ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The development and validation of RP-HPLC assay for cefotaxime sodium.

Alfan Danny Arbianto[1*], Maya Damayanti Rahayu[2] and Susi Kusumaningrum[3]

[1,2,3]Laboratory of Modelling and synthetic for drug discovery, Center for Pharmaceutical and Medicinal Technology, BPPT

ABSTRACT

An analytic high-pressure liquid chromatographic (HPLC) procedure for assay of cefotaxime sodium has been developed and validated. However, a procedure, which is simple and accurate, required to be developed to be easily employed for quality control. This project aimed to develop and validate a rapid method to analyse cefotaxime sodium using RP-HPLC. A simple method was successfully developed. The method was carried out on a 5-µm particle octadesyl silane (ODS) column (150 × 4.6 mm) with Methanol: 0.1% TFA as a mobile phase with flow rate of 1 mL/min. In addition, quantification analysis was done at 254 nm using PDA detector. The correlation coefficient (r^2) of this method was 0.995 over the concentration ranging from 6.25 to 200 µg/mL of cefotaxime sodium. This method then was validated for linearity, accuracy, and precision. The limit of detection and quantification were 7.70 and 25.66 µg/mL, respectively. In conclusion, the proposed method had shown to be simple, precise, suitable, and accurate for quantification of cefotaxime sodium as an alternative to the existing methods for the routine analysis of cefotaxime sodium and this method is sensitive enough for analysis cefotaxime sodium during the synthesis process.

Keywords: RP-HPLC, cefotaxime sodium, antibiotics

***Corresponding author**

ISSN: 0975-8585

INTRODUCTION

Cephalosporins, β-lactam antibiotics are widely used as antibiotics. It has been highlighted as an anti-bactericidal, which its mechanism of action is closely related to penicillin and cephamicin that are also β-lactam antibiotics. Cephalosporins are very unique because its main nucleus 7-amino cephalosporanic acid (7-ACA) is a cephem derivative. In the modern era, the used cephalosporins for therapeutic purposes are semisynthetic products [1]. The first cephalosporin of the third generation developed was cefotaxime (7b-(2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino acetamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid or its sodium salt) (structure presented in Figure 1). This antibiotic displays a high antimicrobial potency, a broad antibacterial spectrum, high resistance against the action of b-lactamases, as well as a low index of side effects [2]. For these reasons, it has been largely used in the treatment of several infections including, meningitis, septicemia, peritonitis, infections of the genito-urinary and breathing tracts, infections of the skin, bones, articulations, and many other infectious treatments [3]. Moreover, cefotaxime sodium can be used as the main intermediary in the synthesis of cefpodoxime proxetil, a third generation cephalosporin for oral administration, which is recently introduced into the medical practice [4, 5].

A wide variety of analytical methods has been reported for the determination of cephalosporins in pure form, pharmaceutical preparations and biological fluids. These excisting methods include the use of spectrophotometry [6-9], fluorometry [10, 11], liquid chromatography [11-14], capillary electrophoresis [15, 16], chemiluminescence [17, 18], voltammetric [19-22] and polarographic [23]. The existing method for cefotaxime sodium analysis that published by United State Pharmacopeia and Europe Pharmacopeia are a gradient of phosphate buffer and methanol for 60 minutes with flow rate 1 ml/min. The purpose of this paper, therefore, was to develop and validate RP-HPLC with isocratic system with a short run time that could be applied for the quantification of cefotaxime sodium during synthesis process.

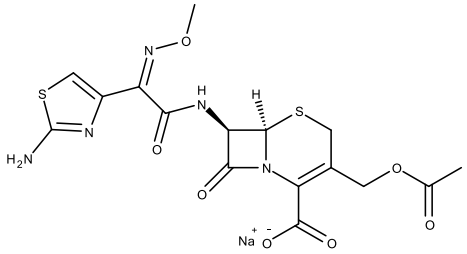

Figure 1: The structure of cefotaxime sodium

MATERIALS AND METHOD

Cefotaxime sodium sample was produced by Modelling and Synthesis Laboratories, BPPT. The comparator (cefotaxime sodium) was produced Hefei JOYE Import and Export Co.,Ltd whereas the standard of cefotaxime sodium was procured from European Pharmacopeia. Methanol (HPLC grade) and Trifluoroacetic acid (AR grade) were procured from E. Merck Ltd. Purified HPLC grade water was obtained by reverse osmosis and filtration through a 0.45-µm membrane filter; all used solutions were prepared using applied purification method.

