SPECTROPHOTOMETERIC DETERMINATION OF SOME CEPHALOSPORINS IN PHARMACEUTICAL FORMULATIONS

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تم وضع طريقة طيف ضوئية بسيطة وحساسة لتقدير سيفادروكسيل (cefadroxil)، سيفوتاكسيم (cefotaxime) و سيفترايا كسون (ceftriaxone) بصورها النقية ومستحضراتها الطبيـــة. وتعتمد الطريقة على مفـــاعـــلة هذه العقاقير مع نترات الحديـــد الثلاثي لتكوين حديـــد ثنائي الـــذي يتفـــاعل مع ســـداسي سيـــانو حديـــد(III) البوتـــاسيوم potassium hexacyanoferrate(III) مكوناً متراكب (مركب معقد) أزرق اللون يمكن قياسه طيفياً عند ٧٧٧ نانومتر. وتم الحصول على أعلى شـــدة لون بالتسخين. والعلاقة الخطية كانت في مدى ٠,٦-١٠,٠ ميكرو جرام/مل، ٠,١-٨,٠ ميكرو جرام/مل و ٠,١-٣,٠ ميكرو جرام/مل للسيفادروكسيل، سيفوتاكسيم و سيفترايا كسون، على التوالي في حدود ٠,١ ميكرو جرام/مل للسيفادروكسيل و ٠,٠٥ ميكرو جرام/مل للعقارين الآخرين. ووجد أن معـــامل الارتبـــاط الخطي (r) > ٠,٩٩٩ (n = 8-9). وكان متوسط الانحراف القياسي (RSD %) في القراءات في حدود ≤ ١,١ ٪ مما يعزز جدوى هذه الطريقة. وكان الاختلاف بين نتائج هذه الطريقة والطرق المرجعية المعتمدة صغيراً يمكن إهماله. وأمكن تطبيق هذه الطريقة بنجاح لتقدير هذه العقاقير في جرعاتها.

A simple and sensitive spectrophotometric method is described for the determination of cefadroxil, cefotaxime and ceftriaxone in pure form and pharmaceutical formulations. The method is based on reacting these drugs with iron(III) nitrate to form iron(II) which reacts with potassium hexacyanoferrate(III) forming a blue colored complex measurable spectrophotometrically at 777 nm. Maximum color formation was obtained through heating. Linearity was in the rang 0.6-10.0 μg ml^{-1}, 0.1-8.0 μg ml^{-1} and 0.1-3.0 μg ml^{-1} for cefadroxil, cefotaxime and ceftriaxone, respectively with limits of detection of 0.1 μg ml^{-1} for cefadroxil and 0.05 μg ml^{-1} for the other two drugs. Linear response was observed over the tested range of each drug with correlation coefficient (r) > 0.999 (n = 8-9). The mean relative standard deviation (RSD %) of the results of within day precision and accuracy of them were ≤ 1.1% which confirmed the reproducibility of the assay technique. The method results showed insignificant difference with those of reference methods. The method was successfully applied to the determination of these drugs in their dosage forms.

Keywords: Spectrophotometric; cefadroxil; cefotaxime; ceftriaxone; hexacyanoferrate(III); pharmaceutical formulations.

INTRODUCTION

Cefadroxil monohydrate (**1**) is one of the first generation of cephalosporin antibiotics. It is a semi-synthetic antibiotic has introduced into clinical practice [1]. The cephalosporins are bactericidal and act similarly to the penicillins, by inhibiting the synthesis of the bacterial cell wall. Cefadroxil is active by oral route on the sensitive gram-positive and gram-negative organisms and it is used for the treatment of urinary tract infections [1-3]. Numerous analytical procedures have been reported for its determination in pure form, pharmaceutical preparations or in biological fluids. The USPXXIII [4] recommends an HPLC assay, for the evaluation of its raw material and dosage forms. Other reported methods included spectrophotometric [5-7], flow-injection spectro-

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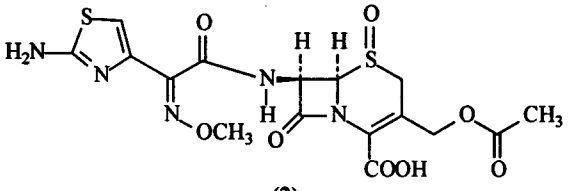
photometric [8], fluorimetric [9], polarographic [10], TLC [11], HPLC [12,13] and flow-injection chemilumenescence methods [14, 15].

