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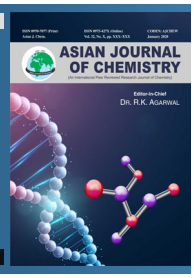

Spectrophotometric and RP-HPLC Methods for Determining Cefotaxime Sodium in Pharmaceutical Preparations

LAMYA A. SARSAM[1,*], SALIM A. MOHAMMED[2] and SAHAR A. FATHE[3]

[1]Department of New and Renewable Energy, College of Science, Mosul University, Mosul, Iraq
[2]Department of Chemistry, College of Science, Mosul University, Mosul, Iraq
[3]General Company for Communications Equipment and Power Al-Kindy Factory, Mosul, Iraq

*Corresponding author: E-mail: luma_sarsam@yahoo.com; lamyasarsam@uomosul.edu.iq

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A rapid, simple and sensitive spectrophotometric and RP-HPLC methods have been developed for the quantitative determination of cefotaxime-Na in both pure and dosage forms. The spectrophotometric method was based on diazotization of cefotaxime-Na and then coupling with 8-hydroxyquinoline in an alkaline medium. The resulting azo dye exhibited maximum absorption at 551 nm with a molar absorptivity of 0.597×10^4 L mol^{-1} cm^{-1}. Beer's law was obeyed over the range 10-700 µg/25 mL (*i.e.* 0.4-28.0 ppm) with an excellent determination coefficient ($R^2 = 0.9993$). The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.0194 and 0.3765 µg mL^{-1}, respectively. The recoveries were obtained in the range 97.3-102.5% and the relative standard deviation (RSD) was better than ± 1.56. The HPLC method has been developed for the determination of cefotaxime-Na. The analysis were carried out on a C18 column and a mobile phase composed of acetonitrile and phosphate buffer solution (0.024M KH_2PO_4 and 0.01M H_3PO_4) at pH 3.5 in the ratio of 60:40 (v:v), with a flow rate of 1.0 mL min^{-1} and UV detection at 258 nm. The proposed method showed good linearity (in a range of concentration 1.0-200 µg mL^{-1}. The recovery percent and a relative standard deviations were found in the range 96 to 104.8% and ± 0.017 to ± 0.031%, respectively. Both methods were applied successfully to the assay of cefotaxime-Na in commercial injection preparations.

Keywords: Cefotaxime-Na, RP-HPLC, Spectrophotometry, Diazometry.

INTRODUCTION

Cefotaxime sodium (Fig. 1) is a sodium (6*R*,7*R*)-3-[(acetyloxy)-methyl]-7-[[(2*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl] amino]-8-oxo-5-thia-1- azabicyclo[4.2.0]oct-2-ene-2-carboxylate (m.w. 477.4 g/mol) belongs to an important class of antibiotics called cephalosporin. It is a white or slightly yellow powder, freely soluble in water, sparingly soluble in methanol [1]. According to its high activity against a large number of Gram-positive and Gram-negative micro-organisms [2,3], cefotaxime sodium considers a broad spectrum of antibiotics and thus widely used to treat bacterial infections of the skin, soft tissues and the urinary tract. This drug is referred to as (lactam antibiotics), considered among the oldest and the most valuable clinical antimicrobial agents [4].

Cefotaxime sodium has positive and negative characteristics that are of considerable clinical and analytical interest. As positive features, it shows resistance to penicillinases and useful to treat infections which are resistant to penicillin derivatives, while as negative features several side effects includes diarrhea and when mixed with alcohol can cause stomach cramps, nausea, vomiting, fainting and headache [5].

Several analytical methods for cefotaxime sodium analysis *viz.* HPLC [6,7], LC-MS [8], HPTLC [9], electrochemical [10], chemiluminescence [11], cyclic voltammetry and adsorptive stripping differential pulse voltammetry [12], flow injection analysis [13] infrared spectroscopy [14], capillary zone electrophoresis [15], spectrophotometric-kinetic [16], turbidimetric-flow injection [17] and extraction-flotation technqiue [18] are reported in the literature. Several UV-visible spectrophotometric methods are also reported however, some of these procedures include diazotization of cefotaxime sodium and then coupling with different coupling agents such as thymol [13], β-naphthol [19], *etc*. Beside this, using the charge-transfer complex [20,21],

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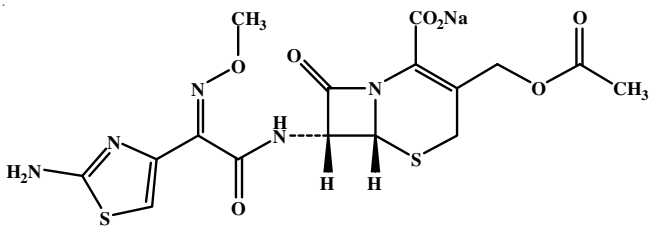


Fig. 1. Structure of sodium cefotaxime

oxidative coupling reaction reduction [22], prussian blue formation [23], ninhydrin reagent [24] and formation of ion-associated complex with Eriochrome black-T in acidic medium [25] are also reported.

