Pharmaceutical Sciences, March 2016, 22, 28-34
doi: 10.15171/PS.2016.06
http://journals.tbzmed.ac.ir/PHARM

Research Article

Determination of Some Cephalosporins in Pharmaceutical Formulations by a Simple and Sensitive Spectrofluorimetric Method

Ali Abdollahi[1], Ahad Bavili Tabrizi[2]*

[1]*Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*
[2]*Biotechnology Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*

Article Info

Article History:
Received: 25 November 2015
Accepted: 13 December 2015
ePublished: 30 March 2016

Keywords:
-Cefixime
-Cefalexin
-Ceftriaxone
-Cerium(IV)
-Spectrofluorimetry
-Pharmaceutical formulation

ABSTRACT

Background: Cephalosporins are among the safest and the most effective broad-spectrum bactericidal antimicrobial agents which have been prescribed by the clinician as antibiotics. Thus, the developing of simple, sensitive and rapid analytical methods for their determination can be attractive and desirable.
Methods: A simple, rapid and sensitive spectrofluorimetric method was developed for the determination of cefixime, cefalexin and ceftriaxone in pharmaceutical formulations. Proposed method is based on the oxidation of these cephalosporins with cerium (IV) to produce cerium (III), and its fluorescence was monitored at 356 ± 3 nm after excitation at 254 ± 3 nm.
Results: The variables effecting oxidation of each cephalosporin with cerum (IV) were studied and optimized. Under the experimental conditions used, the calibration graphs were linear over the range 0.1-4 µg/mL. The limit of detection and limit of quantification were in the range 0.031-0.054 and 0.102-0.172 µg/mL, respectively. Intra- and inter-day assay precisions, expressed as the relative standard deviation (RSD), were lower than 5.6 and 6.8%, respectively.
Conclusion: The proposed method was applied to the determination of studied cephalosporins in pharmaceutical formulations by good recoveries in the range 91-110%.

Introduction

Cephalosporins are among the safest and the most effective broad-spectrum bactericidal antimicrobial agents which have been prescribed by the clinician as antibiotics.[1] All of these semi-synthetic antibiotics are derived from 7-amino-cephalosporanic acid and contain a β-lactam ring fused to a dihydrothiazine ring but differ in the nature of the substituents attached at the 3 and/or 7-positions of the cephem ring.[1-3] Gram positive and Gram negative bacterial infections are commonly controlled by cephalosporin antibacterials. As well as, cephalosporins are the second most important β-lactams after penicillin for treating infectious diseases.[2]

Cefalexin, is a first-generation cephalosporin antibiotic which has Gram-positive and Gram-negative activity and has become the most widely used antibiotic in the world.[4] Cefixime, is a semi-synthetic third-generation oral cephalosporin antibiotic being prescribed for the treatment of susceptible infections such as gonorrhea, otitis media, pharyngitis, lower respiratory tract infections like bronchitis, and urinary tract infections.[5] Ceftriaxone, a third generation parentral cephalosporin, has a relatively long half life and is stable to β-lactamases particularly those produced by Gram-negative organisms. It is excellently effective in Gram-negative bacterial infections. Its ring system contains a highly acidic heterocyclic system on the 3-thiomethyl group, so it is believed the unique pharmacokinetic properties of ceftriaxone is due to this unusual ring system. The bacteria can be killed by its interfering in the synthesis of the cell wall. Ceftriaxone has been effective in treating infections due to other 'difficult' organisms such as multidrug-resistant *Enterobacteriaceae*.[6-8] Figure 1 shows the structure of studied cephalosporins.

The determination of these cephalosporins in their pharmaceutical formulations have been done by variety of methods such as, spectrophotometry,[1,2,10-16] spectrofluorimetry,[4,5,17] chromatography,[6-8,18-22] capillary electrophoresis (CE),[3] near IR[23] and electrochemical methods.[24-27]

The chromatographic and electrophoresis methods are very sensitive and reliable, but they are relatively time-consuming and expensive. The majority of other reported methods utilize expensive or unstable reagents. Thus, the developing of simple, sensitive and rapid analytical methods as practical alternatives to above mentioned methods can be attractive and desirable. Fluorescence spectrometry has great sensitivity and selectivity as well as relatively low cost for the operation, thus it is used in quantitative analysis

***Corresponding Author:** Ahad Bavili Tabrizi, Tel: (+98) 41 33372250, E-mail: a.bavili@tbzmed.ac.ir

of pharmaceuticals. Due to these excellent properties, we propose here a simple, sensitive and inexpensive spectrofluorimetric method for the determination of these drugs in pharmaceutical preparations.