Cefotaxime (2) and ceftriaxone (3) are third-generation cephalosporin antibiotics used in the treatment of infections due to susceptible organisms, especially serious and life-threatening infections, intensive care, Lyme disease, meningitis, peritonitis, pneumonia, septicaemia, surgical infection and typhoid fever [2,3]. Because of the therapeutic importance and wide spread use of these compounds, the literature contains many publications dealing with their determination in pure forms, dosage forms and in biological fluids.



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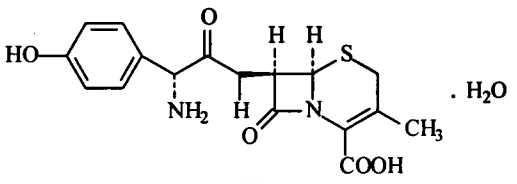

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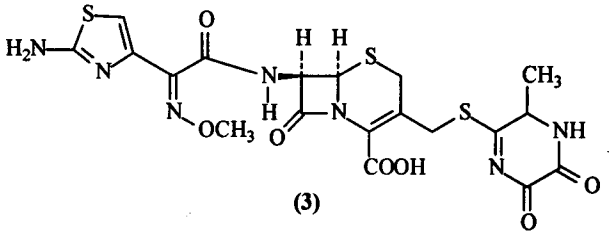

(3)

Most of the methods employed for the determination of cefotaxime and ceftriaxone are spectrophotometric. Among these methods, oxidation with cerium(IV) and chlorobenzotriazole [16], complexation with palladium(II) chloride [17], reaction with DDQ [18], oxidation with cerium(IV) and H_2SO_4 [19], hydrolysis with sodium hydroxide at 80°C followed by oxidation with iron(III) in H_2SO_4 and reaction of the resulted iron(II) with O-phenanthroline [20], reaction with 2,3,5-triphenyl tetrazolium chloride at 50°C [21] and charge transfer complexation with *p*-chloranilic acid [22]. Other methods include: derivative spectrophotometric [23] spectrofluorimetric [19], voltammetric [24,25], polarographic [26,27], TLC [28,29], HPLC [30,31] and capillary zone electrophoresis methods [32]. This work describes a new spectrophotometric method based on the reduction properties of the studied drugs.

EXPERIMENTAL

Apparatus:

A Unicam UV–VIS Spectrometer (Helios Alpha: Helios Beta, Cambridge, Britain) was used equipped with a quarts cell of 1.0 cm width for the λ_{max} determination and all absorbance measurements.

Reagents and Materials:

Analytical reagents grade chemicals and distilled water were used throughout. Pure drug samples were kindly provided by Bristol-Myers, Squibb, Egypt and used as received. Each drug standard solution 1.0 mg ml^{-1} was prepared in distilled water. Working standard solutions were prepared by appropriate dilution immediately before use. Dosage forms containing the studied drugs being purchased from commercial sources. Iron(III) nitrate (Riedel de Haen), 0.1M solution, was prepared by dissolving 4.04 g of $Fe(NO_3)_3$. 9 H_2O in 100 ml of distilled water; working

solutions were also diluted with distilled water. Potassium hexacyanoferrate(III) (BDH), 0.1 M stock solution, was prepared by dissolving 3.29g of $K_3Fe(CN)_6$ in 100 ml of distilled water.

