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Research Article

DEVELOPMENT OF SIMPLE GREEN SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CEFOPERAZONE SODIUM AND CEFEPIME HYDROCHLORIDE IN BULK, PHARMACEUTICAL DOSAGE FORMS AND HUMAN URINE

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ABSTRACT

Objective: To evaluate a validated, simple,sensitive,inexpensive,green method for determination of cefoperazone Na and cefepime hydrochloride in pure form, pharmaceutical dosage form and human urine using ferric chloride and ferricyanide by spectrophotometry.

Method: The estimation is based on the reduction of ferric ions in its salt form to ferrous ion by the drug, which in presence of potassium ferricyanide produces greenish blue colored chromogen measured at 766 nm against blank.The proposed method was applied to the determination of these drugs in pharmaceutical formulations and urine.

Results: Beer's law was obeyed in the concentration range 0.8-8 µg/mL, for both drugs, the limits of detection and quantification were reported. The intensity of the color in case ofcefoperazone Na increases with time at room temperature and so a kinetic method was developed for its determination. The results demonstrate that the method is equally accurate and precise as the reference methods as found from the t- and F-values. The reliability of the method was established by recovery studies using standard-addition technique.

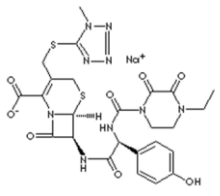
Conclusion: The proposed method has higher sensitivity than many of the reported methods, the method is green analytical methods so, it is inexpensive and ecofriendly. Moreover, the method doesn't require various elaborate treatments and tedious extraction procedures.

Keywords: Kinetic determination; Spectophotometry;cefoperazone Na; Ferricyanide.

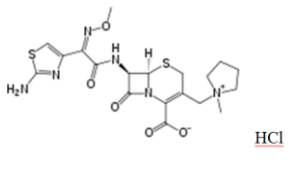
INTRODUCTION

Cephalosporin is the largest and most diverse family of beta-lactam antibiotics. Cephalosporin is indicated for the prophylaxis and treatment of infections caused by bacteria susceptible to this particular form of antibiotic. They are structurally and pharmacologically related to the penicillin.Cephalosporin has a beta-lactam ring structure, infused to a 6-membered dihydrothiazine ring, thus forming the cephem nucleus and interferes with bacterial cell wall synthesis. Cephalosporin distrusts the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity.

It is among the safest and the most effective broad-spectrum bactericidal antibiotics and therefore, it is the most frequently prescribed class of antibiotics [1]. Cephalosporin is divided into first, second, third, and fourth-generation agents. Cefoperazone Na and cefepime hydrochloride are two of third and fourth generation agents respectively.



Cefoperazone Na



Cefepime hydrochloride

The United States Pharmacopoeia (USP)recommended liquid chromatographic method for determination of cefoperazone Na and cefepime hydrochloride[2].Several methods were reported for cephalosporin determination[3,4].A survey of the literature reveals that cefoperazone Na was determined in pure form,pharmaceuticals or biological fluids using spectrophotometric[5-17],voltammetric[18, 19]and chromatographic [20-22] methods.

A number of methods like spectrophotometry [23-25],voltammetry[26,27] and HPLC [21,28],were reported in the literature for the determination of cefepime hydrochloride.

The aim of present work is to develop and validate spectrophotometric method for determination of cefoperazone Na and cefepime HCl in pure form, pharmaceutical preparations and urine using potassium ferricyanide in presence of $FeCl_3$.The proposed method overcomes most ofthe limitations of the existing visible spectrophotometric methods such as extraction with organic solvent, heating, using buffer, the method is very simple in application and of low expenses in comparison to chromatographic technique, as the same time this method offering a high degree of accuracy when compared to the reference methods.

MATERIALS AND METHODS

Instrumentation

Shimadzu recording spectrophotometer UV 1201 equipped with 10 mm matched quartz cells was employed for all absorbance measurements.

