

# Kinetic spectrophotometric determination of certain cephalosporins using iodate/iodide mixture

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## ABSTRACT

A simple, precise and accurate kinetic spectrophotometric method for determination of cefradine anhydrous, cefaclor monohydrate, cefadroxil monohydrate, cefalexin anhydrous and cefixime in bulk and in pharmaceutical formulations has been developed. The method based on a kinetic investigation of the reaction of the free carboxylic acid group of the drug with a mixture of potassium iodate and potassium iodide at room temperature to form yellow coloured triiodide ions. The reaction was followed up spectrophotometrically by measuring the increase in absorbance at 352 nm as a function of time. The initial rate, fixed time, variable time and rate-constant methods were adopted for constructing the calibration curves but fixed time method has been found to be more applicable. The analytical performance of the method, in terms of accuracy and precision, was statistically validated; the results were satisfactory. The method has been successfully applied to the determination of the studied drugs in commercial pharmaceutical formulations. Statistical comparison of the results with a well established reported method showed excellent agreement and proved that there is no significant difference in the accuracy and precision.

**Keywords:** Cephalosporins; Kinetic Spectrophotometry; Iodate/Iodide Mixture; Pharmaceutical Analysis

## 1. INTRODUCTION

Because cephalosporins are among the safest and the most effective broad-spectrum bactericidal antimicrobial agents available to the clinician, they have become the most widely prescribed of all antibiotics. All of these semi-synthetic antibiotics are derived from 7-amino-ce-

phalosporanic acid and contain a  $\beta$ -lactam ring fused to a dihydrothiazine ring (Table 1) but differ in the nature of the substituents attached at the 3 and/or 7-positions of the cephem ring. These substitutions affect either the pharmacokinetic properties (3-position) or the antibacterial spectrum (7-position) of the cephalosporins. Cephalosporins operate by inhibiting bacterial cell wall biosynthesis which grows actively against a wide range of both gram-positive and gram-negative bacteria. The positive results of these drugs include the resistance of penicillinases and ability to treat infections that are resistant to penicillin derivatives. The official methods for analyzing cephalosporins are mostly chromatographic methods [1] which are expensive. Most of the reported methods involve the cleavage of the  $\beta$ -lactam moiety of the cephalosporin structure. These methods include spectrophotometric [2-6] spectrofluorimetric [7-10], and electrochemical methods [11-13]. A direct chemical analysis based on the reactivity of the intact molecule is not frequently encountered.

Kinetic spectrophotometric methods are becoming of great interest in chemical and pharmaceutical analysis [14]. The application of these methods offered some specific advantages [15,16].

1) Simplicity owing to elimination of some experimental steps such as filtration and extraction prior to absorbance measurements.

2) High selectivity due to the measurement of the increase or decrease of the absorbance as a function of reaction time instead of measuring the concrete absorbance value.

3) Avoiding the interference of the coloured and/or turbidity background of the samples, and possibility of avoiding the interference of the other active compounds present in the commercial product if they are resisting the established reaction conditions.

The literatures are still lacking analytical procedures based on kinetics for determination of the investigated drugs in commercial dosage forms. A kinetic spectrophotometric method has been reported for determination of cefadroxil based on its alkaline hydrolysis [17]. With

the exception of cefadroxil, this part represents the first attempt for assaying the investigated drugs without degradation in pure forms and in different pharmaceutical dosage forms using kinetic spectrophotometric method. The literature reveals a kinetic spectrophotometric method for determination of ramipril [18] that based on the reaction of its carboxylic acid group with iodate/iodide mixture in aqueous medium at room temperature to form yellow coloured triiodide ions. The reaction was followed up spectrophotometrically by measuring the increase in absorbance at 352 nm as a function of time.

This reaction drew our attention to investigate it on our studied drugs that contain free carboxylic acid group (**Table 1**). Accordingly, this reaction was studied in order to find out if it would lend itself applicable to the analysis of cefradine anhydrous, cefaclor monohydrate, cefadroxil monohydrate, cefalexin anhydrous and cefixime in pure forms and in pharmaceutical formulations. As a result of these investigations; a simple, rapid and accurate kinetic spectrophotometric method for determination of the aforementioned cephalosporin drugs without degradation was devised. The fixed time method is adopted after full investigation and understanding of the kinetics of the reaction. The proposed method does not require the elaboration of treatment and procedures, which are usually associated with chroma-

tographic methods.

## 2. EXPERIMENTAL

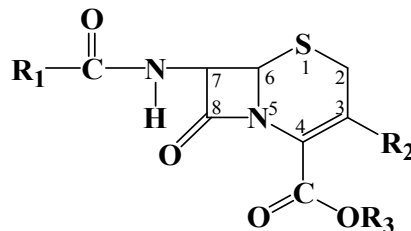
### 2.1. Apparatus

Shimadzu UV-1700 PC, UV-Visible Spectrophotometer (Tokyo, Japan), Ultrasonic cleaner (Cole – Parmer, Chicago, USA) and Sartorius handy balance – H51 (Hanover, Germany).

### 2.2. Materials and Reagents

All solvents used were of analytical-reagent grade, potassium iodide (El-Nasr Chemical Co. Cairo, Egypt) freshly prepared aqueous solution (1.5 M), potassium iodate (El-Nasr Chemical Co. Cairo, Egypt) freshly prepared aqueous solution (0.3 M), cefaclor monohydrate and cefradine anhydrous (Sigma Chemical Co., St. Louis, USA) cefadroxil monohydrate (Amoun Pharmaceutical Industries Co., APIC, Cairo, Egypt), cefalexin anhydrous (GalaxoWellcome, S.A.E., El Salam City, Cairo, Egypt) and cefixime (El-Hekma Co., Cairo, Egypt) were obtained as gifts and were used as supplied and pharmaceutical formulations containing the studied drugs were purchased from local market.

**Table 1.** Chemical structures of the investigated cephalosporin antibiotics.



No.	Name	R <sub>1</sub>	R <sub>2</sub>	Generation
1.	Cefalexin anhydrous		-CH <sub>3</sub>	First
2.	Cefradine anhydrous		-CH <sub>3</sub>	First
3.	Cefadroxil monohydrate		-CH <sub>3</sub>	First
4.	Cefaclor monohydrate		-Cl	Second
5.	Cefixime			Third

## 2.3. Preparation of Standard Solutions

Stock solutions containing 1 mg mL<sup>-1</sup> of each cephalosporin namely, cefradine anhydrous, cefadroxil monohydrate, cefaclor monohydrate, cefalexin anhydrous and cefixime were prepared in methanol. Working standard solutions containing 0.1-0.5 mg mL<sup>-1</sup> (in case of cefixime, working standard solutions containing 0.05-0.25 mg mL<sup>-1</sup>) were prepared by suitable dilution of the stock solution with methanol. The stock and working standard solutions must be freshly prepared.

