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SIMPLE SPECTROPHOTOMETRIC DETERMINATION OF SELECTED CEPHALOSPORINS USING THIONIN

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ABSTRACT

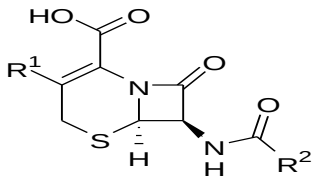
A simple spectrophotometric method for the determination of cephalosporin antibiotics as pharmaceutical ingredient, namely cefotaxime, ceftriaxone, cefadroxil and cefalexin using thionin is described. The method is based on the hydrolysis of β-Lactam ring of with sodium hydroxide which subsequently reacted with potassium iodate in acidic medium to liberate iodine. The liberated iodine bleaches the violet color of thionin, which measured at 600 nm. The decrease in absorbance is directly proportional to the concentration of cefotaxime, ceftriaxone, cefadroxil and cefalexin. Beer's law is obeyed in the range 0.5 - 6.4 μg mL^{-1}, 0.4 - 5.2 μg mL^{-1}, 0.8 - 4.2 μg mL^{-1} and 1.0 - 7.5 μg mL^{-1} for cefotaxime, ceftriaxone, cefadroxil and cefalexin respectively. The molar absorptivity was found to be 7.21×10^4 $Lmol^{-1}cm^{-1}$ for cefotaxime, 1.23×10^5 $Lmol^{-1}cm^{-1}$ for ceftriaxone, 6.91×10^4 $Lmol^{-1}cm^{-1}$ for cefadroxil and 4.08×10^4 $Lmol^{-1}cm^{-1}$ for cefalexin. The method has been successfully applied to the determination of cefotaxime, ceftriaxone, cefadroxil and cefalexin in various pharmaceutical samples.

Keywords: *Cephalosporins Determination, Spectrophotometry, Thionin*

1. INTRODUCTION

The cephalosporins are a class of β-lactam antibiotics originally derived from *Acremonium,*which was previously known as "Cephalosporium"[1-4]. Cephalosporin compounds were first isolated from cultures of *Cephalosporium acremonium* from a sewer in Sardinia in 1948 by Italian scientist Giuseppe Brotzu. He noticed that these cultures produced substances that were effective against *Salmonella typhi*, the cause of typhoid fever, which had beta-lactamase. Guy Newton and Edward Abraham at the Sir William Dunn School of Pathology at the University of Oxford isolated cephalosporin C. The cephalosporin nucleus, 7-aminocephalosporanic acid (7-ACA), was derived from cephalosporin C and proved to be analogous to the penicillin nucleus 6-aminopenicillanic acid, but it was not sufficiently potent for clinical use. Modification of the 7-ACA side-chains resulted in the development of useful antibiotic agents, and the first agent cephalothin (cefalotin) was launched by Eli Lilly and Company in 1964.

The cephalosporin nucleus can be modified to gain different properties. Cephalosporins are sometimes grouped into "generations" by their antimicrobial properties. The first cephalosporins were designated first-generation cephalosporins, whereas, later, more extended-spectrum cephalosporins were classified as second-generation cephalosporins. Each newer generation of cephalosporins has significantly greater Gram-negative antimicrobial properties than the preceding generation, in most cases with decreased activity against Gram-positive organisms. Fourth-generation cephalosporins, however, have true broad-spectrum activity.



Cephalosporin (1)

Cefotaxime[5] pronounced is a third-generation cephalosporin antibiotic. Like other third-generation cephalosporins, it has broad spectrum activity against Gram positive and Gram negative bacteria. In most cases, it is considered to be equivalent to ceftriaxone in terms of safety and efficacy. Cefotaxime sodium is marketed under various trade names including Kefotex by Gepach International, Cefotax by EIPICO and Claforan by Roussel-Uclaf.