Procedures:

General Procedure:

Working solutions of each drug in the range cited in Table 1 were prepared from stock solutions. The drug sample is allowed to react with 2.5 ml of 1×10^{-3} M iron(III) nitrate in a 25-ml volumetric flask. The produced iron(II) is reacted with 2.5 ml of 1×10^{-3} M potassium hexacyanoferrate(III), heating in a water bath at $90^{o}C$ for 60 min then completed to volume with distilled water. The absorbance of the resulting blue solution is measured at 777 nm against a blank solution treated similarly. Calibration graphs were prepared by plotting the absorbance against the drug concentration over the ranges cited in Table 1 for each drug.

Determination of Accuracy, Precision and Reproducibility:

To assess the precision of the within-day method, six measurements of each of the concentrations range, cited in Table 1, for the three drugs, were performed on a single day. The reproducibility of the assay (within-day and between-day) was evaluated by comparing the linear regression analysis of three standard plots obtained at three different days over two week's period.

Procedure for Capsules and Suspensions:

An accurately weighed amount of the contents of 10 capsules or an accurately measured volume of oral suspension equivalent to 10.0 mg of the drug, was transferred into a beaker with 50 ml distilled water sonicated for 5 min and filtered into a 100-ml volumetric flask then completed to volume with distilled water.

The method of standard additions was followed for the determination of cefadroxil in dosage forms using the general procedure described above.

Procedure for Ampoules:

An accurately weighed amount of mixed contents of 10 ampoules equivalent to 10.0 mg of the drug was transferred into a 100 ml volumetric flask and completed to the mark with distilled water and the measurement carried out as described earlier under general procedure. The nominal content was calculated from the corresponding calibration graph.

RESULTS AND DISCUSSION

Absorption Spectra of the Coloured Complexes:

Each of the studied cephalosporins reacts with iron(III) to produce iron(II) which in the presence of potassium hexacyanoferrate(III) forms a blue compound measurable at 777 nm (Fig. 1).

The color of the complex is related to the charge transfer excitation where the electron is transferred from iron(II) to iron(III). This complex is known as Turnbull blue, $[KFe(CN)_6Fe]$, but when iron(III) salts are added to potassium hexacyanoferrate(II), $[Fe(CN_6)]^{-4}$, the resulted blue complex is known as Prussian blue, $[KFe(CN_6)Fe]$. The chemical structures for both complexes are similar but in Prussian blue complex the iron(III) is bonded with the cyanide molecule through the nitrogen atom while in Turnbull blue complex, the iron(II) is bonded to cyanide molecule through the carbon atom [33]. Because of their strong absorbance, the complexes of Turnbull blue and Prussian blue were used to determine many organic and inorganic compounds such as tenoxicam [34], phenothiazines [35], sugars [36], and persulphate [37].

The absorbance of the blue complex is directly related to the concentration of the cephalosporin and can be used for its spectrophotometric determination.

The development of the color depends very much on the reaction conditions. Therefore it is very important to optimize the reaction conditions.

Effect of Iron(III) Nitrate:

Iron(III) is reduced to iron(II) by the cephalosporin, which reacts with $K_3Fe(CN)_6$ to form a blue color.

The effect of different concentrations of iron(III) nitrate was investigated in the range 1×10^{-5} - 5×10^{-4} M. A concentration of 1×10^{-4} M gave the highest absorbance and thus was chosen for further use (Fig. 2).

