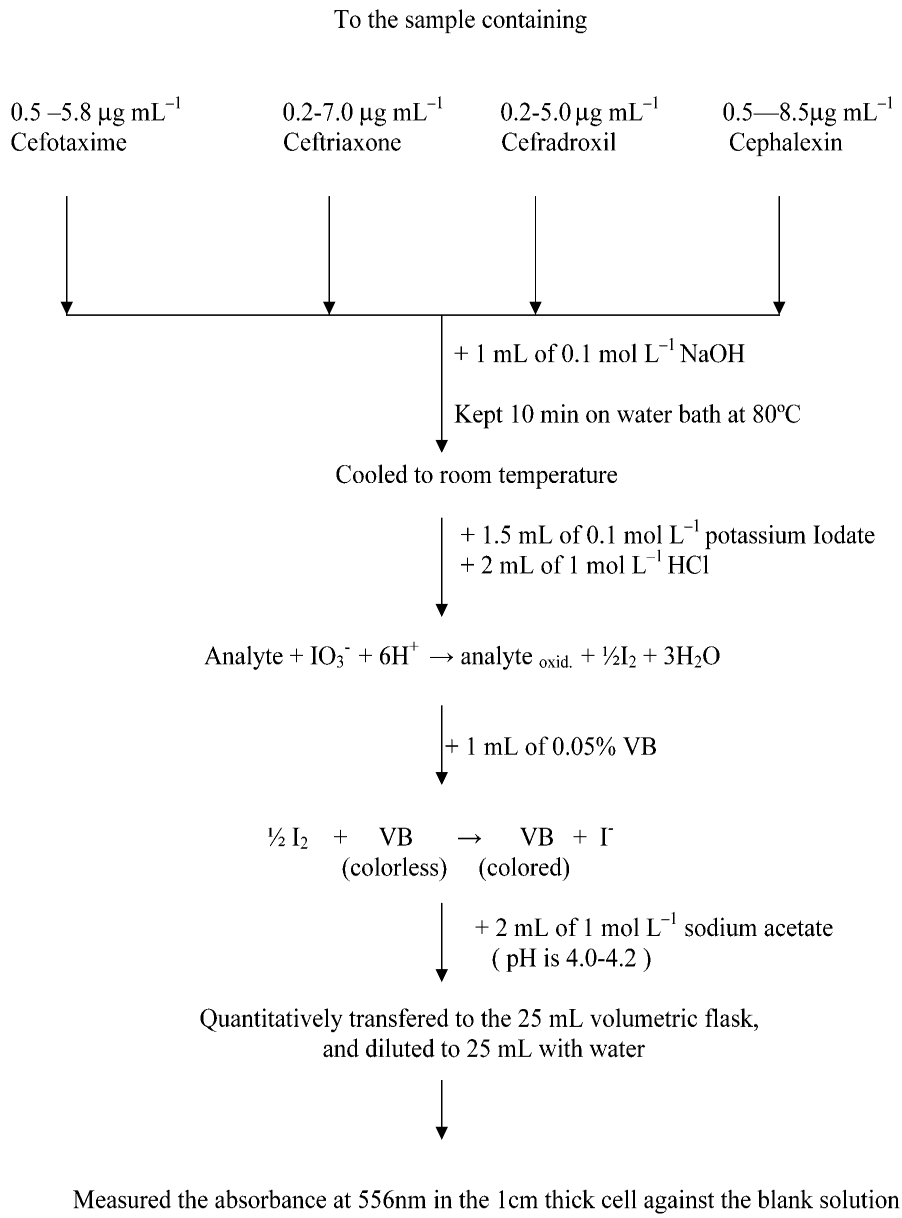


Figure 2. Absorption spectra of colored species of variamine blue with cefotaxime, ceftriaxone, cefadroxil and cephalexin against reagent blank: c (variamine blue) = 0.05%, c (cephalosporins) = 2 μg mL^{-1}.