HPLC Instrumentation And Chromatographic Conditions:

The HPLC analysis was accomplished using KNAUER®– ASI – 1998 – 2005. The used column was Inertsil ODS-3, C8 (150 × 4.6 mm) packed with 5 µm particles. The injection volume, Twenty µL of sample, was applied for all experiments in a gradient mobile phase containing methanol and 0.1% TFA (35 : 65) that pumped through the column with a flow rate of 1 mL/min. Furthermore, quantification was calculated at 254 nm using PDA detector. Lastly, before employed to the machine, the mobile phase was filtered through a 0.45-µm membrane filter and degassed. The optimized chromatographic method was completely validated according to the procedure described in ICH guidelines Q2 (R1) for the validation of analytical methods.

ISSN: 0975-8585

Table 1 Optimized chromatographic conditions

Stationary phase (column)	**Inertsil ODS-3 C-8, (150 x 4.6 mm) packed with 5µm particles**
Mobile phase	Methanol : 0.1% TFA
Detection wavelength (nm)	254
Run time (min)	7 minutes
Flow rate (mL/min)	1
Volume of injection loop (µl)	20
Column temperature	25°C
Cefotaxime sodium Rt (min)	4.65

Preparation of stock and standard solutions:

A stock solution of 1000 µg/mL was prepared by transferring 23.87 mg of cefotaxime sodium into a 20-mL volumetric flask. Then, 15 mL of methanol was added and mixtured with sonicator to dissolve. The final volume of the solution was made up with HPLC grade methanol. The standard stock solutions of cefotaxime sodium were transferred using a grade bulb pipettes into 5-mL volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations in the range of 6.25, 12.50, 25.0, 50.0, 100 and 200 µg/mL.

Linearity and range:

Standard stock solution was diluted to prepare solutions containing 1.56 to 200 µg/mL of the cefotaxime sodium. The solutions were injected in triplicate into the HPLC column, retaining the injection volume constant (20 µL).

System suitability:

Twenty microliters of the standard solution (200 µg/mL) was injected six times under optimized chromatographic conditions to evaluate the system suitability.

Precision:

Three injections of three different concentrations (50, 100 and 200 µg/mL) were given on the same day and the values of percent relative standard deviation (%RSD) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

Accuracy:

The accuracy of an analytical method is the closeness of the obtained test results to the true value. Accuracy may frequently be expressed as percent recovery by the assay of known, added amounts of analyte. The accuracy of assay method of cefotaxime sodium was determined by analyzing the samples to cover both above and below the expected normal levels in samples. The recovery should be in the range of 98.0% to 102.0%.

$$Recovery = \frac{Analytical\ result}{True\ value} \times 100\%$$

Specificity:

Specificity study of analytical method checks the positive or negative interference due to diluents on final results of the analytical method. Specificity study of the cefotaxime sodium assay was established by analysing the diluents in duplicate as per method is calculating the same interference.

ISSN: 0975-8585

Limit of detection and quantification:

The limit of detection (LOD) is the lowest amount of analyte can be detected in a sample, but not necessarily quantified, under the stated experimental conditions. The limit of quantification (LOQ) was identified as the lowest concentration of the standard curve that could be quantified with acceptable accuracy, precision, and variability. Both limits are determined by the signal to noise method.

Assay:

Cefotaxime sodium samples were accurately weighed and transferred to a 20-mL volumetric flask containing 15.0 mL of methanol. The mixture was sonicated to dissolve, made up the volume with methanol 5 mL and filtered through a 0.45-µm membrane filter. Filtered solution were transferred using A-grade bulb pipettes into 5-mL volumetric flasks, and the solutions were made up to volume with mobile phase to achieve final concentration of 200 µg/mL. The above mentioned solution was, then, analyzed for the content of cefotaxime sodium using the proposed method.