## 2.4. Preparation of Sample Solutions

### 2.4.1. Tablets and Capsules

Twenty tablets or the contents of 20 capsules were weighed, finely powdered and mixed thoroughly. An accurately weighed amount of the powder obtained from tablets or capsules equivalent to 250 mg of each drug was transferred into a 50-mL volumetric flask, dissolved in about 25 mL methanol, sonicated for 15 min, diluted to the mark with methanol, mixed well and filtered; the first portion of the filtrate was rejected. Further dilutions with methanol were made to obtain sample solution containing 0.3 mg mL<sup>-1</sup> (in case of cefixime, further dilutions with methanol were made to obtain sample solution containing 0.15 mg mL<sup>-1</sup>) and then the general procedure was followed.

### 2.4.2. Powder for Oral Suspension

An accurately weighed amount of powder equivalent to 250 mg of each drug was transferred into a 50 mL volumetric flask, then the procedure was followed as under tablets and capsules beginning from (dissolved in about 25 mL methanol).

## 2.3. General Procedure

Accurately measured one millilitre aliquot volume of the standard or sample solutions was transferred into 10- mL volumetric flask. One millilitre of 0.3 M of potassium iodate was added followed by 1 mL of 1.5 M of potassium iodide. The content of the flask was mixed well and diluted to volume with methanol. The increase in absorbance was measured at 352 nm against reagent blank treated similarly. The four kinetic methods namely, initial rate, fixed time, variable time and rate constant methods were used for construction of the calibration curves and determination of the studied drugs.

## 3. RESULTS AND DISCUSSION

### 3.1. Absorption Spectra

Absorption spectrum of cefradine anhydrous which was taken as a representative example for all studied drugs is shown in **Figure 1**. This spectrum shows no absorption at 352 nm whereas the absorbance of the reagent solution (KIO<sub>3</sub> and KI in methanol) at 352 nm is about 0.02. The wavelengths of maximum absorption of the interac-

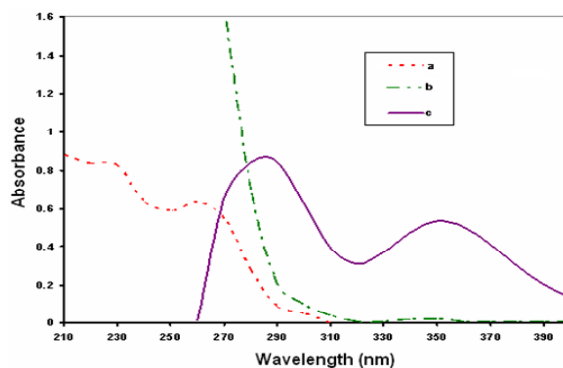
tion coloured product of cefradine anhydrous with KIO<sub>3</sub> and KI are at 298 and 352 nm. It is obvious that at 298 nm there is background absorption from the drug itself and from the reagent blank (**Figure 1**). Therefore, the absorbance measurements for the determination of the studied drugs were made at 352 nm. The equilibrium is attained in ~30 minutes. Therefore, a kinetically based spectrophotometric method was developed for the quantitative determination of the investigated drugs by measuring the increase in absorbance at 352 nm as a function of time.

### 3.2. Optimization of Reaction Conditions

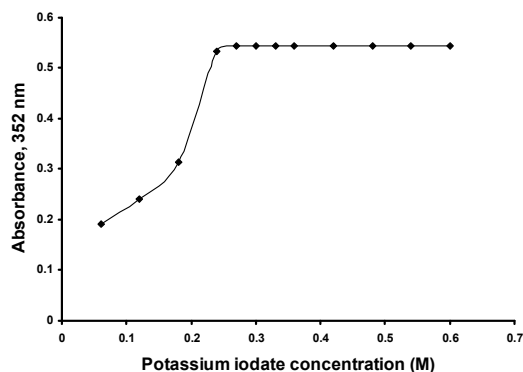
The experimental parameters affecting the reaction between the investigated drugs, potassium iodate and potassium iodide were carefully studied and optimized. Cefradine anhydrous (30 µg mL<sup>-1</sup>) was taken as a representative example for this study. These factors include:

#### 3.2.1. Effect of Potassium Iodate Concentration

The concentration of potassium iodate, for the maximum colour development at 352 nm, was studied in the range of 0.05-0.6 M. From **Figure 2**, it was found that the



**Figure 1.** Absorption spectra of (a) cefradine anhydrous (30 µg mL<sup>-1</sup>); (b) reagent solution (0.3 M potassium iodate and 1.5 M potassium iodide) and (c) the interaction coloured product of cefradine anhydrous with potassium iodate and potassium iodide.



**Figure 2.** Effect of potassium iodate concentration on the absorbance of the reaction coloured product at 352 nm.

absorbance of the interaction coloured product is increased with increasing potassium iodate concentration. Maximum absorbance was attained by using 0.25 M; above this concentration and up to 0.6 M KIO<sub>3</sub>, the absorbance remains constant. Therefore, 1 mL of 0.3 M potassium iodate was selected during subsequent work.

### 3.2.2. Effect of Potassium Iodide Concentration

The influence of potassium iodide concentration on producing the maximum absorption intensity was investigated using 0.3-2.4 M potassium iodide. Maximum absorption readings were obtained upon using 1 mL of 1.3 M potassium iodide; above this concentration the absorbance remains constant. So, 1 mL of 1.5M of KI was used for further work (Figure 3).

### 3.2.3. Effect of Diluting Solvent

Different solvents were tested in order to select the most appropriate solvent for producing the maximum absorption intensity. The results given in Table 2 show the slight effect on  $\lambda_{\text{max}}$  while the absorption intensity was affected. Methanol was used throughout this work because it gave the highest absorbance readings and the most reproducible results.

### 3.2.4. Effect of Temperature

As expected from the Arrhenius equation [19], the reaction rate is increased with increasing temperature. So, trials have been done to carry out the reaction at higher temperatures. It was found that the studied drugs undergo degradation and iodine is unstable at higher temperatures [20]. Therefore, room temperature ( $25 \pm 5^\circ\text{C}$ ) was recommended as the optimum temperature for this study.

### 3.2.5. Quantitation Methods

The initial rate, fixed time, variable time and rate constant methods [21,22] were tested and the most suitable analytical approach was chosen regarding the applicability, sensitivity, the values of the intercept and correlation coefficient ( $r$ ).

### 3.2.6. Initial Rate Method

Under the optimum experimental conditions, the assay of cefradine anhydrous, cefadroxil monohydrate, cefaclor monohydrate, cefalexin anhydrous and cefixime was performed at different concentration levels for 17 min at intervals of 2 min starting from 1 min at room temperature ( $25 \pm 5^\circ\text{C}$ ). The absorbance at 352 nm was then recorded at each time interval. The assay was carried out in presence of excess concentration of potassium iodate and potassium iodide. Therefore, a pseudo-zero order reaction condition was worked out with respect to the concentration of the reagent.

The kinetic plots are all sigmoid in nature and the initial rate of reaction was obtained by measuring the slopes ( $\Delta A/\Delta t$ ) of the initial tangent to the absorbance-time curves at different concentrations of the inves-

tigated drugs. Figure 4 shows the kinetic plot for cefradine anhydrous as a representative example.