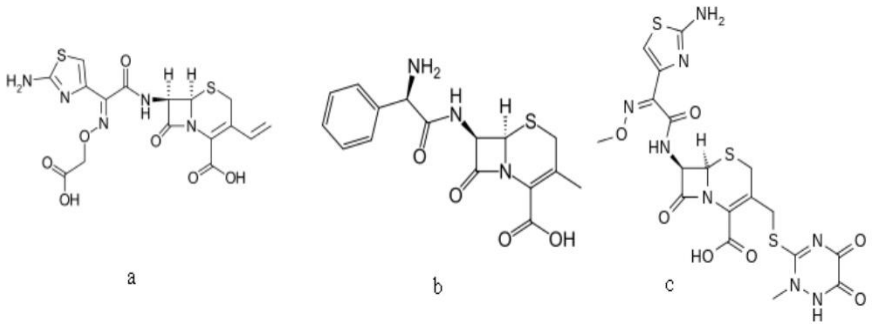

Figure 1. Structure of studied cephalosporins: a) cefixime, b) cefalexin, c) ceftriaxone.

Ce(IV) is a well-known oxidizing agent; it is used for the determination of some drugs.[28-36] It can be easily reduced to Ce(III) that shows a characteristic fluorescence in sulfuric acid medium. The literature survey revealed that this system has not been used for the analysis of these drugs. Thus, in this work Ce(IV) has been used as an oxidizing agent for the spectrofluorimetric determination of studied cephalosporins in their pharmaceutical formulations.

Materials and Methods

Apparatus

Fluorescence measurements were done with a Shimadzu RF-5301 PC spectrofluorophotometer, equipped with a 150 W Xenon lamp and 1.00 cm quartz cells. Both excitation and emission slits were adjusted to 3 nm and the sensitivity adjusted to low.

Reagents

Cefalexin, cefixime and ceftriaxone were obtained as gifts from Danna Pharma Co. (Tabriz, Iran). Sulfuric acid, ethanol and Ce(IV)-sulfate-tetrahydrat were purchased from E. Merck (Darmstadt, Germany).

A stock standard solution of each drug at a concentration of 500 µg/mL was prepared by dissolving appropriate amount of each drug in 5 mL absolute ethanol and diluting to 25 mL with double distilled water. These solutions were stored in refrigerator and kept from light when not in use for two weeks. Working standard solutions were obtained daily by appropriately diluting these stock solutions with double distilled water. Ceric sulfate, 0.01 mol/L was prepared in 2.0 mol/L sulfuric acid and was kept in the refrigerator at 4 °C for two week. All other reagents were of analytical-reagent grade (E. Merck) and all solutions were prepared in doubly distilled water.

Recommended procedure for calibration

An aliquot of sample solution containing each drug in the range 0.1-4.0 µg/mL was transferred into 15-mL calibrated centrifuge tubes. Then, 0.3 mL of 2.0 mol/L sulfuric acid and 60 µL of 0.01 mol/L Ce(IV) was added. The content of each tube was mixed well and diluted to 10 mL with double distilled water. The resultant solutions were left at ambient temperature for 30 min, then the fluorescence intensity of each solution was measured at 356 ± 3 nm while excited at 254 ± 3 nm against reagent's blank.

Preparation of Pharmaceutical Formulations

Tablets and capsules

Ten cefixime tablets (Pars Darou, Tehran, Iran), each containing 400 mg cefixime (as trihydrate), were accurately weighed individually and finely powdered. Powdered sample containing 40 mg cefixime was weighed and placed into a 15-mL glass tube, dissolved in 5-mL absolute ethanol and vigorously shaken on a vortex mixer for 1 min. The solution was then filtered and transferred into a 100-mL volumetric flask. The residue was washed in enough water and the solution was finally made up to the mark with double distilled water. Thus, a 400 µg/mL solution of cefixime was obtained.