However, majority of these analytical procedures are expensive, time-consuming, tedius solvent extraction process or difficult methods of fabrication and have poor sensitivity. The development of the spectrophotometric and RP-HPLC methods for the analytical determination of cefotaxime sodium in pure form and in its pharmaceutical preparations (injections) is considered the main contribution of this study. The diazotization reaction of cefotaxime sodium with $NaNO_2$ in an acidic medium was carried out and then diazonium compound formed was coupled with 8-hydroxyquinoline in the presence of alkaline solution to form a water-soluble azo dye, which is considered as a base of the spectrophotometric methods. This method does not requires the removal of excess $NaNO_2$ by using sulfamic acid since low concentration of $NaNO_2$ is utilized by adding an equimolar solution of cefotaxime sodium and $NaNO_2$.

EXPERIMENTAL

All absorbance measurements were carried out by a digital Cecil double beam UV-visible spectrophotometer (Japan) having 1 cm quartz cells. The pH scale measurements were made with a professional HANNA pH meter 212. Using RP-HPLC methods, the measurements were carried out by a high performance liquid chromatograph model LC -20AD, Shimadzu, with UV detector, SPD-20A. Separation was achieved using a C18 GL science column for the stationary phase (4.6 mm × 250 mm i.d., 3 µm particle size). Ultrasonic 5200 was also used.

All the experiments were performed with analytical grade chemicals and procured from Sigma-Aldrich.

Preparation of stock solutions

(A) For spectrophotometric method

Cefotaxime sodium solution: The standard drug cefotaxime sodium was provided by the State Company for Drug Industries and Medical Appliances (SDI), Sammara, Iraq. A stock solution of cefotaxime sodium (500 µg mL^{-1}) was prepared using double distilled water. Similarly, a working solution of cefotaxime sodium (200 µg mL^{-1} = 4.1894×10^{-4} M) was prepared by appropriate dilution of the stock solution with distilled water.

Sodium nitrite solution: A stock solution of $NaNO_2$ (2.971×10^{-3} M) was prepared by dissolving 0.0205 g of sodium nitrite in100 mL distilled water. Similarly, a working solution (4.1894×10^{-4} M) of $NaNO_2$ was then prepared by dissolving 14.1 mL of the stock solution with distilled water in a 100 volumetric flask.

8-Hydroxyquinoline (0.1%) solution: The solution was prepared by dissolving 0.1 g of 8-hydroxyquinoline reagent in 1 mL of acetic acid and the volume was made up to100 mL with distilled water.

(B) For RP-HPLC method

Phosphate buffer solution (pH 3.5): Potassium dihydrogen phosphate (3.242 g) was dissolved in 400 mL doubled distilled water. The flask was shaken until the salt dissolved. The pH of the solution was adjusted to pH 3.5 by adding 0.01M ortho-phosphoric acid (H_3PO_4) solution and then the solution was made up to 1000 mL with double distilled water.

Cefotaxime sodium (200 µg mL^{-1}) solution: The solution was prepared by dissolving 0.02 g of cefotaxime sodium in a 100 mL of mobile phase solution which consists of acetonitrile-phosphate buffer solution (pH 3.5) in the ratio of 60:40 (v:v) in a 100 mL volumetric flask. A working solution of cefotaxime sodium was prepared by diluting an appropriate volume of the stock solution with the same solvent.

Calibration curve for spectrophotometric method: An increasing volume 0.05-4.0 mL of a standard solution of cefotaxime sodium 200 µg mL^{-1} (4.1894×10^{-4} M) was transferred into a series of 25 mL calibrated flasks. To this solution an equimolar of $NaNO_2$ solution 4.1894×10^{-4} M was added and followed by 1 mL of 1M HCl solution and mixed thoroughly. After 4 min, a 1.5 mL of 0.1% (8-hydroxyquinoline) and 1.4 mL of 1M NaOH solutions were added. The contents are mixed well and diluted to the mark with distilled water. The flasks were kept at room temperature for 10 min and the absorbance of azo dye was measured at 551 nm *versus* the corresponding reagent blank.