The initial rate of reaction would follow a pseudo-first order and obeyed the following rate equation:

$$v = \frac{\Delta A}{\Delta t} = k' C^n \quad (1)$$

whereas  $v$  is the reaction rate,  $A$  is the absorbance,  $t$  is the measuring time,  $k'$  is the pseudo-first order rate constant,  $C$  is the concentration of the drug and  $n$  is the order of the reaction. The logarithmic form of the above equation is written as follows:

$$\log v = \log \frac{\Delta A}{\Delta t} = \log k' + n \log C \quad (2)$$

A calibration curve was constructed by plotting the logarithm of the initial rate of reaction ( $\log v$ ) versus logarithm of initial concentration of the investigated drugs ( $\log C$ ), which showed a linear relationship over concentration range of  $2.59 \times 10^{-5}$  -  $1.44 \times 10^{-4}$  M for cefadroxil monohydrate, cefaclor monohydrate, cefalexin anhydrous and cefradine anhydrous (in case of cefixime,  $1.10 \times 10^{-5}$  -  $5.51 \times 10^{-5}$  M). The regression equations of  $\log$  rate versus  $\log C$  are given in Table 3.

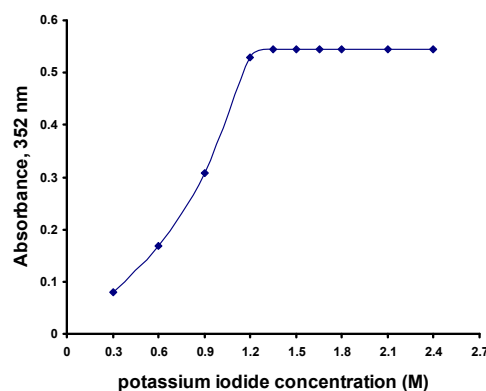


Figure 3. Effect of potassium iodide concentration on the absorbance of the reaction coloured product at 352 nm.

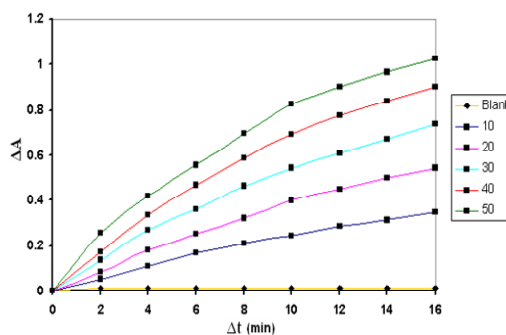


Figure 4. Absorbance-time curve for the reaction of cefradine anhydrous ( $\mu\text{g mL}^{-1}$ ) with potassium iodate and potassium iodide.

**Table 2.** Effect of solvent on  $\lambda_{\max}$  and the absorption intensity of the reaction coloured product of the studied drugs with  $\text{KIO}_3$  and KI.

Drug Solvent	Cefradine anhydrous (30 $\mu\text{g mL}^{-1}$ )		Cefadroxil monohydrate (30 $\mu\text{g mL}^{-1}$ )		Cefaclor monohydrate (30 $\mu\text{g mL}^{-1}$ )		Cefalexin anhydrous (30 $\mu\text{g mL}^{-1}$ )		Cefixime (15 $\mu\text{g mL}^{-1}$ )	
	$\lambda_{\max}$ (nm)	A <sup>a</sup>	$\lambda_{\max}$ (nm)	A <sup>a</sup>	$\lambda_{\max}$ (nm)	A <sup>a</sup>	$\lambda_{\max}$ (nm)	A <sup>a</sup>	$\lambda_{\max}$ (nm)	A <sup>a</sup>
Water	346	0.420	346	0.394	346	0.308	347	0.331	348	0.318
Ethanol	357	0.520	358	0.410	359	0.382	358	0.410	358	0.394
Methanol	<b>352</b>	<b>0.544</b>	<b>352</b>	<b>0.510</b>	<b>352</b>	<b>0.400</b>	<b>352</b>	<b>0.429</b>	<b>352</b>	<b>0.413</b>
Acetone	359	0.400	360	0.385	359	0.294	360	0.315	360	0.303
Acetonitrile	352	0.410	356	0.390	351	0.312	351	0.322	353	0.306
Propan-1-ol	358	0.390	359	0.375	360	0.296	358	0.306	357	0.280
Propan-2-ol	361	0.434	361	0.420	361	0.327	361	0.333	360	0.300
DMF	351	0.380	355	0.360	354	0.286	351	0.290	352	0.260
DMSO	350	0.375	349	0.370	350	0.282	350	0.287	350	0.260

<sup>a</sup> Average of 3 determinations.**Table 3.** Relation between reaction rates and concentrations.

$\log \Delta A/\Delta t$	$\log [\text{Drug}] \text{ (M)}$	Calibration equation $\log v = \log k' + n \log C$	Correlation coefficient ( <i>r</i> )
<b>Cefradine anhydrous</b>			
-1.577	-4.543	$\log v = 2.729 + 0.956 \log C$	<b>0.9867</b>
-1.377	-4.240		
-1.164	-4.066		
-1.066	-3.941		
-0.893	-3.844		
<b>Cefadroxil monohydrate</b>			
-1.577	-4.581	$\log v = 2.765 + 0.956 \log C$	<b>0.9868</b>
-1.377	-4.280		
-1.164	-4.104		
-1.066	-3.979		
-0.893	-3.882		
<b>Cefaclor monohydrate</b>			
-1.699	-4.586	$\log v = 3.441 + 1.122 \log C$	<b>0.9971</b>
-1.377	-4.285		
-1.164	-4.109		
-1.066	-3.984		
-0.893	-3.887		
<b>Cefalexin anhydrous</b>			
-1.553	-4.541	$\log v = 2.976 + 1.002 \log C$	<b>0.9828</b>
-1.268	-4.240		
-1.155	-4.064		
-1.011	-3.939		
-0.801	-3.842		
<b>Cefixime</b>			
-1.523	-4.958	$\log v = 4.011 + 1.126 \log C$	<b>0.9856</b>
-1.314	-4.656		
-1.039	-4.480		
-0.905	-4.355		
-0.738	-4.259		

The correlation coefficients (*r*) of all studied drugs ranged from 0.9828 to 0.9971. The order (*n*) with respect to the studied drugs was evaluated by plotting the logarithm of the initial rate of reaction versus logarithm of the concentrations of the investigated drugs and was found to be approximately one which confirms the first-order reaction with respect to all investigated drug concentrations.

### 3.2.7. Fixed Time Method

In this method, the absorbance changes caused by effect of drug acidity on a mixture of potassium iodate and potassium iodide were recorded at a preselected fixed time at intervals of 2 min. The change in absorbance ( $\Delta A$ ) between the times  $t_1$  (1 min) and  $t_2$  (3, 5, 7, 9, 11,

13, 15 and 17) was computed and plotted against the concentration of each of the studied drugs. The corresponding linear regression equations with correlation coefficients are summarised in **Table 4**. It is evident from the table that the most acceptable linearity was obtained when the calibration graphs were plotted by considering the change in absorbance between 1 and 11 min (*i.e.*  $\Delta A = A_{11} - A_1$ ). It is also clear that the slope increases with time and the most acceptable values of *r* and the intercept were obtained for a fixed time of 10 min, which was therefore chosen as the most suitable time interval for the measurement. The calibration curve was linear in the range of 10 to 50  $\mu\text{g mL}^{-1}$  for cefadroxil



**Table 4.** Calibration equations for the studied drugs of different concentrations at different time intervals using fixed time method.