The contents of five capsules of cefalexin (Jaber Ebne Hayyan, Tehran, Iran), each containing 500 mg cefalexin, were thoroughly mixed. Powdered sample containing 50 mg of cefalexin was weighed and transferred to a 100-mL volumetric flask and dissolved in 5-mL absolute ethanol. Then, the volume was adjusted to the mark with water to obtain a 500 µg/mL solution of cefalexin. Aliquots of 10 µL of these prepared samples were used for cefixime or cefalexin determination as mentioned procedure.

Powder for Oral Suspension

Two brands for oral suspension were studied including cefixime (Exir Pharm Co., Boroojerd, Iran) and cefalexin (Danna Pharma Co., Tabriz, Iran). The contents of each vessel was completed to 100 mL with double distilled water, so each 5 mL of prepared suspension was containing 100 mg of cefixime or 250 mg cefalexin based on labeled amounts, respectively. An aliquot of 1 mL of each prepared suspension was diluted to 100 mL with water, then 40 µL of each diluted sample was used for cefixime or cefalexin determination as mentioned procedure.

Vial

The content of each ceftriaxone vial (Afa Shimi, Tehran, Iran), each containing 1 g ceftriaxone (as sodium), was completed to 3 mL with double distilled water. An aliquot of 250 µL of prepared vial was diluted to 100 mL with water, than 40 µL of this diluted sample was subjected to ceftriaxone determination as mentioned procedure.

Results and Discussion

Certain drugs have been determined based on their reaction with Ce(IV) as an oxidizing agent.[28-36] The produced Ce(III) is usually more fluorescent than the oxidation products and unreacted Ce(IV) and thus the monitoring of its fluorescence has been used as a very sensitive method for determination of certain drugs. In the present work the oxidation reaction of studied drugs with Ce(IV) was performed in sulfuric acid medium and the fluorescence intensity of the produced Ce(III) was monitored in desired wavelengths. Figure 2 shows the excitation and emission spectra obtained in the optimum conditions for cefixime-Ce(IV) system. Similar spectra were obtained for cefalexin or ceftriaxone reaction systems.

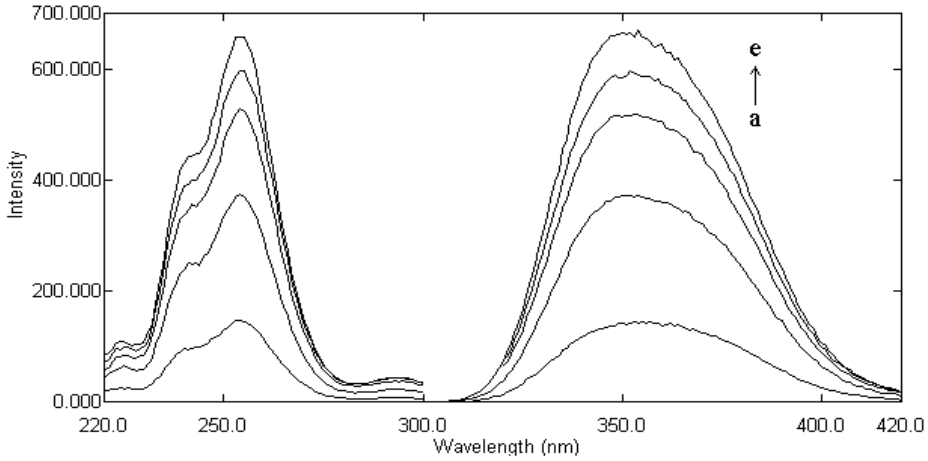

Figure 2. Excitation and emission spectra: (a) reagent's blank, (b) sample prepared from powder for oral suspension (1.4 µg/mL) (c) sample prepared from tablet (1.8 µg/mL), (d) sample (b) spiked with cefixime at 0.8 µg/mL, (e) standard solution of cefixime (2.4 µg/mL); other conditions: Ce(IV) (6.0×10^{-5} mol/L), sulfuric acid (0.06 mol/L).

Effect of Ce(IV) concentration

For investigation the influence of Ce(IV) concentration on the fluorescence intensity of the reaction product, increasing volumes of 0.01 mol/L Ce(IV) solution in the range 10-170 µL were used when other conditions kept constant. As shown in Figure 3, the maximum and constant fluorescence intensities were attained when Ce(IV) concentration was in the range 60-70 µL. (e.g. final concentration of 6.0 -7.0 (×10-5) mol/L).