A calibration curve was obtained over the concentration range 10-700 µg of cefotaxime sodium/25 mL. Higher concentrations show a negative deviation from Beer's law (Fig. 2).

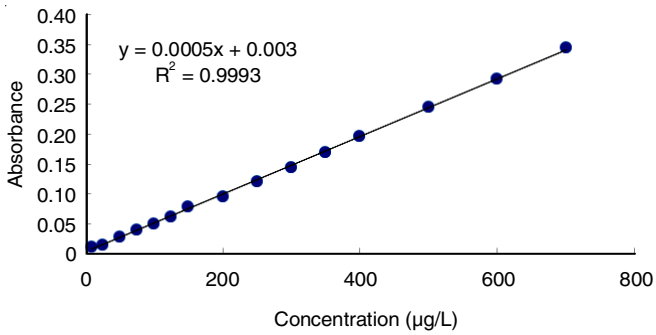


Fig. 2. Calibration curve for sodium cefotaxime determination

Assay of injection: Three types of injections were procured and analyzed by the developed methods. An accurately weighed portion from mixed three vials of powder, equivalent to 0.02 g of cefotaxime sodium was dissolved in distilled water and completed to the mark with the same solvent using a 100 calibrated flask. Aliquot samples were treated exactly as in the procedure described for the spectrophotometric method.

Calibration curve for HPLC method: All the solutions used in the HPLC method were degassed and filtered using a membrane filter. A 100 µL of cefotaxime sodium standard solution (100 µg mL^{-1}) in a concentration range between 1.0 to 300 µg mL^{-1} of cefotaxime sodium was injected under opti-

mum condition of analysis. Cefotaxime sodium was isocratically eluted using 1 mL min^{-1} as a flow rate of mobile phase and the chromatographic separation was performed at room temperature using C18 column as a stationary phase with UV detection at 258 nm. A linear calibration graph was obtained over the concentration range 1.0-200 µg of cefotaxime sodium/25 mL (Fig. 3).

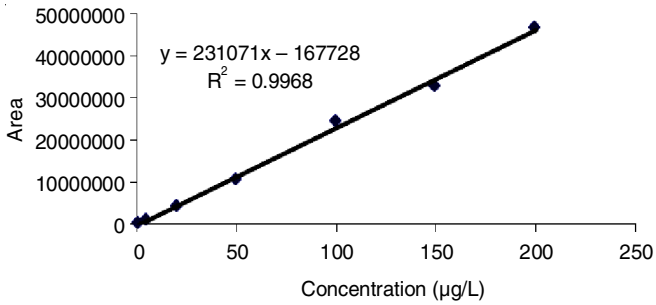


Fig. 3. Calibration graph of sodium cefotaxime by the HPLC method

Procedure for injection analysis: For all injection, an accurately weighed portion from mixed three vials of powder, equivalent to about 0.01 g of cefotaxime sodium was dissolved in the mobile phase (acetonitrile-phosphate buffer solution pH 3.5) sonicated for 10 min using a100 mL calibrated flask. Each mL of this solution contained 100 µg of cefotaxime sodium. Aliquot samples were treated exactly as performed in the recommended procedure.

RESULTS AND DISCUSSION

Spectrophotometric method: Under the reaction condition, cefotaxime sodium was treated with equimolar of $NaNO_2$ solution 4.1894×10^{-4} M in acidic solution, which undergoes diazotization reaction to give the diazonium compound. The diazonium compound was coupled with 8-hydroxyquinoline as a coupling agent in a basic medium of NaOH to form azo dye, which showed maximum absorption at 551 nm *versus* blank reagent (Fig. 4). The intensity of formed dye has been found to be proportional to the amount of cefotaxime sodium originally present in the solution.