Ceftriaxone[6-9] sodium is marketed by Hoffman-La Roche under the trade name Rocephin. Ceftriaxone is often used (in combination, but not direct, with macrolide and/or aminoglycoside antibiotics) for the treatment of community-acquired or mild to moderate health care-associated pneumonia. It is also a choice drug for treatment of bacterial meningitis. In pediatrics, it is commonly used in febrile infants between 4 and 8 weeks of age who are admitted to the hospital to exclude sepsis. The dosage for acute ear infection in the very young is 50 mg/kg IM, one dose only. It has also been used in the treatment of Lyme disease, typhoid fever and gonorrhea.

Several methods have been reported for the quantitative determination of cephalosporins. These include polorographic [10], isotachophoretic [11] and fluorimetry [12]. Some spectrophotometric methods are reported in the literature for the determination of cephalosporins are paramolybdate anion[13], molybdophosphoric acid[14], a reaction with potassium iodate in acidic medium[15] and formation of a complex with Cu(II)[16].

In the present investigation, a simple spectrophotometric determination of cefotaxime, ceftriaxone, cefadroxil and cefalexin with a new reagent thionin is described. The developed method has been successfully applied for the determination of cefotaxime, ceftriaxone, cefadroxil and cefalexin in pharmaceuticals.

2. EXPERIMENTAL

2.1 APPARATUS, CHEMICALS AND REAGENTS

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

All chemicals used were of analytical grade and doubly distilled water was used for dilution of reagents and samples. Cefotaxime, ceftriaxone, cefadroxil, and cefalexin 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 cefalexin (Ranbaxy, India) in water. These compounds chosen to

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 (Merck Limited, Mumbai) 0.1M aqueous solution, hydrochloric acid (Merck Limited, Mumbai) 1M aqueous solution, potassium iodate (S.D. fine – Chem Limited, Mumbai) 0.1M 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. Thionin (S.D. fine – Chem Limited, Mumbai) 0.1% was prepared by dissolving 0.1g of thionin in 25 mL of methanol and made up to 100 mL with distilled water.

2.2 PROCEDURE

An aliquot of a sample solution containing 0.5 – 6.4 μg mL^{-1} of cefotaxime, 0.4 –5.2 μg mL^{-1} of ceftriaxone, 0.8 – 4.2 μg mL^{-1} of cefadroxil and 1.0 – 7.5 μg mL^{-1} of cefalexin was transferred into a series of 25 mL calibrated flasks, 1 mL of 0.1 M sodium hydroxide were added and the mixture was kept in a water bath (80°C) for 10 min. after being cooled to room temperature (27 ± 2°C), 1.5 mL of 0.1 M potassium iodate and 2 mL of 1 M hydrochloric acid were added The mixture was gently shaken until the appearance of yellow color, indicating the liberation of iodine, 1 mL of 0.1 % of thionin was then added to it followed by the addition 2 mL of 1 M 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 resulting solution was measured at 600 nm against distilled water. A blank was prepared by replacing the analyte(cefotaxime, ceftriaxone, cefadroxil and cefalexin) solution with distilled water. The absorbance corresponding to the bleached color that in turn corresponds to the analyte concentration was obtained by subtracting the absorbance of the blank solution from that of test solution. The amount of the cefotaxime, ceftriaxone, cefadroxil and cefalexin present in the volume taken was computed from the calibration graph.

2.3 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.