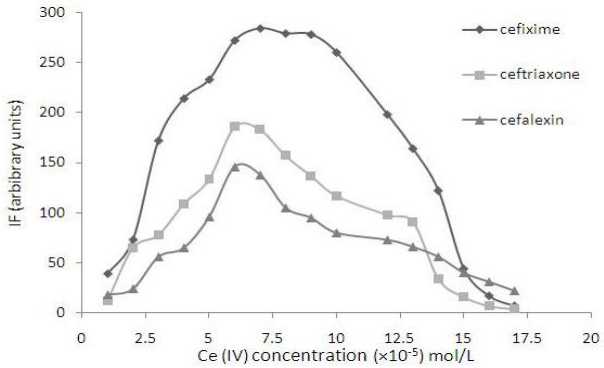

Figure 3. The effect of Ce concentration on the analytical signals, 1.0 µg/mL of each drug was used for optimization; other conditions: sulfuric acid (0.06 mol/L).

At concentrations lower than this range the fluorescence intensity dropped due to insufficient Ce(IV) for oxidation. On the other hand, a quenching effect has been reported in larger volume of Ce(IV).[28-30,33,34] An aliquot of 60 µL of 0.01 mol/L Ce(IV) (equivalent to final concentration of 6.0×10^{-5} mol/L) was used for the oxidation of studied drugs in the rest of work.

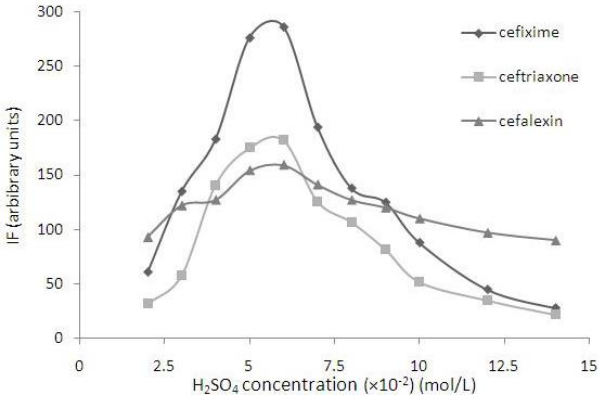

Figure 4. The effect of sulfuric acid concentration on the analytical signals, 1.0 µg/mL of each drug was used for optimization; other conditions: Ce(IV) (6.0×10^{-5} mol/L), sulfuric acid (0.06 mol/L).

Effect of sulfuric acid concentration

The effect of sulfuric acid concentration on the fluorescence intensities was studied in the range 0.02 - 0.14 mol/L and the results were presented in Figure 4. It was found that the fluorescence intensity was rapidly

increasing up to sulfuric acid concentration of 0.05 mol/L, remained approximately constant up to 0.06 mol/L and then decreased gradually. Hence, an aliquot of 300 µL of 2.0 mol/L sulfuric acid (equivalent to final concentration of 0.06 mol/L) was used in other experiments.

Effect of temperature and time

The effect of temperature on the oxidation reaction was studied in different sets ranging from 20-95 °C.

Heating the reaction solution was found to increase the fluorescence intensity, so the maximum signals were obtained at 95 °C, but the results were not repeatable at this temperature. Further experiments showed that oxidation reactions of studied dugs with Ce(IV) were relatively slow but very repeatable and precise at ambient temperature, thus this temperature was used in rest of work. Thus, the oxidation reactions were done in the temperature for periods ranging from 10 to 90 min. The results revealed that standing for 30 min at this temperature was sufficient for the completion of reaction with proper sensitivity.

Analytical characteristics

In the optimum conditions, linear calibration curves (n = 11) with good correlation coefficients (r > 0.9997) were obtained for studied cephalosporin. The characteristics of proposed methods for the determination of cephalosporins have been summarized in Table 1. The limit of detection (LOD) and limit of quantification (LOQ) were defined as $3S_b/m$ and $10S_b/m$, respectively, where S, is the standard deviation of the blank measurements and m is the slope of the calibration line. The precision of the method was determined as intra-day (n = 8) and inter-day precision (n = 4), which found to be lower than 5.6% and 6.8%, respectively. These results have been summarized in Table 2.