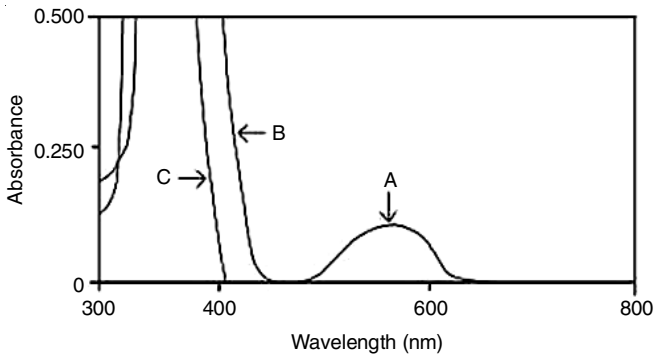


Fig. 4. Absorption spectra of (A) 8 µg mL^{-1} of sodium cefotaxime *versus* reagent blank, (B) 8 µg mL^{-1} of sodium cefotaxime *versus* distilled water, and (C) blank *versus* distilled water

The effect of various parameters on the absorption intensity of the formed product were optimized. The diazonium reaction occurred in an acidic medium. Therefore, the effect of various acidic solutions (1 M) such as HCl, HNO_3, H_2SO_4 and HCOOH have been investigated on the coloured product. The experimental results revealed that HCl was the most suitable acidic medium for a maximum sensitivity and stability. The effect of 0.5-5 mL of 1M HCl volumes was also studied and 1 mL with occasional shaking for 4 min was found to be the optimum.

Effects of 8-hydroxyquinoline amount: The effect of four different amounts *viz*. 1.0, 1.5, 2.0 and 3.0 mL of 0.1% 8-hydroxyquinoline on the absorbance of azo dye have been studied. The results in Fig. 5 indicate that 1.5 mL of 0.1% 8-hydroxyquinoline reagent show a high sensitivity and a good determination coefficient value ($R^2 = 0.9992$), therefore, it was recommended for the subsequent experiments.

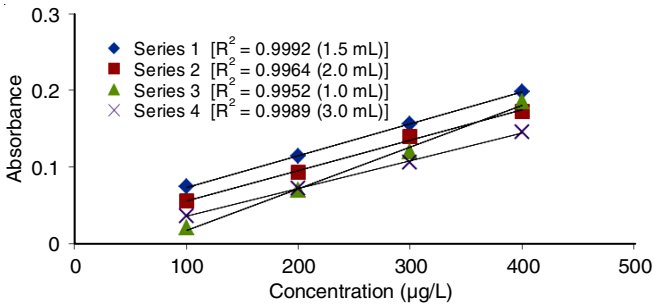


Fig. 5. Effects of 8-hydroxyquinoline amount on absorbance

An absorbance of azo dye formed became more intense and stable in an alkaline solution. Therefore, the effects of various alkaline solutions (1M) were investigated such as NaOH, KOH, Na_2CO_3 and NH_4OH. Maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of NaOH solution. While Na_2CO_3 and NH_4OH exhibited low sensitivity which is apparently due to pH variation. The effect of 1.0-3.0 mL of 1M NaOH was also studied and 1.4 mL was found to be optimum (Fig. 6).

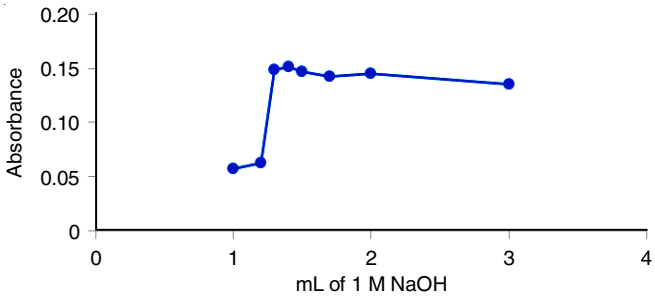


Fig. 6. Effect of NaOH amount on absorbance

Experimental results revealed that the colour intensity reached a maximum after cefotaxime sodium had reacted with 8-hydroxyquinoline in alkaline NaOH for 10 min. Therefore, 10 min development time was suggested as the optimum reaction time and remain stable for at least 90 min at room temperature.

Quantification: The limits of Beer's law, molar absorptivity (λ_{max}), accuracy (recovery %), precision (RSD, LOD and LOQ) values were calculated and are shown in Table-1, which indicates that the proposed method is sensitive, precise and accurate. Linearity was represented by the regression equation and the corresponding determination coefficient (R^2) for cefot-

TABLE-1
SUMMARY OF OPTICAL CHARACTERISTICS AND STATISTICAL DATA FOR THE PROPOSED METHOD

Parameter	Values of method
Beer's law limits (ppm)	0.4-28
Molar absorptivity (L mol^{-1} cm^{-1})	0.597×10^4
LOD (μg mL^{-1})	0.0194
LOQ (μg mL^{-1})	0.3765
Regression equation	Y = 0.0005x – 0.003
Determination coefficient (R^2)	0.9993
Slope	0.0005
Intercept, b	0.003
Range of recovery* (%)	97.3-102.5%
Range of RSD* (%)	Better than ± 1.56

Average of five determinations.

axime sodium determined by the proposed method represents the excellent linearity.