$\Delta t$ (min)	Calibration equation $\Delta A = a + b C$	Correlation coefficient ( $r$ )
<b>Cefradine anhydrous</b>		
2	$\Delta A = -0.008 + 0.005 C$	0.9842
4	$\Delta A = 0.033 + 0.008 C$	0.9989
6	$\Delta A = 0.063 + 0.010 C$	0.9991
8	$\Delta A = 0.871 + 0.012 C$	0.9994
<b>10</b>	<b><math>\Delta A = 0.104 + 0.015 C</math></b>	<b>0.9997</b>
12	$\Delta A = 0.140 + 0.016 C$	0.9988
14	$\Delta A = 0.163 + 0.016 C$	0.9980
16	$\Delta A = 0.196 + 0.017 C$	0.9963
<b>Cefadroxil monohydrate</b>		
2	$\Delta A = -0.008 + 0.005 C$	0.9842
4	$\Delta A = 0.009 + 0.009 C$	0.9975
6	$\Delta A = 0.026 + 0.011 C$	0.9978
8	$\Delta A = 0.044 + 0.013 C$	0.9991
<b>10</b>	<b><math>\Delta A = 0.036 + 0.016 C</math></b>	<b>0.9997</b>
12	$\Delta A = 0.048 + 0.017 C$	0.9995
14	$\Delta A = 0.062 + 0.018 C$	0.9981
16	$\Delta A = 0.089 + 0.019 C$	0.9963
<b>Cefaclor monohydrate</b>		
2	$\Delta A = -0.018 + 0.005 C$	0.9898
4	$\Delta A = -0.019 + 0.009 C$	0.9955
6	$\Delta A = -0.017 + 0.011 C$	0.9967
8	$\Delta A = 0.001 + 0.012 C$	0.9985
<b>10</b>	<b><math>\Delta A = 0.012 + 0.013 C</math></b>	<b>0.9996</b>
12	$\Delta A = 0.006 + 0.015 C$	0.9984
14	$\Delta A = 0.009 + 0.016 C$	0.9989
16	$\Delta A = 0.016 + 0.016 C$	0.9983
<b>Cefalexin anhydrous</b>		
2	$\Delta A = -0.019 + 0.006 C$	0.9666
4	$\Delta A = -0.074 + 0.012 C$	0.9852
6	$\Delta A = -0.048 + 0.013 C$	0.9928
8	$\Delta A = -0.031 + 0.014 C$	0.9954
<b>10</b>	<b><math>\Delta A = -0.012 + 0.015 C</math></b>	<b>0.9991</b>
12	$\Delta A = -0.018 + 0.017 C$	0.9987
14	$\Delta A = -0.014 + 0.017 C$	0.9989
16	$\Delta A = -0.005 + 0.018 C$	0.9971
<b>Cefixime</b>		
2	$\Delta A = -0.038 + 0.015 C$	0.09859
4	$\Delta A = -0.003 + 0.021 C$	0.09994
6	$\Delta A = 0.008 + 0.024 C$	0.9988
8	$\Delta A = 0.005 + 0.026 C$	0.9982
<b>10</b>	<b><math>\Delta A = 0.017 + 0.027 C</math></b>	<b>0.9994</b>
12	$\Delta A = 0.011 + 0.029 C$	0.9992
14	$\Delta A = 0.021 + 0.031 C$	0.9987
16	$\Delta A = 0.020 + 0.033 C$	0.9974

monohydrate, cefaclor monohydrate, cefalexin anhydrous and cefradine anhydrous (in case of cefixime, 5-25  $\mu\text{g mL}^{-1}$ ). The correlation coefficients ( $r$ ) of all studied drugs ranged from 0.9991 to 0.9997. Reasonable values of LOD and LOQ were obtained which ranged from 0.22 to 1.10 and from 0.67 to 3.33  $\mu\text{g mL}^{-1}$ ; respectively as indicated in **Table 5**.

### 3.2.8. Variable Time Method

The general procedure was followed up for each of the studied drugs at different concentration levels by recording the time in seconds required for the absorbance to reach 0.20. This preselected value of the absorbance

was chosen as it gives the widest calibration range. The reciprocal of time ( $1/\Delta t$ ) versus the initial concentration of the studied drugs was plotted and the equations of the calibration graphs are given in **Table 6**. The correlation coefficients ( $r$ ) of all studied drugs ranged from 0.9646 to 0.9873.

### 3.2.9. Rate Constant Method

Under the described experimental conditions, analysis was carried out for each of the studied drugs at different concentration levels starting from 1 min until 17 min at regular intervals of 2 min at room temperature ( $25 \pm 5^\circ\text{C}$ ). Graphs of log absorbance change at 352 nm versus

**Table 5.** Summary of quantitative parameters and statistical data using fixed time method.

Drug	Intercept (a) ± SD <sup>a</sup>	Slope (b) ± SD <sup>a</sup>	Linearity range (µg mL <sup>-1</sup> )	Correlation coefficient (r)	Determination coefficient (r <sup>2</sup> )	LOD (µg mL <sup>-1</sup> )	LOQ (µg mL <sup>-1</sup> )
Cefradine anhydrous	0.104 ± 0.005	0.015 ± 0.002	10-50	0.9997	0.9994	1.10	3.33
Cefadroxil monohydrate	0.036 ± 0.002	0.016 ± 0.002	10-50	0.9997	0.9994	0.41	1.25
Cefaclor monohydrate	0.012 ± 0.001	0.013 ± 0.001	10-50	0.9996	0.9992	0.25	0.80
Cefalexin anhydrous	-0.012 ± 0.001	0.015 ± 0.001	10-50	0.9991	0.9982	0.22	0.67
Cefixime	0.017 ± 0.002	0.027 ± 0.003	5-25	0.9994	0.9988	0.24	0.74

<sup>a</sup> Average of six determinations.