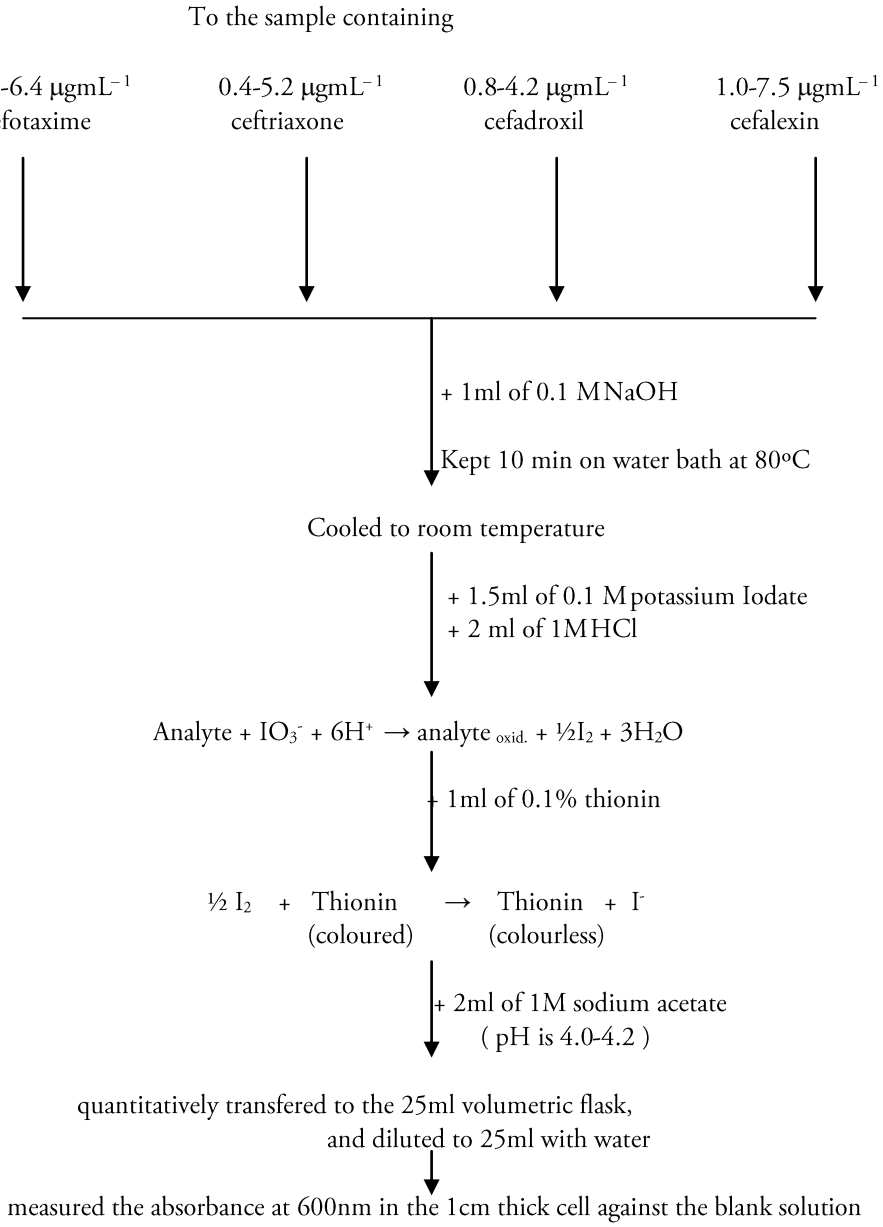
2.4 ANALYSIS OF FORMULATIONS

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.

3. 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 bleaches the violet color of thionin and is measured at 600nm. This decrease in absorbance is directly proportional to the cefotaxime, ceftriaxone, cefadroxil and cefalexin concentration and obeys Beer's law in the range 0.5 – 6.4 μg mL^{-1}, 0.4 – 5.2 μg mL^{-1}, 0.8 – 4.2 μg mL^{-1} and 1.0 – 7.5 μg mL^{-1} for cefotaxime, ceftriaxone, cefadroxil and cefalexin respectively. Determination of cefotaxime, ceftriaxone, cefadroxil and cefalexin are represented in Scheme 1.