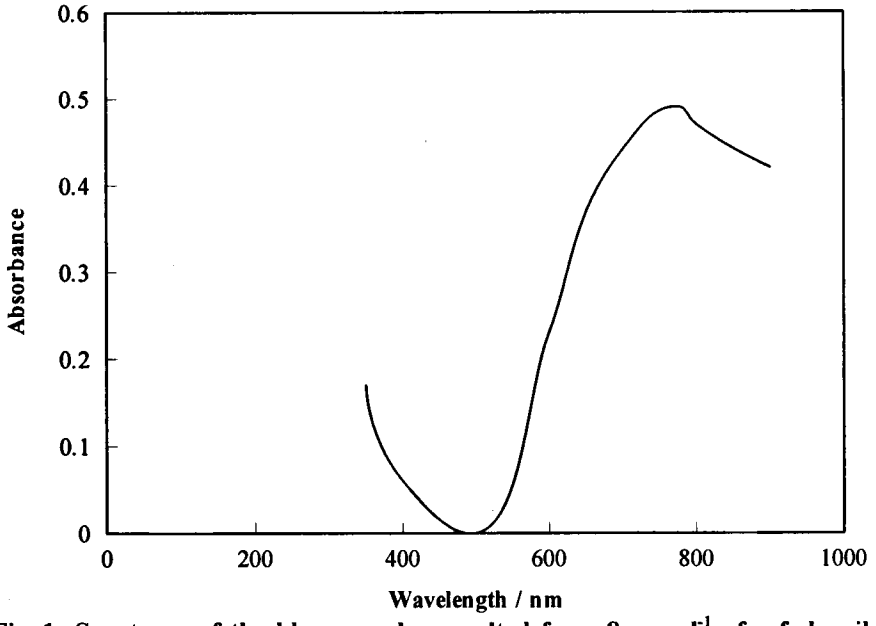

Fig. 1: Spectrum of the blue complex resulted from 8 μg ml^{-1} of cefadroxil with 1 × 10^{-4} M iron(III) nitrate and 1 × 10^{-4} M potassium hexacyanoferrate(III) after heating at 90°C for 60 min.

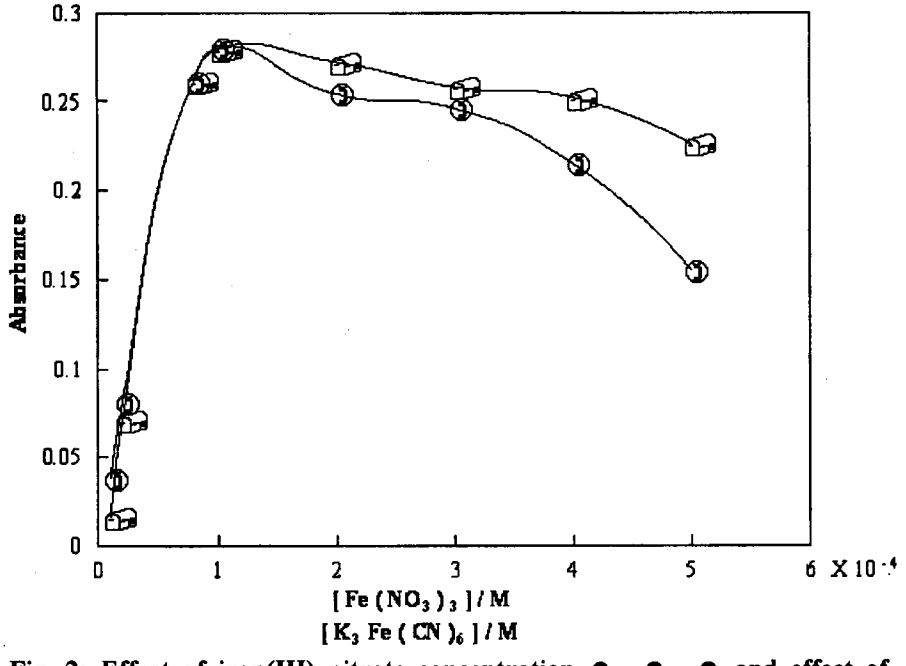

Fig. 2: Effect of iron(III) nitrate concentration ●—●—● and effect of potassium hexacyanoferrate(III) concentration ■—■—■ on the absorbance of the blue complex at 777nm, cefadroxil 8 μg ml^{-1}, temperature 90°C, heating time 60 min.