Materials and reagents

All materials used were of the highest purity available, these included Cefoperzone sodium, Cefozon vials labelled to contain 1000 mg cefoperzone sodium per vial (Egyptian Pharmaceutical Industries Co. E.P.I.C.O. Egypt),cefepime hydrochloride and Wincef vial (labeled to contain1000mg mg of cefepime hydrochloride provided by (Kahera pharm for ADWIA Co. S.A.E.).

- Ferric chloride, (BDH, UK) 0.4% aqueous solution.
- Potassium ferricyanide, (Winlab, Middlesex, England)

0.4% aqueous solution.

Solutions

A 1000µgmL^{-1} standard solution of drug was prepared by dissolving 0.1 gm in 80 mL distilled water ,then dilute the solution to 100 mL,4mL of this standard solution was diluted to 100 mL (40 µgmL^{-1} solution), these solutions were stable for at least a week when kept in the refrigerator and protected from light, solution was preserved without light at 4^{o}C.0.4% solutions of both ferric chloride and potassium ferricyanide were prepared in distilled water.

General procedure

Procedure for calibration graph

Aliquots of working standard drug solution containing (0.8-8 µg/mL) were allowed to react with1.5,1mL of 0.4% ferric chloride for cefoperazone Na and cefepime hydrochloride respectively,and 1 mL of0.4% potassium ferricyanide for both drugs in 10 mL volumetric flasks, the solutions were mixed well.After 45minutefor cefoperazone Na and 15 minute for cefepime hydrochloride at room temperature, the solutions in flasks were made up to 10mL with distilled water. The absorbance was measured at 766nmagainst an appropriate reagent blank prepared simultaneously.

Procedures for determination of the studied drugs in dosage forms

An accurately measured volume of the mixed contents of 10 vials equivalent to 1000µgmL^{-1} were transferred into 100mL volumetric flask and diluted to 100 mL with distilled water,4mL of this standard solution was diluted to 100 mL (40 µgmL^{-1} solutions). The assay was completed as under general procedure by applying standard addition technique.

Procedures for determination of the studied drugs in urine

Human urine samples were collected freshly from healthy adult volunteers and kept frozen until use after gentle thawing. Blank urine pool was diluted 1:1 with double distilled water, appropriate amounts of stock solution was added to 2 mL diluted urine, Then

assay was completed as described in Procedures for calibration graph

Procedures for the kinetic method

Aliquots of (0.8-8µg/mL) of cefoperazone Na were assayed as in the general procedure at different times (20, 35, 45, 60 minutes).

RESULTS AND DISCUSSION

Iron(III) salts play a prominent role in the spectrophotometric determination of some pharmaceutical drugs.[29-32]and in measuring the reducing power activity of some plants[33 -34]

Theoretically, two mechanisms were possible for the ferric ferricyanide reaction. The first one that the ferricyanide ion was reduced to ferrocyanide and react with ferric ions, the second is that the ferric ion was reduced to ferrous and react with ferri-cyanide[35].So drug may reduce ferric ions in its salt form to ferrous ions, which in presence of potassium ferricyanide produces colored chromogen[32] measured at 766 nm. (Figure 1)

The chemical structures for both product complexes were similar but in the first mechanism the iron(lII) is bonded with the cyanide molecule through the nitrogen atom while in the second one, the iron(II) is bonded to cyanide molecule through the carbon atom [36]

The absorbance of the product is directly related to the concentration of the drug and can be used for its spectrophotometric determination.The intensity of the color in case of cefoperazoneNa increases with time at room temperature and so a kinetic method was developed for its determination.The development of the color depends very much on the reaction conditions; therefore it is very important to optimize these conditions.

Optimization of reaction Conditions

Effect of $FeCl_3$ concentration

The maximum absorbance increased with increasing $FeCl_3$ concentration until it reaches 0.4% after which the absorbance is constant. It was found that1.5,1mL of 0.4% $FeCl_3$ was adequate for giving the maximum absorbance for cefoperazone Na and cefepime hydrochloride respectively.