Table 1. Analytical characteristics of the method for studied cephalosporins.

Analyte	C (µg/mL)	r	Calibration equation	LOD (µg/mL)	LOQ (µg/mL)
Cefixime	0.10-4.0	0.9997	272.06C+3.0776	0.031	0.102
Cefalexin	0.17-4.0	0.9998	156.67C+1.5706	0.054	0.172
Ceftriaxone	0.15-4.0	0.9998	178.44C+3.6978	0.047	0.146

C = Concentration

Table 2. Intra- and inter-day precisions for determination of studied cephalosporins.

C	Cefixime		Cefalexin		Ceftriaxone	
(µg/mL)	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
0.1	5.53	5.84	5.32	6.76	5.14	6.68
2.0	3.22	4.25	4.37	5.25	2.63	3.64

Intra- and inter-day precisions expressed as RSD%, and for 8 and 4 replicate determinations, respectively.

Table 3. Analytical characteristics of different methods used for the determination of studied cephalosporins.

Determination Method	Analyte	Sample	Concentration range (µg/mL)	r	RSD%	LOD (µg/mL)	Mean R (%)	Ref.
Kinetic-S	Cfx & Cef	P.F	10-50	0.9991-0.9997	0.53-1.66	0.220-1.100	98.0-101.9	1
S	Cfx & Cef	P.F	0.5-3	0.9993	<2.00	0.120-0.168	96.0-102.3	2
CE	Cfx & Cft	P.F	5-100	0.9970-0.9980	0.75-1.03	1.420-2.730	-	3
F	Cef	P.F &B.S	0.04-0.4	0.9900	<5.00	0.013	98.1-100.7	5
HPLC	Cft	P.F & B.S	2.5-25	0.9997	0.37	0.170-0.450	100.5101.9	6
S	Cft & Cef	P.F	25-60	0.9982-0.9991	0.68-2.69	5.093-6.152	99.7-101.2	10
S	Cfx & Cft	P.F	0.2-85	>0.9991	0.25-3.00	-	97.4-109.8	14
S	Cft	P.F	2-100	0.9998-0.9999	0.26-0.61	-	99.6-100.2	16
F	Cfx & Cef	P.F	10-60 (µg/L)	0.9987-9995	<2.00	2.02-2.09 ($\times10^{-3}$)	95.2-107.0	17
S	Cft	P.F	4-20	0.9992-1.0000	0.13-0.29	0.020-0.048	99.6-100.4	37
HPLC	Cft	P.F &B.S	05-250	0.9998	4.67	0.017	98.0-103.8	38
F	Cef & Cfx & Cft	P.F	0.1-4.0	>0.9997	<5.6	0.031-0.054	91.0-110.0	This work

Cefixime = Cef, cefalexin = Cfx, Ceftriaxone = Cft, R = recovery; S = spectrophotometry; F = spectrofluorimetry; CE = capillary electrophoresis; HPLC = high performance liquid chromatography; B.S = biological sample; P.F = pharmaceutical formulation.

The performance of the proposed method was compared with the performances of other methods to highlight the distinct features of the proposed method (see Table 3). Compared with references which use HPLC or CE for the determination of studied drugs, the proposed method does not require high investment and maintenance costs of the instruments. More importantly, our figures of merit were comparable to or even better than some of these methods.

Table 4. Results of recoveries of spiked samples.

Sample	added (µg/mL)	found ± SD (n = 3), µg/mL	R %
Cefixime			
Tablet	–	0.440 ± 0.014	–
	0.2	0.622 ± 0.022	91
	0.8	1.320 ± 0.047	110
Powder for Oral Suspension			
	–	0.720 ± 0.024	–
	0.2	0.904 ± 0.029	92
	0.8	1.54 ± 0.054	102
Cefalexin			
Capsule			
	–	0.455 ± 0.019	–
	0.2	0.645 ± 0.028	95
	0.8	1.23 ± 0.054	97
Powder for Oral Suspension			
	–	1.85 ± 0.078	–
	0.2	2.04 ± 0.082	95
	0.8	2.66 ± 0.112	101
Ceftriaxone			
Vial			
	–	3.01 ± 0.081	–
	0.2	3.22 ± 0.084	105
	0.8	3.88 ± 0.097	109

Recovery experiments and interference study

Aliquot volumes of each prepared pharmaceutical preparation according to the section of "Preparation of Pharmaceutical Formulations", were transferred to clean centrifuge tubes and spiked with drug at two test concentrations and then analyzed following the optimized procedure. The obtained recoveries have presented in Table 4, which ranged from 91 to 110% and seem to be satisfactory. On the other hand, typical excitation and emission spectra for blank sample, cefixime standard solution, two pharmaceutical preparations and the last spiked with cefixime standard solution were plotted.