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

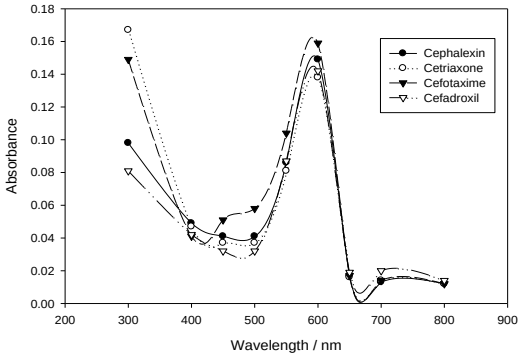
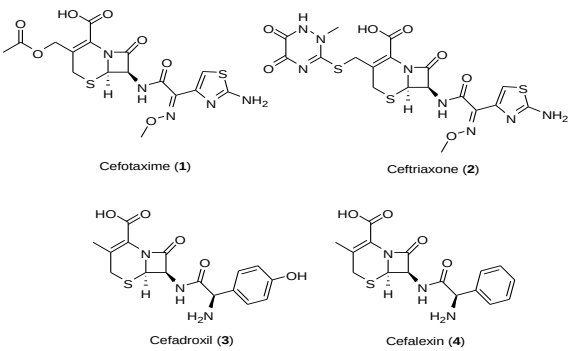


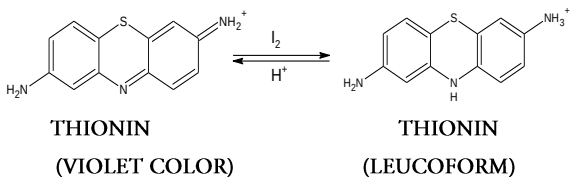
Fig. 1. Absorption spectra of colored species of thionin with cefotaxime, ceftriaxone, cefadroxil and cefalexin. (Cephalosporin concentration = 5 μg mL^{-1})

The absorption spectra of colored species of thionin with cefotaxime, ceftriaxone, cefadroxil and cefalexin against reagent blank in the range 300–800 nm are illustrated in Fig.1. The maximum absorption is at 600 nm and reaction systems are presented in Scheme 2.



$$\text{Analyte} + IO_3^- + 6H^+ \rightarrow \text{analyte}_{\text{oxid.}} + \tfrac{1}{2}I_2 + 3H_2O$$

$$\tfrac{1}{2}\,I_2 + \text{Thionin (coloured)} \rightarrow \text{Thionin (colourless)} + I^-$$



Scheme 2. Scheme of the reaction system

To the aqueous sample solutions containing 0.5 – 6.4 μgmL^{-1}, 0.4 – 5.2 μgmL^{-1}, 0.8 – 4.2 μg mL^{-1} and 1.0 – 7.5 μg mL^{-1} of cefotaxime, ceftriaxone, cefadroxil and cefalexin 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 cefalexin were found to be 0.9990, 0.9992, 0.9981, and 0.9975 respectively. The following regression coefficients were calculated: for cefotaxime a = 0.1563 b = -0.0016, for ceftriaxone a =0.2251 b = 0.0083, for cefadroxil a = 0.1694 b = -0.0013 and for cefalexin a = 0.0966 b = 0.0161. The following relative molar absorption coefficients were obtained: 7.21×10^4 $Lmol^{-1}cm^{-1}$, 1.23×10^5 L mol^{-1} cm^{-1}, 6.91×10^4 L mol^{-1} cm^{-1} and 4.08×10^4 L mol^{-1} cm^{-1} for cefotaxime, ceftriaxone, cefadroxil and cefalexin respectively. Calibration graphs for the determination of cefotaxime, ceftriaxone, cefadroxil and cefalexin are presented in Fig.2.

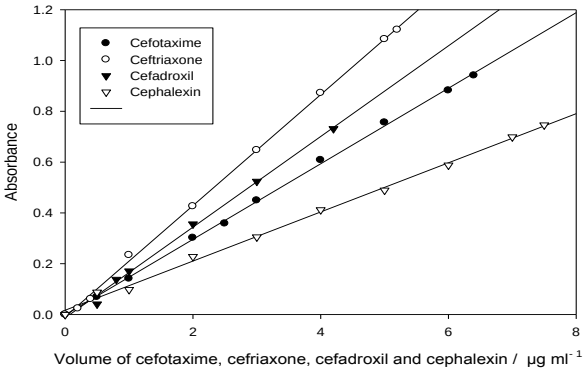


Fig.2. Calibration graphs of cefotaxime, ceftriaxone, cefadroxil and cefalexin.