Stoichiometry: The stoichiometry of the product was investigated using the mole ratio and continuous variation methods. In continuous variation method, 0.5-4.0 mL (4.1894 × 10^{-4} M) portions of cefotaxime sodium (assigned as VS) were diazotized using equimolar of 4.1894 × 10^{-4} M $NaNO_2$ and 2 mL of 1M HCl and reacted according to analytical procedure with the corresponding complementary volume of 4.1894 × 10^{-4} M 8-hydroxyquinoline solution (assigned as VR) to give a total volume of 5 mL for VS + VR in 1.4 mL of 1M NaOH solution and completed to 25 mL with distilled water. In a mole ratio method, 0.3-3.0 mL of 4.1894 × 10^{-4} M 8-hydroxyquinoline solution (VR) were added to a 1 mL of 4.1894 × 10^{-4} M of cefotaxime sodium (VS) which was diazotized by using 2 mL of 4.1894 × 10^{-4} M $NaNO_2$ solution in the presence of 2 mL of 1M HCl solution, 1.4 mL of 1M NaOH solution was added and the absorbances were measured at 551 nm after dilution to the mark with double distilled water. The results (Figs. 7 and 8) show that the ratio of cefotaxime sodium and 8-hydroxyquinoline is 1:1.

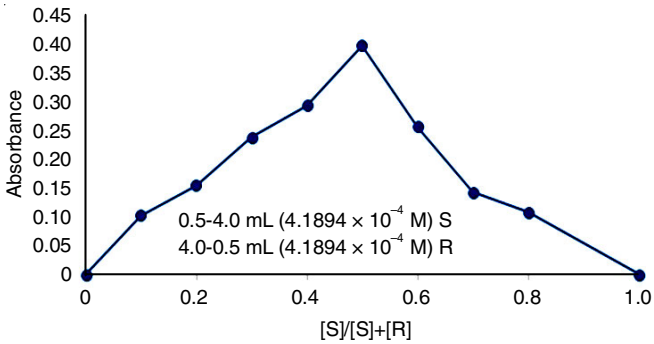


Fig. 7. Continuous variation plot

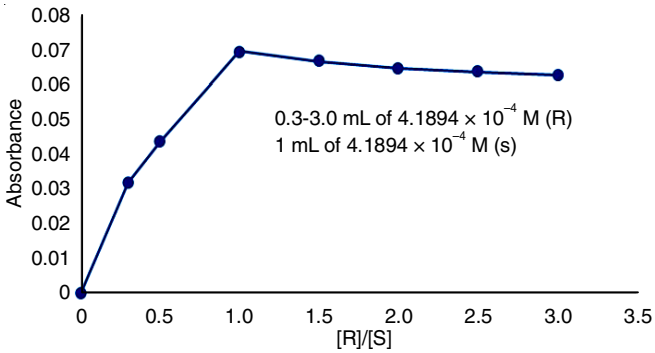
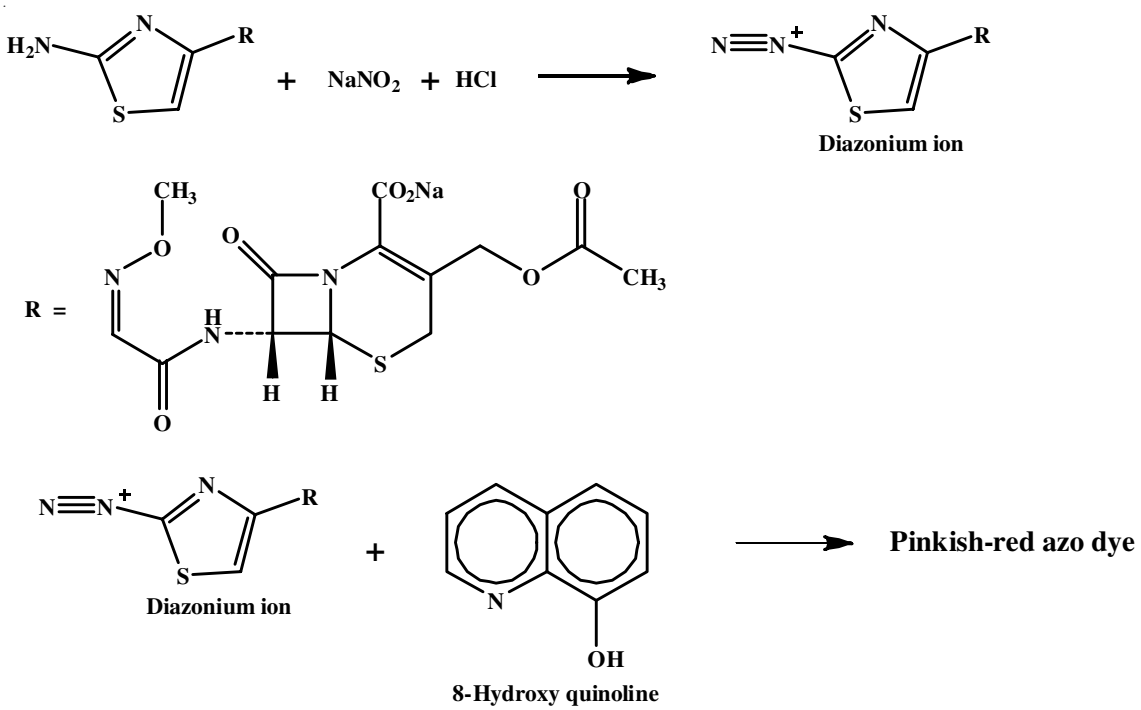