As shown in Figure 1, no additional peaks, caused by interfering compounds, were observed at the emission wavelengths used in this work. Thus, the similarities in the excitation and emission spectra along with reasonable recoveries that were achieved in this work, indicated that there was no significant matrix effect.

As well as, the effect of frequently encountered excipients such as; starch, talc, lactose, glucose, sucrose, magnesium-stearate and gum acacia on the determination of studied drugs were studied. The tolerance limit was taken as the concentration causing an error less than 8% in the determination of the drug. No interference effect from these excipients and additives was observed at concentrations up to at least 500-fold excess related to the studied drug. So, the proposed method can be considered a selective one.

Analysis of Pharmaceutical Formulations

The proposed method was applied successfully for determination of studied drugs in the pharmaceutical formulations and the results are presented in Table 5.

Table 5. Determination of the studied drugs in their pharmaceutical formulations using proposed method.

Sample	Labeled amount(mg)	Found amount ± SD (n = 3) (mg)	Experimental *t*-values	R%
Cefixime				
Tablet	400	432.0 ± 14.0	3.95	108
Powder for Oral Suspension	Each 5 mL containing 100 mg	Each 5 mL containing 92.0 ± 3.6	3.84	92.0
Cefalexin				
Capsule	500	455.0 ± 21.1	3.69	91.0
Powder for Oral Suspension	Each 5 mL containing 250 mg	Each 5 mL containing 231.0 ± 10.2	3.22	92.4
Ceftriaxone				
Vial	1000 mg	943.0 ± 25.7	3.84	94.3

Tabulate *t*-test at P=0.05, $t = 4.3$

Conclusion

This report describes a validated spectrofluorimetric method for the assay of cefixime, cefalexin and ceftriaxone in their pharmaceutical formulations. Method validation using spiked real samples demonstrated that the method is capable of determining studied cephalosporins with adequate trueness and precision. In addition, the obtained LODs and LOQs are comparable or better than those of other methods reported in Table 3. Although, HPLC or CE methods are precise and sensitive (in the case of HPLC), but they use high sophisticated and expensive instruments.

Therefore, from the economical point of view, the proposed method is simple, rapid, sensitive and inexpensive thus can be used as an alternative method for quality control or pharmaceutical analysis purposes.