Effect of sodium hydroxide concentration

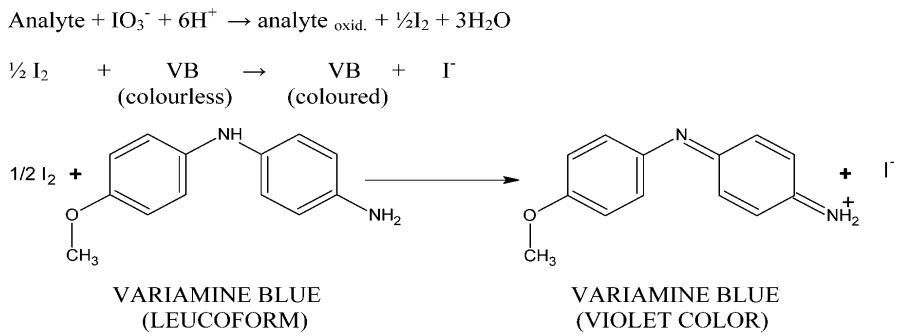
The effect of sodium hydroxide concentration on the absorbance was studied with 2 μg mL^{-1} of cephalosporins. Volumes from 0.5 – 2.0 mL of 0.1 mol L^{-1} NaOH solutions were examined. The investigation showed that 1.0 – 1.5 mL of 0.1 mol L^{-1} NaOH solution gave maximum absorbance and 1.0 mL of 0.1 mol L^{-1} NaOH solution was chosen for the procedure.



Scheme 1. Determination of cefotaxime, ceftriaxone, cefadroxil and cephalexin.

Effect of temperature, time and pH

The effect of different variables such as temperature, time and pH on the coloration was studied with 2 µg mL^{-1} of cephalosporins. It was observed that the optimum reaction temperature is 80°C – 90°C, lower or higher temperature gives inaccurate results, and the reaction time for complete hydrolysis of ß-lactum ring was 10–15 min. Constant and maximum absorbance values were obtained in the pH = 4.0 – 4.2 hence the pH of the reaction system was maintained at pH = 4.0–4.2 throughout the study by adding 2 mL of 1 mol L^{-1} sodium acetate solution. Effect of pH on color stability is presented in figure 3.



Scheme 2.

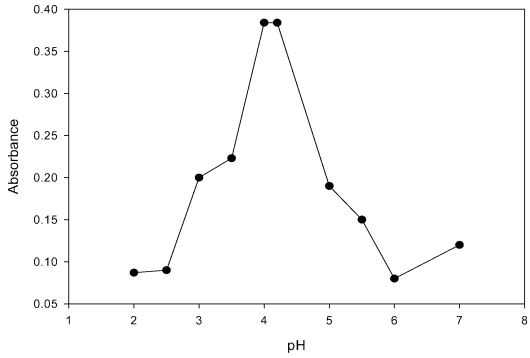


Figure 3. Effect of pH on color intensity for cefotaxime (2 μg mL^{-1}).

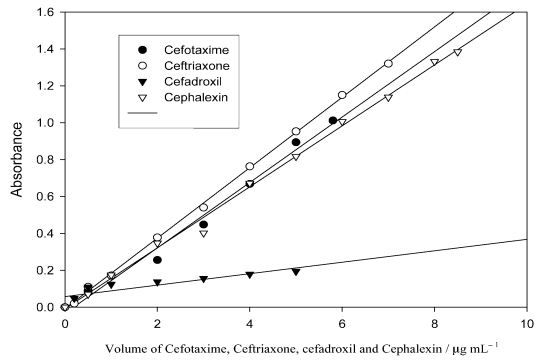


Figure 4. Calibration graphs of cefotaxime, ceftriaxone, cefadroxil and cephalexin:
c (cefotaxime) = 0.5 – 5.8 μg mL^{-1}, c (ceftriaxone) = 0.2 – 7.0 μg mL^{-1}, c (cefadroxil) = 0.2 – 5.0 μg mL^{-1}, c (cephalexin) = 0.5 – 8.5 μg mL^{-1}.