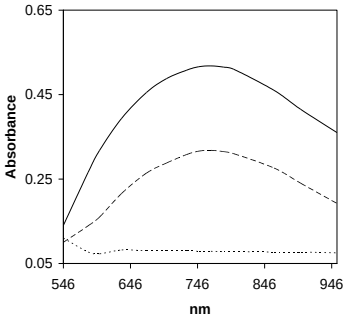


Fig.1: It shows absorption spectra of the reaction between ferric chloride(0.4%),ferricyanidel (0.4% w/v)and 4 µg/mL cefoperazone sodium (___), 4.8 µg/mL cefepime hydrochloride(.-.-.),blank(.....).

Effect of ferricyanide concentration

1 mL of 0.4% ferricyanide was found to be sufficient to give maximum absorbance, after which ferricyanide had no effect on the absorbance.

Effect of pH

The effect of pH was studied; it was found that maximum and constant absorbance was observed in the presence and absence of different concentrations of acetic acid, so addition of acetic acid was not incorporated in the reaction procedure, nitric acid was not used due to its strong oxidizing power. Hydrochloric and sulphuric acids were also tried, but the absorbance reading of the colored complex did not remain stable for more than 10 min.

Effectof diluting solvent

It was found that water was the best solvent for dilution. Using methanol or ethanol however, resulted in precipitation of the colored product.

Effectof temperature

Different temperatures were tested, from 25-100°C, using water bath. It was found that temperature has no effect on absorbance until 60^{o}C, above which precipitation occur.

Effect of time

The effect of time was studied in the range of 10-60 minute.45,15minute were found to give the maximum absorbance for cefoperazone Na and cefepime hydrochloride respectively.

The optical characteristic such as beer's law range, molar absorptivity, relative standard deviation, regression characteristic like slope, intercept, correlation-coefficient and standard error were also calculated and were shown in Tables 1,2.

Under the described experimental conditions, standard calibration curves for the studied drugs were constructed by plotting the absorbance versus concentration;Beer's Law was evident over the concentration range of the final dilution as given in table 1.

Method validation

The method was validated according ICH guidelines on the validation of analytical methods [37].

Quantification, accuracy and precision

Beer's law was obeyed over a concentration range of 0.8-8µg/mL for cefoperazone Na and cefepime hydrochloride. Molar absorptivity, correlation coefficient, intercept and slope for the calibration curve, detection and quantification limit were calculated, Table 1. Also

relative standard deviation,standard error and variance were calculated and listed in Table 2.

Table1: Spectral data for determination of cefoperazone Na and cefepime hydrochloride using potassium ferricyanide and ferric chloride

Items	Cefoperazone Na	Cefepime hydrochloride
Linearity range (µgmL−1)	0.8-8	0.8-8
Apparent molar absorpitivity* (L mol−1 cm−1)	$8.1x10^4$	$3.7x10^4$
Regression equation**		
Intercept (a)	-0.0188	0.0617
Slope (b)	0.1327	0.0544
Correlation coefficient (r)	0.9999	0.9999
Variance	0.14	0.57
Detection limit (µgml−1)	0.22	0.21
Quantification limit (µgml−1)	0.66	0.64

*Calculated on the basis of the molecular weight of the drug.

** A = a + bC

Table 2: Determination of cefoperazone Na and cefepime hydrochloride using potassium ferricyanide and ferric chloride.

Cefoperazone Na		Cefepime hydrochloride	
Taken (µgml-1)	Recovery%	Taken (µgml-1)	Recovery%
0.8	100.60	0.8	99.49
1.2	99.70	1.2	100.03
2.8	100.06	2	101.37
4	100.18	4.8	99.68
4.8	100.13	6.4	99.17
6.4	99.35	7.2	99.90
7.2	100.24	8	100.71
8	100.20		
Mean*±S.D.	100.05±0.377	100.05±0.755	
N		7	
	8		
V	0.142	0.570	
R.S.D.	0.376	0.754	
S.E.	0.133	0.285	

*Mean of three different experiments.

Accuracy and precision

In order to determine the accuracy and precision of the proposed method, solutions containing 3 different concentrations of drug were prepared and analyzed in six replicate. The relative standard deviation as precision,percentage relative error (Er %) as accuracy of the suggested method were calculated at 95% confidence levels and can be considered satisfactory. Precision was carried out by six determinations at three different concentrations; the percentage relative error was calculated according to the following equation:

Er % =[(found -added)/added] x100 (1)

The inter- and intra-day precision and accuracy results are shown in Table3.The analytical results for accuracy and precision show that the proposed method has good repeatability and reproducibility.