**Table 6.** Calibration equations and correlation coefficients using variable time method.

Δt (min)	1/Δt (s <sup>-1</sup> )	[Drug] (M)	Calibration equation 1/Δt = a + b C	Correlation coefficient (r)
<b>Cefradine anhydrous</b>				
7.5	2.22 × 10 <sup>-3</sup>	2.86 × 10 <sup>-5</sup>	1/Δt = -0.001 + 73.720 C	0.9646
5	3.33 × 10 <sup>-3</sup>	5.73 × 10 <sup>-5</sup>		
3	5.56 × 10 <sup>-3</sup>	8.59 × 10 <sup>-5</sup>		
2.5	6.67 × 10 <sup>-3</sup>	1.15 × 10 <sup>-4</sup>		
1.5	11.11 × 10 <sup>-3</sup>	1.43 × 10 <sup>-4</sup>		
<b>Cefadro × il monohydrate</b>				
11	1.52 × 10 <sup>-3</sup>	2.62 × 10 <sup>-5</sup>	1/Δt = -0.001 + 85.911 C	0.9754
5	3.33 × 10 <sup>-3</sup>	5.24 × 10 <sup>-5</sup>		
3	5.56 × 10 <sup>-3</sup>	7.87 × 10 <sup>-5</sup>		
2.5	6.67 × 10 <sup>-3</sup>	1.05 × 10 <sup>-4</sup>		
1.5	11.11 × 10 <sup>-3</sup>	1.13 × 10 <sup>-4</sup>		
<b>Cefaclor monohydrate</b>				
16	1.04 × 10 <sup>-3</sup>	2.80 × 10 <sup>-5</sup>	1/Δt = -0.002 + 94.370 C	0.9793
6	2.78 × 10 <sup>-3</sup>	5.18 × 10 <sup>-5</sup>		
3.5	4.76 × 10 <sup>-3</sup>	7.78 × 10 <sup>-5</sup>		
2.5	6.67 × 10 <sup>-3</sup>	1.04 × 10 <sup>-4</sup>		
1.5	11.11 × 10 <sup>-3</sup>	1.30 × 10 <sup>-4</sup>		
<b>Cefale × in anhydrous</b>				
16	1.04 × 10 <sup>-3</sup>	2.88 × 10 <sup>-5</sup>	1/Δt = -0.003 + 114.352C	0.9724
6	2.78 × 10 <sup>-3</sup>	5.76 × 10 <sup>-5</sup>		
3.5	4.76 × 10 <sup>-3</sup>	8.64 × 10 <sup>-5</sup>		
1.5	11.11 × 10 <sup>-3</sup>	1.15 × 10 <sup>-4</sup>		
1.25	13.33 × 10 <sup>-3</sup>	14.40 × 10 <sup>-4</sup>		
<b>Cefi × ime</b>				
16	1.04 × 10 <sup>-3</sup>	1.10 × 10 <sup>-5</sup>	1/Δt = -0.003 + 346.347C	0.9873
6	4.17 × 10 <sup>-3</sup>	2.21 × 10 <sup>-5</sup>		
2.5	6.67 × 10 <sup>-3</sup>	3.31 × 10 <sup>-5</sup>		
1.5	11.11 × 10 <sup>-3</sup>	4.41 × 10 <sup>-5</sup>		
1	16.67 × 10 <sup>-3</sup>	5.51 × 10 <sup>-5</sup>		

time in seconds for each of the studied drugs were constructed. Pseudo first-order rate constants ( $k'$ ) corresponding to different investigated drugs concentrations ( $C$ ) were calculated from the slopes, multiplied by -2.303. Pseudo first-order rate constant ( $k'$ ) versus the initial concentration of the studied drugs was then plotted and the equations of the calibration graphs are given in Table 7. The correlation coefficients ( $r$ ) for all the studied drugs ranged from 0.8742 to 0.9290. These low values of  $r$  may be due to slight changes in temperature.

### 3.3. Method Validation Study

Fixed time method was chosen to carry out the valida-

tion study as it gives the highest values of correlation coefficients. The proposed method was validated according to ICH (International Conference on Harmonization) guidelines on the validation of analytical methods [23] and complied with USP 31 validation guidelines [1]. All results were expressed as percentages, where  $n$  represents the number of values. For the statistical analysis Excel 2003 (Microsoft Office) was used. A 5% significance level was selected.

#### 3.3.1. Accuracy

The accuracy of the method was determined by investigating the recovery of each of the studied drugs at three

concentration levels covering the specified calibration range (six replicates of each concentration). The results shown in **Table 8** depict good accuracy and recovery percentage ranged from 98.0 to 101.9%.

### 3.3.2. Precision

As indicated in **Table 9**, the results of SD and % RSD can be considered to be very satisfactory which prove the precision of the proposed method.

### 3.3.3. Selectivity

The selectivity of the proposed method for determination of the studied drugs in the presence of frequently en-

countered excipients such as; starch, talc, lactose, glucose, sucrose, magnesium-stearate and gum acacia was studied. It was found that there is no interference from these excipients and additives. So, the proposed method can be considered a selective one.

### 3.3.4. Robustness

Robustness was examined by evaluating the influence of small variation of method variables including; potassium iodate concentration, potassium iodide concentration, measurement time on the method suitability and sensitivity. It was found that none of these variables significantly affected the performance of the method (**Table 10**).

**Table 7.** Values of  $k'$  calculated from slopes of log A versus t graphs multiplied by -2.303 for different concentrations of the studied drugs.

$k'$ ( $s^{-1}$ )	[Drug] (M)	Calibration equation $k' = a + b C$	Correlation coefficient (r)
<b>Cefradine anhydrous</b>			
$-1.79 \times 10^{-3}$	$2.86 \times 10^{-5}$	$k' = -0.002 + 1.942 C$	<b>0.9256</b>
$-1.77 \times 10^{-3}$	$5.73 \times 10^{-5}$		
$-1.63 \times 10^{-3}$	$8.59 \times 10^{-5}$		
$-1.67 \times 10^{-3}$	$1.15 \times 10^{-4}$		
$-1.57 \times 10^{-3}$	$1.43 \times 10^{-4}$		
<b>Cefadro × il monohydrate</b>			
$-1.85 \times 10^{-3}$	$5.24 \times 10^{-5}$	$k' = -0.002 + 4.112 C$	<b>0.9290</b>
$-1.71 \times 10^{-3}$	$7.87 \times 10^{-5}$		
$-1.72 \times 10^{-3}$	$9.20 \times 10^{-5}$		
$-1.72 \times 10^{-3}$	$1.05 \times 10^{-4}$		
$-1.49 \times 10^{-3}$	$1.31 \times 10^{-4}$		
<b>Cefaclor monohydrate</b>			
$-1.61 \times 10^{-3}$	$2.59 \times 10^{-5}$	$k' = -0.002 + 3.203 C$	<b>0.8731</b>
$-1.50 \times 10^{-3}$	$5.18 \times 10^{-5}$		
$-1.32 \times 10^{-3}$	$7.78 \times 10^{-5}$		
$-1.43 \times 10^{-3}$	$1.04 \times 10^{-4}$		
$-1.23 \times 10^{-3}$	$1.30 \times 10^{-4}$		
<b>Cefale × in anhydrous</b>			
$-1.43 \times 10^{-3}$	$2.88 \times 10^{-5}$	$k' = -0.002 + 5.458 C$	<b>0.8742</b>
$-1.26 \times 10^{-3}$	$5.76 \times 10^{-5}$		
$-1.41 \times 10^{-3}$	$8.64 \times 10^{-5}$		
$-0.90 \times 10^{-3}$	$1.15 \times 10^{-4}$		
$-0.83 \times 10^{-3}$	$1.44 \times 10^{-4}$		
<b>Cefi × ime</b>			
$-1.17 \times 10^{-3}$	$1.10 \times 10^{-5}$	$k' = -0.001 + 7.215 C$	<b>0.9147</b>
$-1.14 \times 10^{-3}$	$2.21 \times 10^{-5}$		
$-0.94 \times 10^{-3}$	$3.31 \times 10^{-5}$		
$.00 \times 10^{-3}$	$4.41 \times 10^{-5}$		
$0.84 \times 10^{-3}$	$5.51 \times 10^{-5}$		

**Table 8.** Accuracy of the proposed kinetic spectrophotometric method for analysis of the studied drugs at three concentration levels.