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. El-Shaboury SR, Mohamed FA, Saleh GA, Rageh AH. Kinetic spectrophotometric determination of certain cephalosporins using iodate/iodide mixture. Nat Sci. 2010;2(5):432-43. doi:10.4236/ns.2010.25053
2. Ali Ahmed SM, Elbashir AA, Aboul-Enein HY. New spectrophotometric method for determination of cephalosporins in pharmaceutical formulations. Arabian J Chem. 2015;8(2):233–9. doi:10.1016/j.arabjc.2011.08.012
3. Hancu G, Kelemen H, Rusu A, Gyéresi Á. Development of a capillary electrophoresis method for the simultaneous determination of cephalosporins. J Serb Chem Soc. 2013;78(9):1413-23. doi:10.2298/jsc121117028h
4. Zhang J, Wang Z, Mi T, Wenren L, Wen K. A Homogeneous fluorescence polarization immunoassay for the determination of cephalexin and cefadroxil in milk. Food Anal Methods. 2014;7(4):879-86. doi:10.1007/s12161-013-9695-4
5. Manzoori JL, Amjadi M, Soltani N, Jouyban A. Spectrofluorimetric determination of cefixime using terbium-danofloxacin probe. Iran J Basic Med Sci. 2014;17(4):256-61.
6. Sultana N, Arayne MS, Shahzad W. Simultaneous determination of ceftriaxone sodium and non steroidal anti-Inflammatory drugs in pharmaceutical formulations and human serum by RP-HPLC. J Chin Chem Soc 2010;57(6):1278-1285. doi:10.1002/jccs.201000189
7. Sultana N, Arayne MS, Shahzad W. Simultaneous determination of ceftriaxone sodium and statin drugs in pharmaceutical formulations and human serum by RP-HPLC. J Chil Chem Soc. 2010;55(2):193-8. doi:10.4067/s0717-97072010000200010
8. Sultana N, Arayne MS, Shahzad W, Shah SN. Simultaneous determination of ceftriaxone sodium and H_2 receptor antagonists in in pharmaceutical formulations and human serum by RP-HPLC. Asian J Pharm Res Dev. 2013;1(1):57-65.
9. United States Pharmacopoeia 31 and NF 26. Washington: American Pharmaceutical Association; 2008.
10. Adegoke OA, Quadri MO. Novel spectrophotometric determinations of some cephalosporins following azo dye formation with p-dimethylaminobenzaldehyde. Arabian J Chem. 2012; in press. doi:10.1016/j.arabjc.2012.02.005
11. Agbaba D, Eric S, Karljikovic-Rajic K, Vladimirov S, Zivanov-Stakic D. Spectrophotometric determination of certain cephalosporins using ferrihydroxamate method. Spectrosc Lett. 1997;30(2):309-19. doi:10.1080/00387019708006990
12. Ramadan AA, Mandil H, Dahhan M. UV-Vis spectrophotometric study for determination of cefixime in pure form and in pharmaceuticals through complexation with Cu (II) using acetate-NaOH buffer in water:methanol. Int J Pharm Pharm Sci. 2013;5(1):428-33.
13. Suddhasattya D, Prasanna KP, Upadhayay, UM, Shah S, Kuntal G. UV Spectrophotometric Determination of Cefixime in Bulk and its Dosage Form. J Pharm Res. 2012;5(12):5419-22.
14. Pasha C, Narayana B. A simple method for the spectrophotometric determination of cephalosporins in pharmaceuticals using variamine blue. Ecletica Quim. 2008;33(2):41-6. doi:10.1590/s0100-46702008000200006
15. Durairaj S, Annadurai T, kumar BP, Arunkumar S. Simultaneous estimation of ceftriaxone sodium and sulbactam sodium using multi-component mode of analysis. Int J ChemTech Res. 2010;2(4):2177-81.
16. Patel KR, Patel VD, Patel KP, Patel VG. Development and validation of spectrophotometric method for determination of ceftriaxone sodium in pharmaceutical dosage forms. Der Pharma Chemica. 2010;2(5):255-9.
17. Elbashir AA, Ali Ahmed SM, Aboul-Enein HY. New spectrofluorimetric method for determination of cephalosporins in pharmaceutical formulations. J Fluoresc. 2012;22(3):857–64. doi:10.1007/s10895-011-1021-1
18. Talebpour Z, Pourabdollahi H, Rafati H, Abdollahpour A, Bashour Y, Aboul-Enein HY. Determination of cefixime by a validated stability-indicating HPLC method and identification of its related substances by LC-MS/MS studies. Sci Pharm. 2013;81(2):493-503. doi:10.3797/scipharm.1301-15
19. Khan A, Iqbal Z, Khan MI, Javed K, Khan A, Ahmad L, et al. Simultaneous determination of cefdinir and cefixime in human plasma by RP-HPLC/UV detection method: Method development, optimization, validation, and its application to a pharmacokinetic study. J Chromatogr B. 2011;879(24):2423-9. doi:10.1016/j.jchromb.2011.06.040
20. Dhoka MV, Sandage SJ, Dumbre SC. Simultaneous determination of cefixime trihydrate and dicloxacillin sodium in pharmaceutical dosage form by reversed-phase high-performance liquid chromatography. J AOAC Int. 2010;93(2):531-5.
21. Raj KA, Yada D, Yada D, Prabu C, Manikantan S. Determination of cefixime trihydrate and cefuroxime axetil in bulk drug and pharmaceutical dosage forms by HPLC. Int J ChemTech Res. 2010;2:334-6.
22. Siddiqui MR, Alothman ZA, Wabaidur SM, Khan, MA, Alam, MDS, Ali MDS. High performance liquid chromatographic method for the quantitative analysis of cefuroxime in pharmaceutical