Table3: The inter-day precision and accuracy data for the studied drugs obtained by the proposed method.

drug	Intra-day				Inter-day			
Cefoperazone	Added µgmL-1	Found ± SE* µgmL-1	Precision RSD %	Accuracy ER %	Added µgmL-1	Found ± SE* µgmL-1	Precision RSD %	Accuracy ER %
	1.2	1.18±0.373	1.007	-1.600	1.2	1.19±0.476	1.169	-0.660
	2.8	2.78±0.181	0.444	-0.710	2.8	2.76±0.118	0.292	-1.428
Cefepime	4.8	4.80±0.145	0.355	0.208	4.8	4.77±0.117	0.288	-0.625
hydrochloride	1.2	1.19±0.528	1.300	-0.833	1.2	1.19±0.572	1.408	-0.833
	2	1.99±0.463	1.131	-0.500	2	1.99±0.564	1.384	-0.500
	4.8	4.73±0.318	0.787	-1.450	4.8	4.73±0.263	0.652	-1.450

Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation of method variables including, volume of analytical reagents(ideal volume ±0.2) and time of reaction(ideal time ±2minute). In these experiments, one parameter was changed whereas the other was kept unchanged;I t was found that noneof these variables significantly affect the method. This provided as indication for the reliability of the proposed method during its routine application for analysis of the investigated drug.

Ruggedness was tested by applying the proposed method to the assay drug using the same operational conditions but using two different instruments. Results obtained were found to be reproducible, as RSD did not exceed 2% (Table 4).

The % recoveries of the pure drug using the proposed method compared with that given by the reference methods[16,24] were illustrated in (Table 5). The Student t-test and F-test values of 95% confidence level did not exceed the theoretical values indicating no significant difference between the accuracy and the precision of the two methods.

Determination of cefoperazone Na and cefepime hydrochloride in pharmaceutical preparations

Table4: Evaluation results of the ruggedness of the proposed spectrophotometric method for determination of cefoperazone Na and cefepime hydrochloride

Drug	Shimadzu UV-1800	Shimadzu UV-260
Cfoperazone(2.8µgmL-1)	99.61±0.41	99.34±0.15
Cefepime hydrochloride(4.8 µgmL-1)	99.3±0.38	
		98.53±0.38

*Mean recovery % ±S.D.

The proposed methods were applied for determination of the studied drugs in their pharmaceutical preparations using the standard addition technique (Table 6).

Application to spiked human urine

As another application of the proposed method, recovery from human urine samples was carried out.Urine samples were prepared for analysis of the recovery of the studied drugs using the proposed method. The results were incorporated in table7.High accuracy and good recoveries were obtained which indicates that the proposed method can be successfully applied to recover cefoperazone Na and

cefepime hydrochloride in urine samples.Their values confirm the sensitivity of the proposed method in human urine.

Table 5: Statistical Data for Determination of the studied drugs through reaction with ferric chloride and ferricyanide.

Drug	Cefoperazone Na		Cefepime hydrochloride	
Items	**Reference method(16)**	**proposed method**	**Reference method(24)**	**proposed method**
Mean±S.D	100.3±0.638	100.05±0.377	100.01±0.409	100.05±0.755
N	7	8	7	7
V	0.407	0.142	0.167	0.57
t	---	0.940(2.160)	----	0.123(2.179)
F	---	0.265(3.870)	---	3.40(4.28)

* Theoretical values of t and F at P = 0.05.