Table1: Performance data for spectrophotometric determination of the studied cephalosporins

Compound	Linear calibration Range (μg ml^{-1})	Limit of detection (μg ml^{-1})	Linear regression		
			Intercept (a)	Slope (b)	Correlation coefficient (r)
Cefadroxil	0.6-10.0*	0.10	0.015	0.033	0.9999
Cefotaxime	0.1-8.0*	0.05	0.012	0.0397	0.9994
Ceftriaxone	0.1-3.0**	0.05	-3.87x10^{-5}	0.105	0.9995

* n = 9
** n = 8

Effect of Potassium Hexacyanoferrate(III):

The effect of potassium hexacyanoferrate(III) concentration was similarly studied in the range 1 x 10^{-5} – 5 × 10^{-4} M. The absorbance increased with increasing $K_3Fe(CN)_6$ concentration up to 1 × 10^{-4}M, above which it decreased due to precipitate formation, as shown in Fig. 2. The working solution chosen was 1 × 10^{-4} M.

Effect of Temperature:

Temperature greatly enhances the reaction. Different temperatures were tested, from 25-100 °C, using the water bath. 90 °C gave the best results as shown in Fig. 3. Temperatures higher than 90 °C gave low absorbance readings due to precipitate formation.

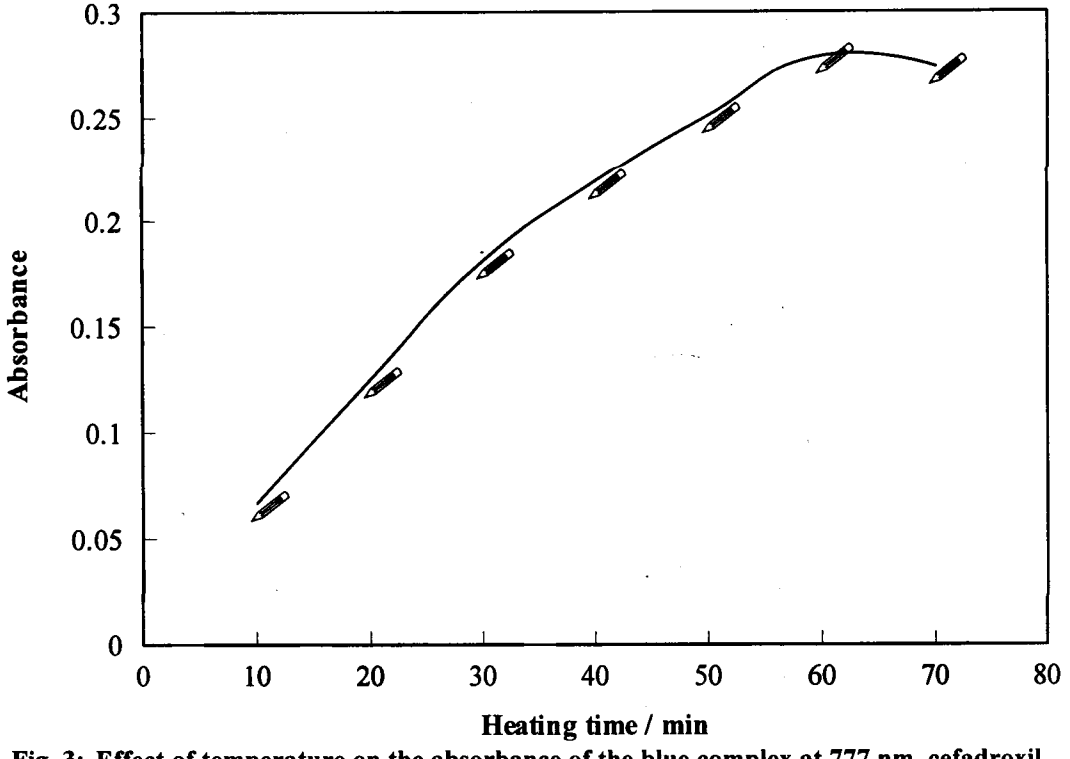

Fig. 3: Effect of temperature on the absorbance of the blue complex at 777 nm, cefadroxil 8 μg ml^{-1}, iron(III) nitrate 1 × 10^{-4} M, potassium hexacyanoferrate (III) 1 × 10^{-4} M, heating time 60 min.

Effect of Heating Time:

The effect of heating time was studied in the range 10-70 min as shown in Fig. 4. At the beginning of the reaction, a green color is observed, the development of the blue color started after 40 min of the reaction and completed after 60 min in the water bath at 90 °C, after which the absorbance started to decrease.