Fig. 8. Mole ratio plot

The subsequent reactions according to the above results is shown in **Scheme-I**.

Applications: The proposed method was applied to assay three different injections containing cefotaxime sodium. The results in Table-2 indicate that the method is satisfactory.



Scheme-I

TABLE-2
ANALYSIS OF CEFOTAXIME SODIUM (CTXNa) IN INJECTIONS

Pharmaceutical injection	Conc. of CTXNa		Relative error* (%)	Recovery* (%)	RSD*
	Taken (µg)	Found (µg)			
Cefotaxime (1g of ÇTXNa), LDP Laboratorios TORLAN, Barcelona, Spain	100	97.3	-2.7	97.3	± 1.21
	250	251.1	0.4	100.4	± 0.61
	500	506.8	1.3	101.2	± 0.35
Foxime (1g ÇTXNa) Tabuk Pharmaceutical, (Tabuk, Saudi Arabia)	100	102.5	2. 5	102.5	± 1.56
	250	246.0	-1.6	98.4	± 0.45
	500	508.7	1.74	101.7	± 0.21
Belaksim (1g ÇTXNa) Mustaea Nevzat Pharmaceuticals Co. Istanbul. (Turkish)	100	97.9	-2.1	97.9	± 1.32
	250	244.2	-2.32	97.7	± 1.01
	500	497.9	-0.42	99.6	± 0.48

*Average of three determinations

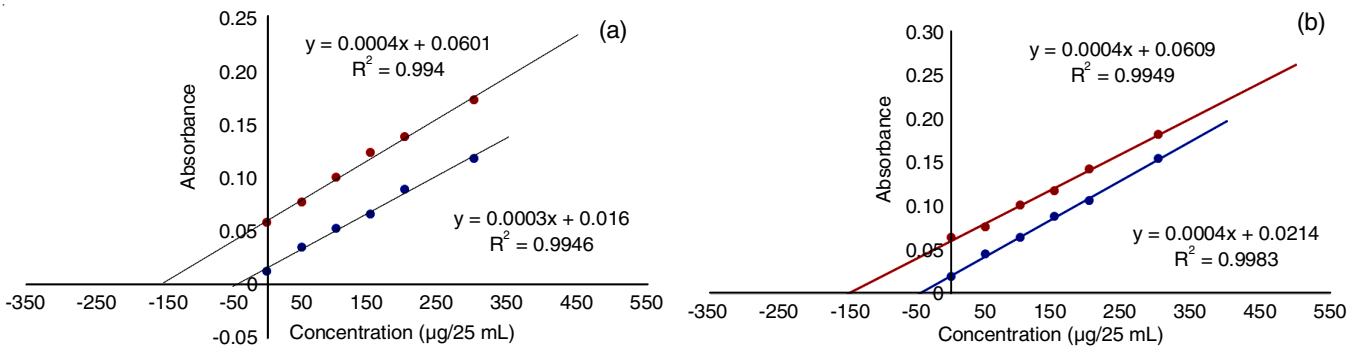


Fig. 9. Graphs of standard addition method for determination of sodium cefotaxime in (a) cefotaxime and (b) Belaksim injections

Evaluation of proposed method: The standard additions method was followed to check the validity of the proposed method, which proved that the proposed method was applied successfully for the determination of cefotaxime sodium (Fig. 9 and Table-3).