Table 6:Application of standard addition technique for determination of cefoperazone Na and cefepime hydrochloride in their pharmaceutical formulations using potassium ferricyanide and ferric chloride

	Cefoperazone Na			Cefepime hydrochloride		
	Taken µg/mL	**Added µg/mL**	**Recovery***	**Taken µg/mL**	**Added µg/mL**	**Recovery***
	1.2	---	99.09	1.2	----	100.03
		0.8	97.77		0.8	97.19
		1.2	98.46		2	99.54
		2	100.90		2.4	99.80
		2.8	98.98		3.6	99.72
		3.6	97.92		4	98.94
		4	99.62		4.8	98.92
		4.8	99.81			
Mean±S.D.	99.06±1.12			99.01±0.97		
N	7			6		
V	1.26			0.94		
S.D.	1.12			0.97		
S.E.	0.42			0.39		

* Mean of three different experiments

Table7: Application of the proposed method to cefoperazone Na and cefepime hydrochloride concentrations measurements in spiked urine.

Drug	added, µg mL^{-1}	found * µg mL^{-1}	Recovery (Percent±S.D.)
Cefoperazone Na	1.2	1.19	99.16±0.835
	2.8	2.78	99.28±0.36
	4.8	4.79	99.92±0.23
Cefepime hydrochloride	1.2	1.23	102.49±1.66
	2	1.97	98.5±1.00
	2.4	2.50	104.16±0.83

Evaluation of the kinetic method (for cefoperazone Na)

The rate of reaction was found to be dependent on drug concentration. The rate was followed at room temperature with various concentrations in the range of0.8-8 µg/mL, keeping $FeCl_3$ and ferricyanide concentrations constant (Figure 2). The reaction rate was found to obey the following equation:

Rate = K'[drug]n (2)

Where K` is the pseudo order constant of the reaction, and n is the order of the reaction.

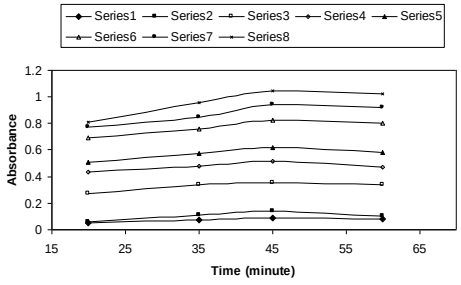


Fig. 2: It Shows absorbance versus time graphs for the reaction between cefoperazone sodium,ferric chloride and ferricyanide at different concentrations of drug:(a)1.23 x 10^{-6} M.(b)1.85 x 10^{-6} M (c) 4.33 x 10^{-6} M (d) 6.19x 10^{-6} M (e) 7.43 x 10^{-6} M (f) 9.91 x 10^{-6} M (g) 1.115 x 10^{-5} M (h)1.239 x 10^{-5} M

The rate of the reaction may be estimated by the variable time method [38]. In this method the reaction rate was followed by measuring the change of absorbance at different time intervals. Taking logarithms of rates and concentration(Table 8) Equation 2 is transformed into:

log (rate)=log ΔA/Δt= log K` + n log [drug] (3)

Where A is the absorbance and t is the time in seconds.

Regression of log (rate) versus log (drug) gave the regression equations:

Log(rate)= 1.1056 +0.6 log C(r=0.898), K` = 12.75 S-1 (4)

Hence the reaction is first order (n ≈ 1) with respect to drug concentration.

Table 8: Logarithms of the rates for different concentrations of cefoperazone Na at constant concentration of potassium ferricyanide and $FeCl_3$.

Log (rate), log ΔA/Δt	Log (drug) (M)
-4.590	-5.91
-4.27	-5.732
-4.280	-5.363
-4.270	-5.208
-4.120	-5.129
-4.050	-5.000
-3.955	-4.952
-3.809	-4.906

Evaluation of the kinetic methods

The quantitative determination of the studied drugs underthe optimized experimental conditions outlined before wouldresults in; a pseudo-first order reaction with respect to theirconcentration. However, the rates will be directly proportionalto drug concentration in a pseudo-first order rate equation asfollow:

Rate=K` [Mdrug] (5)

Where K` is the pseudo-first order constant. Equation 5 was thebases for several experiments, which were run to obtain drugconcentration using the rate data. Rate constant, constantconcentration and fixed-time [39,40] were tried and the mostsuitable analytical method was selected taking into account theapplicability, the sensitivity, the correlation coefficient (r) andthe intercept.