Drug	Recovery (%) $\pm$ SD <sup>a</sup>		
	20 $\mu\text{g mL}^{-1}$	30 $\mu\text{g mL}^{-1}$	40 $\mu\text{g mL}^{-1}$
Cefradine anhydrous	99.3 $\pm$ 0.72	98.0 $\pm$ 0.40	101.9 $\pm$ 1.00
Cefadroxil monohydrate	100.3 $\pm$ 1.13	101.4 $\pm$ 1.00	98.7 $\pm$ 0.54
Cefaclor monohydrate	101.1 $\pm$ 1.14	99.0 $\pm$ 0.22	99.7 $\pm$ 1.39
Cefalexin anhydrous	98.5 $\pm$ 1.16	99.1 $\pm$ 1.25	98.6 $\pm$ 0.82
Cefixime	Recovery (%) $\pm$ SD <sup>a</sup>		
	10 $\mu\text{g mL}^{-1}$	15 $\mu\text{g mL}^{-1}$	20 $\mu\text{g mL}^{-1}$
Cefixime	100.3 $\pm$ 0.91	101.6 $\pm$ 1.43	100.7 $\pm$ 0.88

<sup>a</sup> Average of six replicates.



### 3.4. Applications to the Analysis of Pharmaceutical Dosage Forms

The proposed method (fixed time) was applied successfully for determination of the studied drugs in their pharmaceutical dosage forms. The results obtained (Table 11) were satisfactory compared to those given by a previously reported method [24]. Recovery studies were also carried out by standard addition method [25]. Good recoveries (96.3 to 102.8%) were obtained and these values confirmed the absence of interference due to common excipients (Table 12). The proposed method couldn't be applied to pharmaceutical formulations containing L-arginine as it is a basic amino acid (its side chain contains a strongly basic guanidine group, pKa = 13.2 [26]) and so, interferes with iodine liberation from the studied drug.

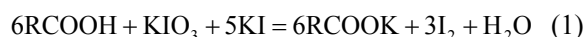
### 3.5. Suggested Reaction Mechanism

It has been suggested that water-soluble acidic compounds liberate iodine from a solution containing both KIO<sub>3</sub> and

KI according to the reaction [27];



Yellowing of the solution reveals the occurrence of the reaction. The yellow colour of the solution is due to the formation of I<sub>2</sub>, which immediately converted into triiodide ions (I<sub>2</sub> + I<sup>-</sup> → I<sub>3</sub><sup>-</sup>) exhibiting absorption maxima at 290 nm and 360 nm [18]. The chemical structure of investigated cephalosporins contains -COOH group in its moiety and hence possibly undergo a similar reaction with iodide-iodate mixture resulting in the production of iodine. The liberated iodine immediately reacts with potassium iodide to give triiodide ions showing absorption maxima at 298 nm and 352 nm. The reaction sequence is shown in Formula (1).



Formula (1) suggested reaction sequence of the proposed method.

**Table 9.** Intra- and inter-day precision of the proposed kinetic spectrophotometric method.

Drug	Drug Conc. (µg mL <sup>-1</sup> )	Intra-day precision		Inter-day precision	
		Mean ± SD <sup>a</sup>	% RSD	Mean ± SD <sup>a</sup>	% RSD
Cefradine anhydrous	20	98.5 ± 0.90	0.91	99.5 ± 0.81	0.81
	30	98.6 ± 1.54	1.57	99.7 ± 1.17	1.17
	40	99.8 ± 1.02	1.03	99.6 ± 1.48	1.48
Cefadroxil monohydrate	20	99.4 ± 0.99	1.00	100.6 ± 1.63	1.62
	30	98.9 ± 1.12	1.13	101.0 ± 1.27	1.26
	40	99.7 ± 0.67	0.67	100.8 ± 1.15	1.14
Cefaclor monohydrate	20	101.0 ± 1.27	1.26	100.6 ± 1.63	1.62
	30	100.6 ± 1.36	1.35	100.5 ± 1.15	1.14
	40	99.8 ± 1.02	1.03	100.9 ± 0.99	0.98
Cefalexin anhydrous	20	100.7 ± 1.12	1.12	99.8 ± 1.65	1.65
	30	98.6 ± 0.52	0.53	101.1 ± 1.20	1.19
	40	100.0 ± 1.56	1.56	98.6 ± 0.94	0.95
Cefixime	10	100.0 ± 1.15	1.15	100.7 ± 1.12	1.12
	15	100.7 ± 0.87	0.87	99.0 ± 0.97	0.98
	20	99.9 ± 1.65	1.66	99.8 ± 1.85	1.85

<sup>a</sup> Average of six determinations.

**Table 10.** Robustness of the proposed kinetic spectrophotometric method.

Experimental parameter variation	Recovery (%) ± SD <sup>a</sup>					
	Cefradine anhydrous (30 µg mL <sup>-1</sup> )	Cefadroxil monohydrate (30 µg mL <sup>-1</sup> )	Cefaclor monohydrate (30 µg mL <sup>-1</sup> )	Cefalexin anhydrous (30 µg mL <sup>-1</sup> )	Cefixime (15 µg mL <sup>-1</sup> )	
No variation <sup>b</sup>	97.9 ± 1.20	100.5 ± 1.23	101.5 ± 1.32	99.5 ± 0.47	99.4 ± 1.31	
1 - Potassium iodate concentration	0.28M	98.0 ± 1.35	101.9 ± 0.79	99.8 ± 1.37	99.4 ± 1.29	101.9 ± 1.45
	0.32M	97.7 ± 1.65	102.4 ± 0.85	100.9 ± 0.99	98.5 ± 0.90	100.8 ± 0.88
2 - Potassium iodide concentration	1.45M	101.4 ± 0.99	98.7 ± 1.45	98.7 ± 1.21	99.0 ± 1.36	102.1 ± 0.73
	1.55M	102.1 ± 1.13	97.8 ± 0.64	102.3 ± 1.56	98.7 ± 1.23	100.7 ± 0.92
3 - Measurement time	8 min	98.7 ± 1.53	99.0 ± 1.75	98.6 ± 0.84	98.6 ± 0.64	100.7 ± 0.61
	12 min	101.6 ± 1.45	100.0 ± 0.47	99.9 ± 1.38	97.6 ± 0.88	97.8 ± 0.81

<sup>a</sup> Average of three determinations.

<sup>b</sup> Following the general assay procedure conditions.