RP-HPLC method

Selection of analytical wavelength: An absorption spectrum of 100 µg mL^{-1} of cefotaxime sodium prepared in various dissolution solvents as a mobile phase has been taken. Fig. 10 shows that cefotaxime sodium exhibited a maximum absorption intensity at 258 ± 2 nm *versus* its dissolution solvent. Therefore, 258 nm has been used throughout for UV detection.

Selection of type and composition of mobile phase: The effects of various polar solvents with different compositions have been used as a mobile phase on the shape of chromatogram. The sample was isocratically eluted with 1.0 mL min^{-1} as a flow rate of mobile phase to construct the optimum composition. Chromatograms were followed at room temperature and the results are shown in Table-4, which indicated that acetonitrile: phosphate buffer solution at pH 5.2 (50:50) (v:v) is the best mobile phase with UV detection at 258 nm.

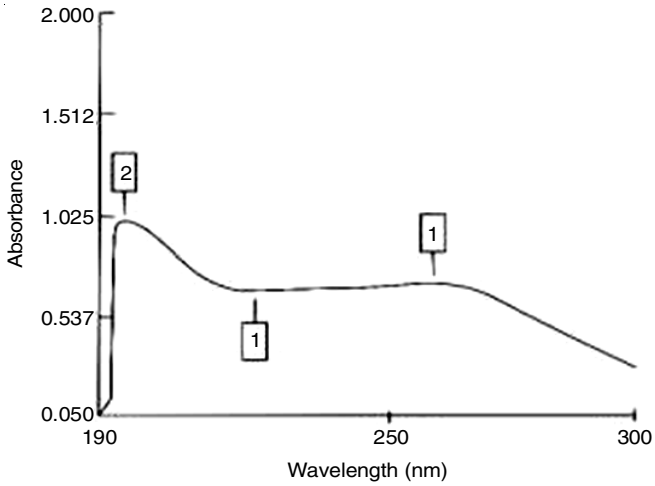


Fig. 10. UV-Absorption spectrum of sodium cefotaxime

The effect of different volumes ratio of acetonitrile:phosphate buffer solution (pH 5.2) as mobile phase was also studied. The results in Table-5 show that acetonitrile and phosphate buffer solution of the ratio 60:40 (v:v) at pH 5.2 is the best and most useful mobile phase as indicated by the resolution obtained.

TABLE-3
ANALYSIS OF CEFOTAXIME SODIUM (CTXNa) IN INJECTIONS BY USING STANDARD ADDITION METHOD

Formulation injection	Concentration of cefotaxime sodium		Recovery (%)
	Taken (µg/25 mL)	Found (µg/25 mL)	
Cefotaxime (1 g CTXNa) LDP laboratories Torlan, S.A., Ctra. De Barcelona, 135-B 08290 Cerdanyola del Valles Barcelona, Spain	50	53.33	106.7
	150	150.2	100.1
Belaksim (1 g, CTXNa) Mustaea Navzat (Turkish)	50	52.50	105.0
	150	154.3	102.9

TABLE-4
SELECTION OF MOBILE PHASE AND ITS COMPOSITION

Mobile phase	Ratio	λ_{max}	Retention time (min)	Notes
Methanol	100%	–	–	Slightly soluble
Acetonitrile:H_2O	50:50	260	2.6	Unclear peak
Methanol:H_2O	50:50	235	–	No peak
Acetonitrile:Acetic acid:H_2O	36:1: 63	237	14.4	Unclear peak
Acetonitrile:Phosphate buffer solution (pH 5.2)	50:50	258	3.97	Sharp peak
Acetonitrile:Ammonium acetate buffer (pH 3.4)	50:50	237	–	No peak

TABLE-5
SELECTION OF THE VOLUME RATIO OF MOBILE PHASE

Acetonitrile:Phosphate buffer solution ratio at pH 5.2	Retention time (min)	k'
30:70	4.100	1.19
40:60	3.767	0.87
50:50	3.804	0.82
60:40	3.942	0.95
70:30	3.544	0.91

Therefore, the conditions reported in Fig. 11 were recommended because it gave an ideal capacity factor (k′ = 0.95) (accepted range 0.5 < k′ < 20) [26], therefore, it was selected.