The blue color of the complex was stable for one hour, after that turbidity of solution was observed.

Effect of Acids:

The effect of addition of some acids, including nitric acid, hydrochloric acid, sulphuric acid, perchloric acid, phosphoric acid, formic acid and acetic acid on the absorbance of the blue complex were investigated. All these acids were found to decrease the absorbance so they were avoided.

Effect of Order of Addition:

After studying the optimum conditions for the reaction of cefadroxil with the reagnts, a series of experiments were performed to test for the order of addition of the reactants which gave the highest absorbane of the coloured complex. It was found that the best order of addition is to add iron(III) nitrate to cefadroxil solution followed by the addition of potassium hexacyanofferrate(III) as shown in Table 2.

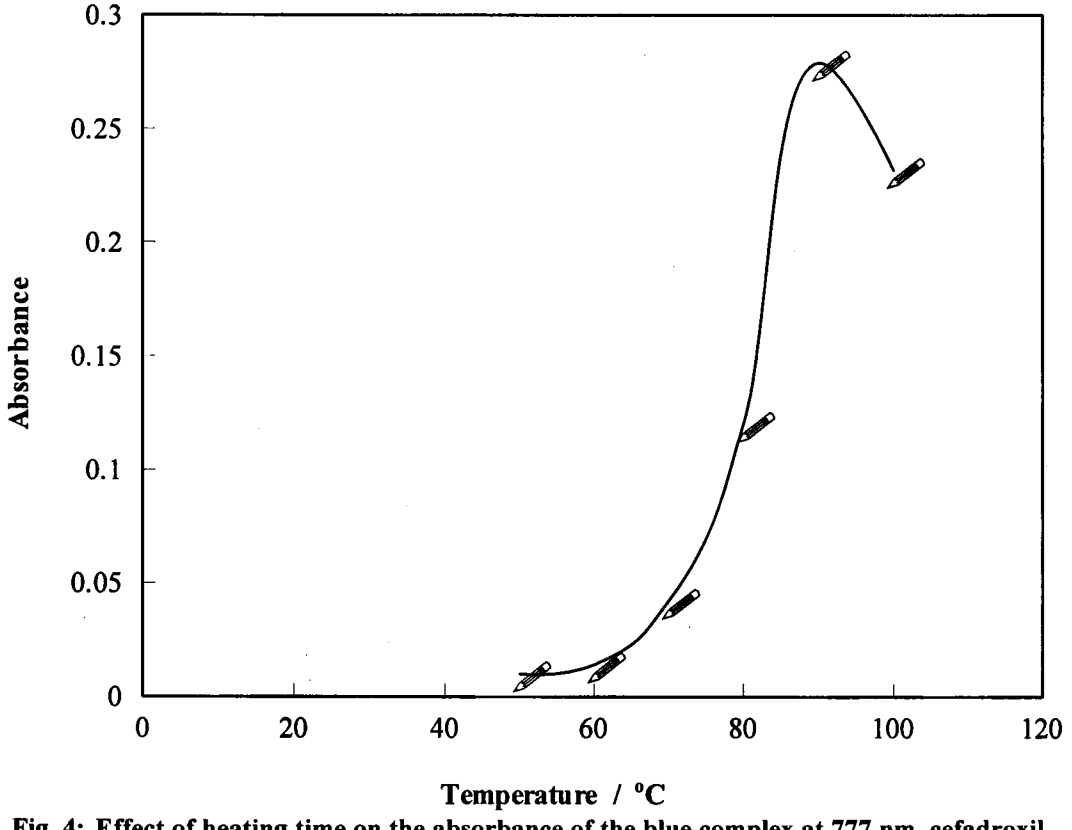

Fig. 4: Effect of heating time on the absorbance of the blue complex at 777 nm, cefadroxil 8 μg ml^{-1}, iron(III) nitrate 1 × 10^{-4} M, potassium hexacyanoferrate(III) 1 × 10^{-4} M, temperature 90°C.