RESULTS AND DISCUSSION

Method development:

Development of new HPLC methods are often useful in regular quality control assessment of pharmaceuticals, which may convey relevant information in establishing optimal experimental conditions for better usage of drugs. In this study, a simple, specific, selective, and accurate RP-HPLC method to quantify cefotaxime sodium was developed and validated according to ICH guidelines. Methanol and 0.1% of trifluoroacetic acid in different proportions were optimised, and finally, a ratio of methanol - 0.1% of trifluoroacetic acid (35:65) - was selected as an appropriate combination, which resulted in better resolution and acceptable system suitability parameters. The working standard chromatogramof cefotaxime sodium solution was shown in Figure 2. Optimized chromatographic conditions were given in Table 1.

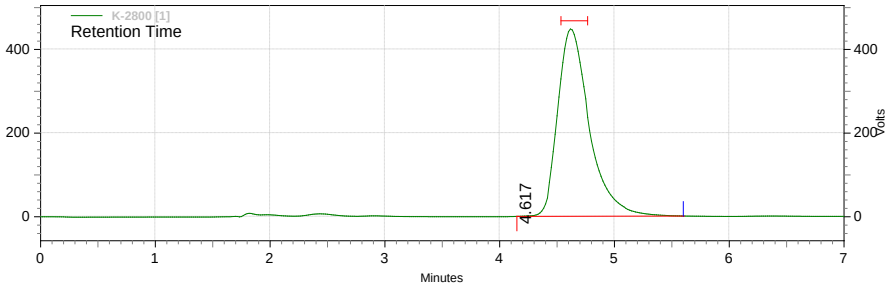

Figure 2: Typical chromatogram of cefotaxime sodium standard

Linearity:

The required test samples were prepared freshly using the stock solution ranging from 6.25 to 200 µg/mL (cefotaxime sodium). Triplicate of 20-µL injections were made for each concentration andanalyzed under the optimized conditions of chromatographic. A calibration curve was obtained by plotting the response (peak area) versus concentration of drug (Figure 3). In addition, table 2 shows linearity parameter for cefotaxime sodium.

ISSN: 0975-8585

Table 2: Linearity parameter for cefotaxime sodium

Conc. (µg/mL)	Area
6.25	271595
12.50	554442
25.00	1136011
50.00	2322253
100.00	4830991
200.00	8462574

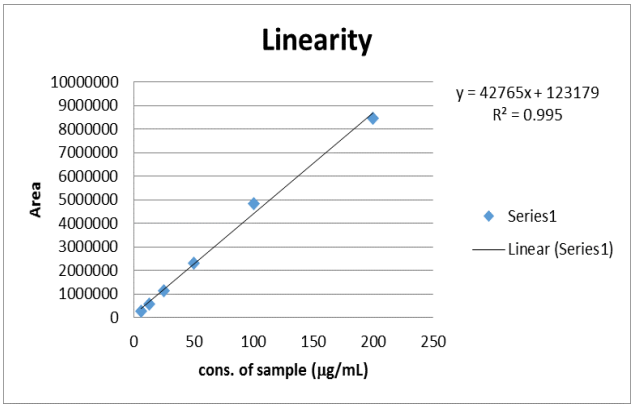

Figure 3: Standard graph of cefotaxime sodium in mobile phase

System suitability:

System suitability tests were accomplished on freshly prepared standard stock solutions of cefotaxime sodium and it was calculated by determining the standard deviation of cefotaxime sodium standards by injecting in six replicates at short time intervals and the peak areas were recorded and represented in Table 3.

Table 3: System suitability parameters

Concentration	Injection	Area	R_t (min)
200 mg/mL	Inj-1	10470789	4.72
	Inj-2	10560651	4.70
	Inj-3	10485459	4.72
	Inj-4	10391313	4.70
	Inj-5	10364407	4.70
	Inj-6	10537423	4.70
	Mean	10468340.33	4.71
	SD	77882.05	0.01
Statistic analysis	%RSD	0.74	0.22
	Tailing factor	1.47	
	Plate count	1002.03	

Precision:

The precision of the method was demonstrated by inter-day and intra-day variation. In the intra-day studies, standard solutions and the comparator were repeated twice on a day, and %RSD for the response factor was calculated (Table 4). The %RSD values in the two cases were < 2%, which indicate the method was sufficiently precise.