Calibration Graph

To the aqueous sample solutions containing 0.5 – 5.8 μg mL^{-1}, 0.2-7.0 μg mL^{-1}, 0.2-5.0 μg mL^{-1} and 0.5-8.5 μg mL^{-1} of cefotaxime, ceftriaxone, cefadroxil and cephalexin respectively, the reagents were added as described above. Within the studied concentration ranges, the measured absorbance values changed linearly. The correlation coefficients for cefotaxime, ceftriaxone, cefadroxil and cephalexin were found to be 0.9980, 0.9992, 0.9996 and 0.9991 respectively. The following regression coefficients were calculated: for cefotaxime α = 0.2239 b = 0.014, for ceftriaxone ? =0.1809 b = 0.0129, for cefadroxil α = 0.1622 b = 0.0065 and for cephalexin α = 0.1630 b = 0.0080. The following relative molar absorption coefficients were obtained: 1.07 x 10^5 $Lmol^{-1}cm^{-1}$, 1.02 x 10^5 $Lmol^{-1}cm^{-1}$, 2.68 x 10^4 $Lmol^{-1}cm^{-1}$ and 5.90 x 10^4 $Lmol^{-1}cm^{-1}$ for cefotaxime, ceftriaxone, cefadroxil and cephalexin respectively. Calibration graphs for the determination of cefotaxime, ceftriaxone, cefadroxil and cephalexin are presented in figure 4.

Effect of foreign substances

The influence of foreign substances was examined by the proposed method. The maximum tolerance (in mg) in the determination of 100 μg mL^{-1} cephalosporins was 54.0 for glucose, 35.5 for fructose, 56.5 for lactose, 32.4 for sucrose, 8.4 for starch and 22.0 for calcium. The tolerance limits of foreign substances are summarized in table 2.

Table 2. Maximum amount tolerance of excipients for the determination of cephalosporins.

Common excipient	Tolerance limit (mg)
Glucose	54.0
Fructose	35.5
Lactose	56.5
Sucrose	32.4
Starch	8.4
Calcium	22.0

Table 3. Determination of cefotaxime, ceftriaxone, cefadroxil, cephalexin and cephalexin in pharmaceuticals preparations.

Pharmaceutical	Declared Quantity ($\mu g\ mL^{-1}$)	Found in the sample a ($\mu g\ mL^{-1}$) ± S.D
TAXIM 1	2.00	1.984 ± 0.03
TAXIM 2	4.00	3.975 ± 0.02
TAXIM 3	5.50	5.454 ± 0.02
MONOCEF 1	1.00	0.986 ± 0.03
MONOCEF 2	3.00	2.992 ± 0.04
MONOCEF 3	5.00	4.954 ± 0.02
MONOCEF 4	7.00	6.966 ± 0.025
CEFADROX 1	2.00	1.994 ± 0.01
CEFADROX 2	4.00	3.986 ± 0.02
SPORIDEX 1	2.00	1.984 ± 0.015
SPORIDEX 2	4.00	3.896 ± 0.01
SPORIDEX 3	6.00	5.982 ± 0.04
SPORIDEX 4	8.00	7.940 ± 0.08

[a] Average of three determinations.

Application

The proposed method has been successfully applied to the determination of studied antibiotics in pharmaceuticals. Cefotaxime was determined in 1 g- vials of taxim, ceftriaxone in 250 mg vials of monocef, cefadroxil in 250 mg tablets of cefodrox and cephalexin in 125 mg tablets of sporidex. Their contents in the investigated drug samples were calculated from the calibration curves mentioned above are found to be in a good agreement with the labelled amounts (table 3). The results, listed in the table 3 compared favorably with those from a reference method [26]. The precision of the proposed method was evaluated by replicate analysis of 3 samples containing cephalosporins at different concentrations.

Conclusions

A simple method for the determination of ß -lactum antibiotics is described. The method is based on the reaction of iodate with the hydrolysed product of ß -lactum antibiotics which liberates iodine, subsequently oxidizes variamine blue into violet colored species, and measured at 556nm. The developed method does not involve any stringent reaction conditions and offers the advantages of high stability of the reaction system (4 hours). The proposed method was applied to the determination of cephalosporins in pharmaceuticals.

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