Rate-constant method

Graphs of log (absorbance) versus time for the studieddrug concentrations in the range of 1.23 x 10^{-6} to 1.239 x 10^{-5}M, were plotted and all appeared to berectilinear. Pseudo-first order rate constants corresponding todifferent drugs concentrations (C) were calculated from theslopes multiplied by -2.303 and are presented in (Table 9).

Regression of (C) versus K` gave the equation:

K`= -2.952x10-4+19.973C (r=0.730) (6)

Table 9: Values of K′ calculated from slope of logA vs. t graphs multiplied by -2.303 for different concentrations of cefoperazone Na and constant concentration of reagents.

K′ (S^{-1})	(M)
$-3.78x10^{-4}$	$1.23x10^{-6}$
$-2.57x10^{-4}$	$1.85x10^{-6}$
$-8.367x10^{-5}$	$4.33x10^{-6}$
$-1.2129x10^{-4}$	$6.19x10^{-6}$
$-1.316x10^{-4}$	$7.43x10^{-6}$
$-1.366x10^{-4}$	$9.91x10^{-6}$
$1.66x10^{-4}$	$1.115x10^{-5}$
$7.676x10^{-8}$	$1.239x10^{-5}$

Fixed-concentration method

Reaction rate was determined for different concentrations of drug in the range of 9.91 x 10^{-6} to 1.239 x 10^{-5}M. A pre-selected value of the absorbance was fixed and the time was measured in second.The reciprocal of time (i.e. 1/t) versus the initial concentration of the studied drug (Table 10) was plotted.

The following equations for calibration graphs were worked out by linear regression:

1/ t = $-1.491x10^{-3}$+ 186.57C (r=0.9956) (7)

The range of the concentration of the studied drug giving the most acceptable calibration graph with the above equation was very limited, which could be disadvantage.

Table 10: Values of reciprocal of time taken at fixed absorbance for different rates of variable concentration of cefoperazone Na.

1/t (S^{-1})	M
$8.33x10^{-4}$	$1.239x10^{-5}$
$5.64x10^{-4}$	$1.115x10^{-5}$
$3.703x10^{-4}$	$9.91x10^{-6}$

Fixed time method

Reaction rates were determined for different concentrations of drug. At a preselected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus initial concentrations of cefoperazone Na were established at fixed times of 20, 35, 45 and60 min. with the regression equations assembled in (Table 11). It is clear that the slope increases with time and the most acceptable values of the correlation coefficient(r) and the intercept were chosen as the most suitable time interval for measurement.

The most acceptable values of the correlation coefficient and more reaction products (indicated by higher absorbance readings) as shown in (Figure 2) were obtained for a fixed time of 45 min, which was, therefore chosen as the most suitable time interval for measurements.

Table 11: Calibration equations for cefoperazone Na at different fixed time over the range of $1.23x10^{-6}$ – $1.239x10^{-5}$ M in presence of constant concentration of reagents.

Time (min)	Calibration equation	Correlation coefficient (r)
20	A=-0.0418+0.1116C	0.9969
35	A=-0.02312+0.12264C	0.9995
45	A=-0.0188+0.1327C	0.9999
60	A=-0.0430+0.1323C	0.9993

CONCLUSIONS

The proposed method is simple, green, accurate and precise in determining the cited drugs in their pharmaceutical formulations and human urine. The proposed method as higher sensitivity than many of the reported methods. In contrast to HPLC, there is no need for special hardware, or expensive solvent.The method is green analytical methods so, it is inexpensive and ecofriendly. Moreover, the method doesn't require various elaborate treatments and tedious extraction procedures, In addition to the satisfactory, sensitivity and reproducibility as well as the convenience and simplicity. So, the proposed method is suitable for routine analysis of the cited drugs in control laboratories.