**Table 11.** Determination of the studied drugs in their pharmaceutical dosage forms using fixed time method.

Drug	Pharmaceutical product	Recovery % $\pm$ SD	
		Proposed method (n = 6)	Reported method <sup>a</sup> (n = 6)
Cefaclor monohydrate	Ceclor <sup>®</sup> suspension <sup>c</sup>	99.2 $\pm$ 0.60 $t = 1.225^b$ $F = 1.778^b$	98.7 $\pm$ 0.80
	Bactiolor <sup>®</sup> suspension <sup>d</sup>	101.2 $\pm$ 0.60 $t = 1.569$ $F = 1.440$	100.7 $\pm$ 0.50
	Duricef <sup>®</sup> tablets <sup>e</sup>	98.9 $\pm$ 0.60 $t = 2.038$ $F = 2.250$	99.8 $\pm$ 0.90
Cefadroxil monohydrate	Duricef <sup>®</sup> suspension <sup>e</sup>	102.2 $\pm$ 1.60 $t = 0.630$ $F = 1.129$	101.6 $\pm$ 1.70
	Duricef <sup>®</sup> capsules <sup>e</sup>	98.9 $\pm$ 1.30 $t = 0.831$ $F = 1.173$	99.5 $\pm$ 1.20
	Biodroxil <sup>®</sup> capsules <sup>f</sup>	101.4 $\pm$ 1.00 $t = 1.275$ $F = 1.234$	100.7 $\pm$ 0.90
Cefalexin anhydrous	Biodroxil <sup>®</sup> suspension <sup>f</sup>	99.1 $\pm$ 0.90 $t = 0.906$ $F = 2.250$	98.7 $\pm$ 0.60
	Ceporex <sup>®</sup> tablets <sup>g</sup>	97.8 $\pm$ 1.10 $t = 1.054$ $F = 1.860$	98.6 $\pm$ 1.50
	Ceporex <sup>®</sup> suspension <sup>g</sup>	100.5 $\pm$ 1.20 $t = 1.470$ $F = 1.778$	99.6 $\pm$ 0.90
Cefradine anhydrous	Ospexin <sup>®</sup> suspension <sup>h</sup>	99.7 $\pm$ 1.50 $t = 1.153$ $F = 3.516$	100.5 $\pm$ 0.80
	Velosef <sup>®</sup> capsules <sup>e</sup>	101.2 $\pm$ 1.50 $t = 0.432^b$ $F = 3.516^b$	100.9 $\pm$ 0.80
	Velosef <sup>®</sup> tablets <sup>e</sup>	99.9 $\pm$ 1.20 $t = 1.307$ $F = 1.778$	100.7 $\pm$ 0.90
Cefixime	Ximacef <sup>®</sup> capsules <sup>i</sup>	98.7 $\pm$ 0.40 $t = 1.644$ $F = 4.000$	99.0 $\pm$ 0.20

<sup>a</sup> Reference 24.<sup>b</sup> Theoretical value for  $t$  and  $F$  at 95% confidence limit,  $t = 2.228$  and  $F = 5.053$ .<sup>c</sup> Egyptian Pharmaceuticals and chemicals industries Co., S.A.E., Bayad El-Arab, Beni Suef, Egypt.<sup>d</sup> Pharco Pharmaceuticals, Alexandria under license from Ranbaxy UK.<sup>e</sup> Bristol-Myers Squibb Pharmaceutical Co., Cairo, Egypt.<sup>f</sup> Kahira Pharm. & Chem. Ind. Co. under license from Novartis Pharma S.A.E., Cairo, Egypt.<sup>g</sup> GlaxoSmithKline, S.A.E., El Salam City, Cairo, Egypt.<sup>h</sup> Pharco Pharmaceuticals, Alexandria under license from Biochemie GmbH., Vienna, Austria.<sup>i</sup> Sigma pharmaceutical industries, S.A.E., Egypt.

The confirmatory test for the presence of iodine in the final solution of the drug is established by the blue colour, which appears on addition of starch solution. In case

of cefixime, it may be suggested that 3 mole of cefixime instead of six react with iodate/iodide mixture as it contains 2 carboxylic acid groups.

**Table 12.** Standard addition method for the assay of the studied drugs in their pharmaceutical dosage forms using fixed time method.

Drug	Pharmaceutical formulation	Authentic drug added ( $\mu\text{g mL}^{-1}$ )	Authentic drug found ( $\mu\text{g mL}^{-1}$ )	Recovery (%) $\pm$ SD <sup>a</sup>
Cefaclor monohydrate	Ceclor <sup>®</sup> suspension	10.00	9.95	99.5 $\pm$ 1.40
		15.00	15.15	101.0 $\pm$ 1.10
		20.00	19.80	99.0 $\pm$ 1.70
	Bacti-clor <sup>®</sup> suspension	10.00	10.07	100.7 $\pm$ 1.10
		15.00	14.95	99.7 $\pm$ 1.00
		20.00	20.19	100.9 $\pm$ 1.50
Cefadroxil monohydrate	Duricef <sup>®</sup> tablets	10.00	9.87	98.7 $\pm$ 1.20
		15.00	15.25	101.7 $\pm$ 1.50
		20.00	19.60	98.0 $\pm$ 1.70
	Duricef <sup>®</sup> suspension	10.00	9.75	97.5 $\pm$ 1.60
		15.00	14.90	99.3 $\pm$ 1.40
		20.00	20.40	102.0 $\pm$ 1.50
Cefadroxil monohydrate	Duricef <sup>®</sup> capsules	10.00	9.75	97.5 $\pm$ 1.20
		15.00	14.85	99.0 $\pm$ 0.90
		20.00	20.30	101.5 $\pm$ 1.00
	Biodroxil <sup>®</sup> capsules	10.00	9.87	98.7 $\pm$ 1.10
		15.00	14.85	99.0 $\pm$ 0.80
		20.00	20.21	101.1 $\pm$ 0.70
Cefadroxil monohydrate	Biodroxil <sup>®</sup> suspension	10.00	10.23	102.3 $\pm$ 1.30
		15.00	15.30	101.0 $\pm$ 1.20
		20.00	19.85	99.3 $\pm$ 0.80
	Ceporex <sup>®</sup> tablets	10.00	10.13	101.3 $\pm$ 0.40
		15.00	14.63	97.5 $\pm$ 0.60
		20.00	20.16	100.8 $\pm$ 1.70
Cefalexin anhydrous	Ceporex <sup>®</sup> suspension	10.00	9.85	98.5 $\pm$ 1.30
		15.00	15.09	100.6 $\pm$ 0.90
		20.00	19.86	99.3 $\pm$ 1.80
	Ospexin <sup>®</sup> suspension	10.00	10.22	102.3 $\pm$ 0.70
		15.00	15.11	100.7 $\pm$ 1.90
		20.00	20.18	100.9 $\pm$ 1.50
Cefradine anhydrous	Velosef <sup>®</sup> capsules	10.00	9.90	99.0 $\pm$ 0.90
		15.00	14.67	97.8 $\pm$ 1.10
		20.00	20.19	101.0 $\pm$ 0.80
	Velosef <sup>®</sup> tablets	10.00	10.14	101.4 $\pm$ 1.30
		15.00	14.73	98.2 $\pm$ 0.60
		20.00	20.26	101.3 $\pm$ 0.90
Cefixime	Ximacef <sup>®</sup> capsules	10.00	9.89	98.9 $\pm$ 0.90
		12.50	12.25	98.0 $\pm$ 1.40
		15.00	20.15	100.8 $\pm$ 0.70

<sup>a</sup>Average of six determination.