Table 2: Effect of order of addition of reactants on the reaction of 8 μg ml^{-1} cefadroxil with 1×10^{-4} M $Fe(NO_3)_3$ and 1×10^{-4} M $K_3Fe(CN)_6$; temp 90°C and heating time 60 min.

Arrangement	Absorbance at λ = 777 nm
Cefadroxil + $Fe(NO_3)_3$ + $K_3Fe(CN)_6$	0.279
$K_3Fe(CN)_6$ + cefadroxil + $Fe(NO_3)_3$	0.255
$Fe(NO_3)_3$ + cefadroxil + $K_3Fe(CN)_6$	0.238
Cefadroxil + $K_3Fe(CN)_6$ + $Fe(NO_3)_3$	0.226
$Fe(NO_3)_3$ + $k_3Fe(CN)_6$ + cefadroxil	0.219
$K_3Fe(CN)_6$ + $Fe(NO_3)_3$ + cefadroxil	0.199

Determination of the Studied Cephalosporins:

Under the described experimental conditions, standard calibration curves for the studied cephalosporins were constructed by plotting the absorbance versus concentration. Conformity to Beer's Law was evident over the concentration range of the final dilution as given in Table 1. The relative standard deviations (%RDS) were 1.05% for 3.0 μg ml^{-1} cefadroxil, 2.26% for 2.0 μg ml^{-1} cefotaxime and 0.63% for 1.0 μg ml^{-1} ceftriaxone based on 10 replicate determinations of each drug.

The reaction pathway of oxidation of çephalosporins was suggested as: the oxidation occurs at the sulfur moiety of the β–lactam ring and the oxidation number of sulfur atom could be changed from +2 to +4 as connected to oxygen through oxidation with Fe^{+3} in the presence of water.

Least-squares regression calibration curves of the three drugs were found to be linear at the studied concentration ranges with the mean correlation coefficients, r, was > 0.999 as shown in Table 1. It also shows the mean linear regression equations of the three drugs. The detection limit of the method (S:N = 2:1) was 50 ng/ml for Cefotaxime and Ceftriaxone and 100 ng/ml for Cefadroxil.

The precision of the proposed method was evaluated by analysing standard solutions of the studied cephalosporins from replicate analysis (n = 3) at concentrations within the linear range of the method for each drug. The results in Table 3 were in accord with those obtained by reference methods [18,19].

Statistical analysis [38] of these results using student't t–test and the variance ratio F–test showed no significant difference between the performances of the methods as regards to accuracy and precision.

The reproducibility of the method was evaluated by comparing the linear regressions of three standard plots prepared at three different days over two week's period for each drug. The mean correlation coefficient was ≥ 0.999 with RSD% of the slopes of the three lines was ≤ 1.1%. Analysis of variance of the data indicated that there was no significant difference ($p > 0.05$) in the slopes, within-day and between-day, of the calibration curves of each drug. The results confirmed the reproducibility of this method.

Pharmaceutical Applications:

In order to evaluate the analytical usefulness of the proposed method, it was applied to the determination of studied cephalosporins in pharmaceutical formulations. The results were listed in Table 4 and agreed well with the USPXXIII specifications that the drug content is within 90 to 120, 90 to 110, and 90 to 115% of the labeled amount of cefadroxil, cefotaxime and ceftriaxone, respectively. The results showed excellent recoveries were obtained. To avoid the effect of the matrix interference, i.e. reduction of the absorbance signal by other sample components in the dosage forms of cefadroxil, the method of standard additions was constructed to overcome these interferences.

Table 3: Analysis of the studied cephalosporins by the proposed and reference methods.