REFERENCES

1. Williams JD,Naber KG,BryskierA, HoibyN, Gould IM,Periti P, et al. Classification of oral cephalosporins. A matter for debate. Int JAntimicrob Agents.2001; 17 : 443–50.
2. United States Pharmacopoeia 36, United States Pharmacopoeia Convention, Washington. 2013.
3. KoranyMA,El-sayedMA,GalalSM .Use of second derivative spectrophotometryfor the determination of certain cephalosporins and their acid-induced degradation products in combination. Anal Lett. 1989; 22(1):159-75.
4. Korany MA,El-Sayed MA,Galal SM.Utility of Derivative Spectrophotometry For the Determination of Certain Cephalosporins and Their Alkali-Induced Degradation Products In Combination.Anal Lett. 1989;22(1):141-57.
5. El Walily AM, Gazy AA, Belal SF, Khamis EF.Use of Cerium (IV) in the Spectrophotometric and Spectrofluorimetric Determinations of Penicillins and Cephalosporins in Their Pharmaceutical Preparations.SpectroscLett. 2000;33:931-48.
6. Saleh GA,Askal HF, Radwan MF, Omar MA. Use of charge-transfer complexation in the spectrophotometric analysis of certain cephalosporins.Talanta. 2001; 54: 1205–15.
7. .Salem H, Askal H. Colourimetric and AAS determination of cephalosporins using Reineck's salt.J Pharm Biomed Anal 2002;29:347–54.
8. Salem H, Saleh GA. Selective spectrophotometric determination of phenolic β-lactam antibiotics.J Pharm Biomed Anal 2002; 28: 1205–13.
9. SalemH. Selective spectrophotometric determination of phenolic β-lactam antibiotics in pure forms and in their pharmaceutical formulations.Anal ChimActa..2004; 515: 333–41.
10. El-Azazy M S,Shalaby A,EL-Bolkiny M N,Khalil H M.Spectrophotometric determination of cefepime hydrochloridehydrochloride, cefoperazone Na,ceftazidimepentahydrate, cefuroxime sodium and etamsylate using ammonium molybdate.Sci Pharm.2003; 71: 211-28.
11. Saleh GA,El-Shaboury SR,Mohamed FA, Rageh AH. Kinetic spectro-photometric determination of certain cephalosporins using oxidized quercetin reagent.SpectrochimActa Part A.2009;73: 946–54.
12. Rageh A H, El-Shaboury SR,Saleh GA, Mohamed FA. Spectophotometric method for determination of certain

cephalosporins using 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole.Nat Sci.2010; 2:828-40.