#### 4. CONCLUSIONS

The developed kinetic spectrophotometric technique is precise, selective and accurate. The proposed method is applicable in aqueous medium at room temperature and thus there is no fear of decomposition of the drug due to heat, acid or base. Statistical analysis proves that the method is repeatable and selective for the analysis of cefadroxil monohydrate, cefaclor monohydrate, cefalexin

anhydrous, cefradine anhydrous and cefixime in bulk drug and in pharmaceutical formulations and can be used for routine quality control analyses of active drug in the laboratories of hospitals, pharmaceutical industries and research institutions.

#### REFERENCES

- [1] United States Pharmacopoeia 31 and NF 26. (2008)

- American Pharmaceutical Association, Washington, DC.
- [2] El-Obeid, H.A., Gad-Kariem, E.A., Al-Rashood, K.A., Al-Khames, H.A., El-Shafie, F.S. and Bawaseer, G.A.M. (1999) A selective colorimetric method for the determination of penicillins and cephalosporins with  $\alpha$ -aminoacyl functions. *Analytical Letters*, **32(14)**, 2809-2823.
- [3] Metwally, F.H., Alwarthan, A.A. and Al-Tamimi, S.A. (2001) Flow-injection spectrophotometric determination of certain cephalosporins based on the formation of dyes. *Il Farmaco*, **56(8)**, 601-607.
- [4] Sastry, C.S.P., Rao, S.G., Naidu, P.Y. and Srinivas, K.R. (1998) New spectrophotometric method for the determination of some drugs with iodine and wool fast blue BL. *Talanta*, **45(6)**, 1227-1234.
- [5] Ivama, V.M., Rodrigues, L.N.C., Guaratini, C.C.I and Zanoni, M.V.B. (1999) Spectrophotometric determination of cefaclor in pharmaceutical preparations. *Quimica Nova*, **22(2)**, 201-204.
- [6] Al-Momani, I.F. (2004) Flow-injection spectrophotometric determination of amoxicillin, cefalexin, ampicillin, and cefradine in pharmaceutical formulations. *Analytical Letters*, **37(10)**, 2099-2110.
- [7] Yang, J., Zhou, G.J., Cao, X.H., Ma, Q.L. and Dong, J. (1998) Study on the fluorescence characteristics of alkaline degradation of cefadroxil, cefradine, cefotaxime sodium and amoxicillin. *Analytical Letters*, **31**, 1047-1060.
- [8] Aly, F.A., Hefnawy, M.M. and Belal, F. (1996) A selective spectrofluorimetric method for the determination of some  $\alpha$ -aminocephalosporins in formulations and biological fluids. *Analytical Letters*, **29(1)**, 117-130.
- [9] Yang, J.H., Zhou, G.J., Jie, N.Q., Han, R.J., Lin, C.G. and Hu, J.T. (1996) Simultaneous determination of cefalexin and cefadroxil by using the coupling technique of synchronous fluorimetry and h-point standard additions method. *Analytica Chimica Acta*, **325(3)**, 195-200.
- [10] Yang, J.H., Ma, Q.L., Wu, X., Sun, L.M., Cao, X.H. (1999) A new luminescence spectrometry for the determination of some  $\beta$ -lactamic antibiotics. *Analytical Letters*, **32(3)**, 471-480.
- [11] Chailapakul, O., Aksharanandana, P., Frelink, T., Einaga, Y. and Fujishima, A. (2001) The electrooxidation of sulfur-containing compounds at boron-doped diamond electrode. *Sensors and Actuators B*, **80(3)**, 193-201.
- [12] Chailapakul, O., Fujishima, A., Tiphara, P. and Siriwongchai, H. (2001) Electroanalysis of glutathione and cefalexin using the boron-doped diamond thin-film electrode applied to flow-injection analysis. *Analytical Sciences*, **17(ICAS2001)**, i417-i422.
- [13] Li, Q.L. and Chen, S. (1993) Studies on electrochemical behaviour of cefalexin. *Analytica Chimica Acta*, **282(1)**, 145-152.
- [14] Crouch, S.R., Cullen, T.F., Scheeline, A. and Kirkor, E.S. (1998) Kinetic determinations and some kinetic aspects of analytical chemistry. *Analytical Chemistry*, **70(12)**, 53R-106R.
- [15] Perez-Bendito, D., Gomez-Hens, A. and Silva, M. (1996) Advances in drug analysis by kinetic methods. *Journal of Pharmaceutical and Biomedical Analysis*, **14(8-10)**, 917-930.
- [16] Espinosa-Mansilla, A., Acedo Valenzuela, M.I., Salinas, F. and Canada, F. (1998) Kinetic determination of ansamicins in pharmaceutical formulations and human urine; manual and semiautomatic (stopped-flow) procedures. *Analytica Chimica Acta*, **376(3)**, 365-375.
- [17] Helaleh, M.I.H. and Abu-Nameh, E.S.M. (1998) A kinetic approach for determination of cefadroxil in pharmaceuticals by alkaline hydrolysis. *Journal of AOAC International*, **81(3)**, 528-533.
- [18] Rahman, N., Ahmad, Y. and Azmi, S.N.H. (2005) Kinetic spectrophotometric method for the determination of ramipril in pharmaceutical formulations. *AAPS Pharm-SciTech*, **6(3)**, E543-E551.
- [19] Neil, S.I. (1987) Physical organic chemistry. John Wiley & Sons, New York, 93.
- [20] Kelly, F.C. (1953) Studies on the stability of iodine compounds in iodized salt. *Bulletin of World Health Organization*, **9(2)**, 217-230.
- [21] Yatsimirskii, K.B. (1966) Kinetic methods of analysis. Pergamon Press, London, 43.
- [22] Laitinen H.A., Harris, W.E. (1975) Chemical analysis. 2nd Edition, McGraw-Hill, New York.
- [23] (2005) Topic Q2 (R1): Validation of analytical procedures: text and methodology. *International Conference on Harmonization*, Foster. <http://www.ich.org/LOB/media/MEDIA417.pdf>
- [24] Saleh, G.A., Askal, H., Darwish, I. and El-Shorbagi, A. (2003) Spectroscopic analytical study for the charge-transfer complexation of certain cephalosporins with chloranilic acid. *Analytical Sciences*, **19(2)**, 281-287.
- [25] Harvey, D. (2000) Modern analytical chemistry. Boston, McGraw-Hill, Massachusetts, 108.
- [26] The Merck index (2001) An encyclopedia of chemicals, drugs and pharmaceuticals. 13th Edition, Merck & Co., INC., New Jersey, 133.
- [27] Svehla, G. (1979) Vogel's textbook of macro and semi-micro qualitative inorganic analysis. 5th Edition, the Chaucer Press, Great Britain, 342.