Compound	Found (%)	Reference method [18,19]
	Proposed method	
Cefadroxil	98.5	
	100.0	
	101.5	
	100.0	
	100.4	
	99.3	
	100.0	
Mean ± S. D.	100.0 ± 0.93	100.4 ± 1.07 [a] [18]
Student's t – test	0.691(2.228)[b]	
Variance F – test	1.32 (6.16)[c]	
Cefotaxime	99.0	
	98.7	
	102.0	
	98.6	
	100.5	
Mean ± S. D.	99.8 ± 1.47	100.3 ± 0.72 [d] [18]
Student's t test	0.399 (2.447) [b]	
Variance F – test	4.17(19.2) [c]	
Ceftriaxone	99.4	
	100.9	
	100.8	
	98.6	
Mean ± S. D.	99.9 ± 1.12	100.0 ± 1.56 [e] [19]
Student's t - test	0.104 (2.447) [b]	
Variance F - test	1.94 (9.28)[c]	

[a] Analysed by spectrophotometric method (n = 5) [18]
[b] Tabulated t-Values at (p = 0.05) [38]
[c] Tabulated F-Values at (p = 0.05) [38]
[d] Analysed by spectrophotometric method (n = 3) [18]
[e] Analysed by spectrophotometric method (n = 4) [19]

Table 4: Analysis of the studied cephalosporines in pharmaceutical preparations by the proposed method

Preparation	Cephalosporin (mg)			Recovery%
	Concentration taken (μg ml^{-1})	Claimed	Found	
Roxil capsules[a] (500mg cefadroxil / capsule)	2.0	500.0	500.0	100.0
	3.2	500.0	500.0	100.0
	4.0	500.0	512.5	102.5
Mean ± S.D.				100.8±1.4
Roxil suspension[a] (250mg cefadroxil / 5ml)	2.0	250.0	256.3	102.5
	3.0	250.0	250.0	100.0
	4.0	250.0	256.2	102.5
Mean ± S.D.				101.6±1.4
Ultracef capsules[b] (500mg cefadroxil /capsule)	2.0	500.0	500.0	100.0
	3.0	500.0	500.0	100.0
	4.0	500.0	500.0	100.0
Mean ± S.D.				100±0.0
Ultracef suspensio [b] (250mg cefadroxil / 5ml)	2.0	250.0	250.0	100.0
	3.0	250.0	250.0	100.0
	4.0	250.0	250.0	100.0
Mean ± S.D.				100±0.0
Droxil capsules[c] (500mg cefadroxil / capsule)	2.0	500.0	525.0	105.0
	3.0	500.0	500.0	100.0
	4.0	500.0	512.5	102.5
Mean ± S.D.				102.5±2.5
Rocephin ampoules[d] (500mg ceftriaxone/ampoule)	0.5	500.0	490.0	98
	1.0	500.0	496.0	99.2
	1.5	500.0	490.5	98.1
	2.0	500.0	506.5	101.3
	2.5	500.0	496.5	99.3
Ceftax ampoules[e] (100mg cefotaxime/ampoule)	2.0	100.0	98.0	98.0
	3.5	100.0	98.3	98.3
	4.0	100.0	100.6	100.6
	5.0	100.0	100.4	100.4
	6.0	100.0	100.8	100.8
	7.0	100.0	97.1	97.1
Mean ± S.D.				99.2±1.6

[a]Product of Tabok Pharmaceutical Manufacturing Company , Saudi Arabia .
[b]Product of Bristol-Myers Squibb Company Princeton, New Jersey , U.S.A
[c]Product of United Company of Pharmaceutical Industries , Jordan .
[d]Product of F. Hoffman-La Roche Ltd , Basel , Switzerland .
[e]Product of HIKMA Pharmaceuticals , Amman , Jordan .

Conclusion:

The new method provides a simple and sensitive means of determining the studied cephalosporins in pharmaceutical preparations. It has also the advantages of high accuracy and precision with low detection limit compared with other spectrophotometric methods (LOD > 2.5 μg ml^{-1} [22]). This method is also easier and cheaper to perform than HPLC separations and do not require expensive reagents or organic solvents. These advantages coupled with acceptable within-day and day-to-day precision make the method suitable for routine quality control. The cephalosporin reacts with iron(III) nitrate and the resulting iron(II) reacts with potassium hexacyanoferrate(III) and a blue complex is resulted .

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