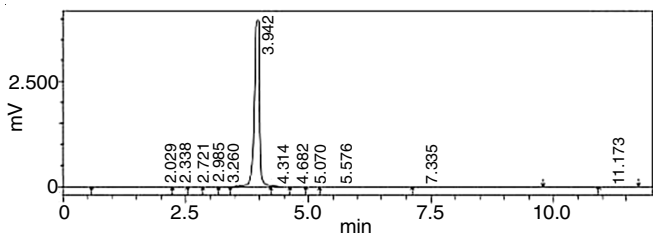


Fig. 11. Chromatogram of sodium cefotaxime using acetonitrile:phosphate buffer solution pH 5.2 (60:40) (v:v) as a mobile phase

Effect of flow rate: The effect of 0.5-1.3 mL min^{-1} as flow rate on the peak shape and retention time and capacity factor has been studied. It was found that 1 mL min^{-1} gave the optimum capacity factor (k′ = 0.85) with clear chromatography and good sharpness (Fig. 12).

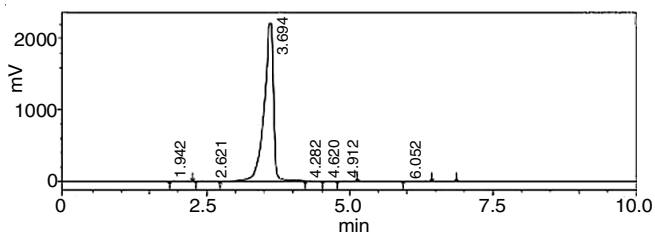


Fig. 12. Chromatogram of sodium cefotaxime using 1 min mL^{-1} as flow rate

Effect of pH of buffer solution: A different pH range (2.5-4.0) of phosphate buffer solutions were prepared and mixed with acetonitrile as a mobile phase using the following ratio acetonitrile: phosphate buffer solution (60:40) (v:v). The data in Table-6 indicates that phosphate buffer solution at pH 3.5 gives the best retention time (t_R = 3.275 min) together with the best capacity factor (k′ = 0.78) (Fig. 13).

TABLE-6
SELECTION OF THE pH OF PHOSPHATE BUFFER SOLUTION

pH of phosphate buffer solution	Retention time (min)	k'
2,50	3.279	0.76
3.06	2.950	0.63
3.30	3.295	0.70
3.50	3.275	0.78
4.00	2.970	0.65

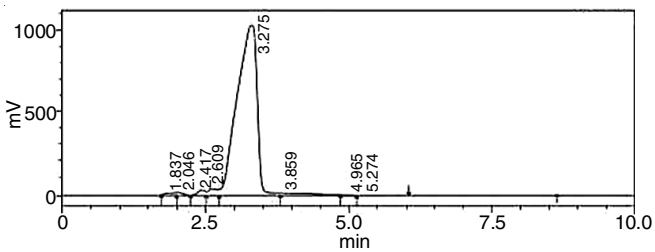


Fig. 13. Chromatogram of sodium cefotaxime using phosphate buffer solution at pH 3.5 with acetonitrile as mobile phase

Applications: A 100 µL of sample solution was injected under optimum analysis conditions and the results are shown in Table-7, which validates the applicability of the proposed method.

Conclusion

A simple and sensitive spectrophotometric and RP-HPLC methods to assay cefotaxime sodium in pharmaceutical injections are developed. Both methods have an advantage of being accurate, did not require organic solvents, any chemical sample pretreatment, temperature control, expensive reagents and solvent extraction step. HPLC method has been validated for their sensitivity, specificity and linearity. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respe-

TABLE-7
ANALYSIS OF CEFOTAXIME SODIUM (CTXNa) IN PHARMACEUTICAL FORMULATION

Pharmaceutical injection	Concentration of CTXNa (µg/25 mL)		Recovery* (%)	RSD*
	Taken	Found		
Belaksim (1g CTXNa) Mustaea Nevzat	50	52.4	104.8	± 0.031
Pharmaceuticals	100	96.4	96.4	± 0.021
Co. Istanbul. (Turkey)	150	154.5	103.0	± 0.017

*Average of five determinations.

ctive label claims and therefore both methods are recommended for routine and quality control analysis of cefotaxime sodium.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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