3.1 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 M NaOH solutions were examined. The investigation showed that 1.0 – 1.5 mL of 0.1 M NaOH solution gave maximum absorbance and 1.0 mL of 0.1 M NaOH solution was chosen for the procedure.

3.2 Effect of temperature, time and pH

The effect of different variables such as temperature, time and pH on the decolorization 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 M sodium acetate solution.

3.3 Study of the proposed experimental conditions

The proposed method was successfully applied to the determination of cephalosporins in pharmaceutical preparations. The results were listed in Table 1. The precision and accuracy of the proposed method was checked. The effect of different variables such as temperature, time, pH and reagent concentration has been evaluated to permit selection of the most advantageous technique. 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. The pH of the reaction system was maintained at 4.0 – 4.2 throughout the study. This could be achieved by the addition of 2 mL of 1 M sodium acetate solution in a total volume of 25 mL. Under the optimum reaction conditions, the colored final product was found to be stable for a period of 8 hours and its absorbance was measured at 600 nm. The reagent blank had negligible absorbance at this wavelength.

Table 1: Determination of cefotaxime, ceftriaxone, cefadroxil and cefalexin in exemplary pharmaceuticals preparations

Pharmaceutical	Declared Quantity (μg mL^{-1})	Found in the sample[a] (μg mL^{-1}) ± S.D
TAXIM 1	2	1.896 ± 0.04
TAXIM 2	4	3.936 ± 0.015
TAXIM 3	6	5.898 ± 0.03
MONOCEF 1	1	0.982 ± 0.04
MONOCEF 2	3	3.010 ± 0.02
MONOCEF 3	5	4.924 ± 0.025
CEFADROX 1	2	1.962 ± 0.015
CEFADROX 2	4	3.891 ± 0.02
SPORIDEX 1	2	1.994 ± 0.05
SPORIDEX 2	4	3.985 ± 0.03
SPORIDEX 3	6	5.954 ± 0.02

[a] Average of three determinations.

3.4 Effect of foreign substances

The influence of foreign substances was examined by the proposed method. The maximum tolerance 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.

4. APPLICATION

The 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 cefalexin in 125 mg tablets of Sporidex. The results, listed in the table 1 compared favorably with those from a reference method[17]. The precision of the proposed method was evaluated by replicate analysis of 3 samples containing cephalosporins at different concentrations.

5. CONCLUSION

A simple and sensitive 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 bleaches the violet coloured species, and measured at 600 nm. The proposed method is applied to the determination of cephalosporins in pharmaceuticals.

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طريقة فوتوميتر طيفية مبسطة لتحديد مركبات معينة من سيفالوسبورين بإستخدام الثيونين

تشاندباشا[1] و ب. نارايانا[2]

[1] قسم تقنية الهندسة الكيميائية، كلية ينبع الصناعية، المملكة العربية السعودية
[2] كلية الدراسات العليا والبحث في الكيمياء، جامعة مانجالور، كارناتكا، الهند

الملخص:

تصف هذه الدراسة طريقة فوتوميتر طيفية لتحديد تركيز المضاد الحيوي (Cephalosporin) في المركبات الدوائية وهي (cefotaxime) و (ceftriaxone) و (cefadroxil) و (cefalexin) بإستخدام مادة ثيونين. هذه الطريقة تعتمد على تميه (β-Lactam) الحلقية الموجودة في مركب (cephalosporins) مع هيدروكلوريد الصوديوم والتي تتفاعل عبد ذلك مع مادة يوديد البوتاسيوم في الوسط الحامضي والتي ينتج عنها اليود .

إن اليود الناتج سيقوم بإلغاء لون الثيونين البنفسجي والذي يمكن قياسه على طول موجي 600 . nm . إن التناقص في الإمتصاص يتناسب طردياً مع تراكيز (cefotaxime) و (ceftriaxone) و (cefadroxil) و (cefalexin) . وهذه الطريقة تم تطبيقها بنجاح لتحديد كميات (cefotaxime) و (ceftriaxone) و (cefadroxil) و (cefalexin) في عينات دوائية مختلفة .