13. Sayed RA, Hassan WS, El- Mammli MY,Shalaby A. Spectrophotometric method for the determination of cefotaximesodiumand cefoperazone Na in Pure and Pharmaceutical osage Forms. Am ChemSci J. 2013;3: 514-25.
14. Sayed RA, Hassan WS, El- Mammli MY, Shalaby A.Use of silver–gelatin complex For the determination of cefoperazone Na Sodium, ceftazidimepentahydrate and cefotaximesodium in Pure and Pharmaceutical.CheSci Rev Lett.2012;1: 10-7.
15. El-Didamony AM, Saad MZ, El-Shaprawy DS.Direct and indirect spectrophotometric determination of some selected antibiotics using potassium permanganate.Journal main group chemistry. 2013;12:139-52.
16. Sayed R A, Hassan W S, El- Mammli MY, Shalaby A. Development of simple green spectrophotometric and conductometricmethods for determination of Cephalosporins in Pure, Pharmaceutical Dosage forms and Human Urine. Journal of Advances in Chemistry. 2013;4:532-47.
17. Hoang VD, LoanNT,Tho VT. UV spectrophotometric simultaneous determination of cefoperazone Na and sulbactam in pharmaceutical formulations by derivative, Fourier and wavelet transforms.SpectrochimActa Part A.2014;121:704–14.
18. Abo El-Maali N, Ali AM, Ghandour MA. Electrochemical reduction and oxidation of two cephalosporin(polarography – voltammetry) antibiotics: Ceftriaxone (rocephin) and cefoperazone Na (cefobid).Electroanalysis.1993; 5: 599-604.
19. Hammam E, El-Attar MA, Beltagi AM.Voltammetric studies on the antibiotic drug cefoperazone Na: Quantification and pharmacokinetic studies.J Pharm Biomed Anal. 2006;42: 523–27.
20. El—Shanawani AA.HPLC determination of sulbactam,sultamicillintosylate,cefaclor,ampicillin and cefoperazone in pharmaceutical preparations. Acta Pol Pharm. 1998;55 :9-14.
21. Elkady EF, Abbas SS.Development and validation of a reversed-phase column liquid chromatographic method for the determination of five cephalosporins in pharmaceutical Preparations. J. AOAC Int. 2011; 94:1440-46.
22. ShettySK,Yashwanth R, Manzoor AB. Development and validation of RP-HPLC method for quantitative estimation of cefoperazone Na in bulk and pharmaceutical dosage forms. Int. J. ChemTech Res.2011; 3: 1075-80.
23. Ródenas V, Parra A, Villanova J, Gomez M.Simultaneous determination of cefepime hydrochloride and l-arginine in injections by second-derivative spectrophotometry.J Pharm Biomed Anal.1995;13: 1095–1099.
24. Elazazy MS, Shalaby A. Validated Spectrophotometric Assay of Cefepime hydrochlorideHydrochloride and Cefuroxime Sodium Using a Tetrazolium Salt.E-J.Chem. 2012;9:2261-67.
25. Chafle DM.Development and validation of spectrophotometric method for the estimationof cefepime hydrochloride in bulk and dosage form. Der Pharma Chem. 2013;5:127-32.
26. Jiménez FJ,Mochón MC, Sánchez JC, Carranza JH.Adsorptive stripping voltammetric determination of cefepime hydrochloride at the mercury electrode in human urine and cerebrospinal fluid, and differential pulse polarographic determination in serum.J.Pharm Sci. 2003; 92:1854–59.
27. Jain R, Gupta VK,Jadon N, Radhapyari K.Voltammetric determination of cefixime in pharmaceuticals and biological fluids.Anal Biochem2010;407:79–88.
28. TamboliSR,Patil DD. RP-HPLC method for simultaneous estimation of cefepime hydrochloridehydrochloride andtazobactamsodium in bulk and pharmaceuticals.journal of Chemistry. 2013;2013:1-6.
29. Basavaiah K,Chandrashekar U, Prameela HC.Sensitive spectrophotometric determination of amlodipine and felodipine using iron(III) and ferricyanide.IlFarmaco.2003;58:141–48.
30. Ibrahim FA, Ali FA, Ahmed SM, Tolba MM.Kinetic and spectrophotometric determination of thioctic Acid in bulk and pharmaceutical formulations. J. ChinChem Soc. 2007; 54: 365-74
31. Guo L, Zhang Y, Li Q. Spectrophotometric determination of dopamine hydrochloride in pharmaceutical,banana, urine and serum samples by potassium ferricyanide-Fe(III). Anal Sci. 2009 ;25:1451-55.
32. Ramaa CS, Chothe PP, Naik AA, Kadam VJ. Spectrophotometric method for the estimation of Oxcarbazepine in tablets. Indian J. Pharm Sci.2006 ;68:265-266.
33. Rahman MH, Alam MB, Hossain MS, Jha MK, Islam A. Antioxidant,analgesic and toxic potentiality of methanolic extract of Stephania japonica (Thunb) Miers leaf. Asian J Pharm Clin Res.2011; 4(3): 38-41.
34. Ponmozhi P, Geetha M, Saravanakumar M, Suganyadevi P. Extraction of anthocyanin and analysing its antioxidant properties from PithecellobiumDulce fruit pericarp. Asian J Pharm Clin Res.2011; 4(1): 41-5.
35. Lillie RD, Donaldson PT.The mechanism of the ferric ferricyanide reduction reaction.Histochem J. 1974; 6:679-84
36. Huhee JE, Keiter EA,KeiterRL.Inorganic Chemistry. 4th Ed. Harper CollinsCollege Publishers;1993.
37. Topic Q2 (R1). Validation of analyticalprocedures: Text and methodology. InternationalConference on Harmonisation, 2005.
38. Weisberger A,Friess S L, Lewis E S. Techniques of OrganicChemistry.vol.3Interscience, New York; 1953.
39. Yatsimirskii K B. Kinetic Methods of Analysis.Pegamon Press, Oxford;1966
40. Laitinen HA, Harris W E. Chemical Analysis. 2nd ed. McGraw-Hill,New York; 1975.