preparations. J Chil Chem Soc. 2015;60(2):2869-71. doi:10.4067/s0717-97072015000200001
23. Gong L-P, Wang W-J, Yang N, Zhang Z-H, Xie Y-C. Development of NIR method for rapid determination of cefalexin tablet. Chin J Pharm Anal. 2011;31:1571-4.
24. Kulapina OI, Makarova NM, Kulapina EG. Potentiometric sensors for the determination of some cephalosporin antibiotics in biological fluids and medicinal preparations. J Anal Chem. 2015;70(4):477-84. doi:10.1134/s1061934815040073
25. Jain R, Gupta VK, Jadon N, Radhapyari K. Voltammetric determination of cefixime in pharmaceuticals and biological fluids. Anal Biochem. 2010;407(1):79-88. doi:10.1016/j.ab.2010.07.027
26. Ensafi AA, Allafchian AR. Multiwall carbon nanotubes decorated with $NiFe_2O_4$ magnetic nanoparticles, a new catalyst for voltammetric determination of cefixime. Colloids Surf B Biointerfaces. 2013;102:687-93. doi:10.1016/j.colsurfb.2012.09.037
27. Majidi MR, Asadpour-Zeynali K, Hafezi B. Electrocatalytic oxidation and determination of ceftriaxone sodium antibiotic in pharmaceutical samples on a copper hexacyanoferrate nanostructure . Anal Methods. 2011;3(3):646-52. doi:10.1039/c0ay00582g
28. Bavili Tabrizi A. A simple spectrofluorimetric method for determination of mefenamic acid in pharmaceutical preparation and urine. Bull Korean Chem Soc. 2006;27(8):1199-1202. doi:10.5012/bkcs.2006.27.8.1199
29. Bavili Tabrizi A. A new spectrofluorimetric method for determination of nifedipine in pharmaceutical formulations. Chem Anal (Warsaw). 2007;52(4):635-43.
30. Bavili Tabrizi A. A simple spectrofluorimetric method for determination of piroxicam and propranolol in pharmaceutical preparations. J Food Drug Anal. 2007;15(3):242-8.
31. Tavallali H, Dezfoli E. Spectrofluorimetric method for determination of low concentration of Minoxidil in pharmaceutical Formulations. Der Pharma Chemica. 2010;2(5):344-50.
32. Ibrahim FA, Ali FA, Ahmed SM, Tolba MM. Kinetic spectrofluorometric determination of thioctic acid in bulk and pharmaceutical preparations *via* its oxidation with Cerium(IV). J Chin Chem Soc. 2007;54(4):925-32. doi:10.1002/jccs.200700133
33. Khashaba PY. Spectrofluorimetric analysis of certain macrolide antibiotics in bulk and pharmaceutical formulations. J Pharm Biomed Anal. 2002;27(6):923-32. doi:10.1016/s0731-7085(01)00609-4
34. Mohamed FA, Mohamed HA, Hussein SA, Ahmed SA. A validated spectrofluorimetric method for determination of some psychoactive drugs. J Pharm Biomed Anal. 2005;39(1):139-46. doi:10.1016/j.jpba.2005.03.024
35. Askal HF, Abdelmegeed OH, Ali SMS, Abo El-Hamd M. Spectrophotometric and spectrofluorimetric determination of 1,4-ihydropyridine drugs using potassium permanganate and cerium (IV) ammoniumsulphate. Bull Pharm Sci. 2010;33(2):201-15.
36. Darwish IA, Khedr AS, Askal HF, Mahmoud RM. Simple fluorimetric method for determination of certain antiviral drugs via their oxidation with cerium (IV). Il Farmaco. 2005;60(6):555-62. doi:10.1016/j.farmac.2005.04.003
37. Hassan EM, Mahrous MS, Shdeed RN. Stability-indicating spectrophotometric methods for the determination of ofloxacin and ceftriaxone and their degradation products. J Pharm Biomed Sci. 2012;18(3):1-13.
38. Shah J, Rasul Jan M, Shah S, Naeem Khan M. Development and validation of HPLC method for simultaneous determination of ceftriaxone and cefaclor in commercial formulations and biological samples. J Mex Chem Soc. 2013;57:314-20.