ISSN: 0975-8585

Table 4: Reproducibility and precision data evaluated through intra-day and inter-day studies

Conc. (µg/mL)	Intra-day (*n = 3*)		Inter-day (*n = 3*)	
	Mean peak area ± SD (*n = 3*)	%RSD	Mean peak area ± SD (*n = 3*)	%RSD
50	3439244 ± 41699	1.21	3521032 ± 49530	1.41
100	5758075 ± 91494	1.59	5864111 ± 48537	0.83
200	10407596 ± 151826	1.46	10463800 ± 111164	1.06

Accuracy:

The accuracy of the method was determined by recovery experiments. The recovery studies were performed using the comparator. The comparator was accurately weighed at different concentration levels (100 and 200 µg/mL). To calculate the percent recovery was calculated by comparing the area standard and comparator. The recovery studies were performed in triplicate. The method resulted percent recovery within the range of 98% to 102% (Table 5) which indicates an accuracy.

Table 5: Recovery studies

Actual conc. (µg/mL)	Calculated conc. (mg/mL) + SD (n=3)	%RSD	%Recovery
100	98 ± 0.37	0.39	98
200	197 ± 1.05	0.53	98

Specificity and selectivity:

Specificity was tested against standard compounds. A possible interference peak in the presence of diluents under optimized test conditions was also studied. There was no observed interference from the diluents at the retention time of cefotaxime sodium (Figure 4 (A) and (B)). Therefore, it was concluded that the method is very specific and can assess unequivocally interested analyte in the presence of possible interferences.

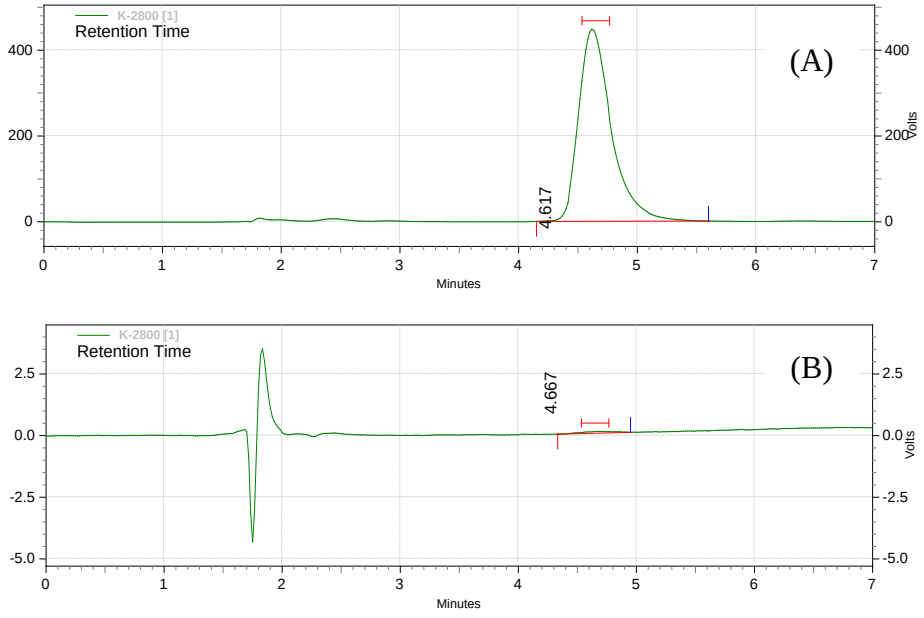

Figure 4: chromatogram of cefotaxime sodium comparator (A), chromatogram of diluents (B)

Limit of detection and limit of quantification:

Standard stock solutions of cefotaxime sodium (6.25, 12.50, 25, 50, 100, and 200 µg/mL were prepared) by diluting the standard stock solution (1000 µg/ml) with mobile phase. The LODs and LOQs of cefotaxime sodium under the present chromatographic conditions were estimated at a signal-to-noise ratio

(S/N) of 3:1 and 10:1, respectively, by injecting a series of diluted solutions with known concentrations. The LOD and LOQ for cefotaxime sodium was 7.70 and 25.66 µg/mL, respectively.

Robustness:

Robustness of the method was checked by making small changes in the chromatographic conditions such as column temperature and flow rate. The observed results found no noticeable changes in chromatograms, which demonstrated a robust method of developed RP-HPLC (Table 6).

Table 6: Robustness study

System suitability parameters (variation)		% RSD peak area (n=6)	Mean tailing factor	Mean Rt (min) (n=6)
Varied column temperature	25°C	0.74	1.42	4.71
	40°C	1.62	1.46	4.35
Flow rate	1.0 mL/min	1.05	1.44	4.67
	0.8 mL/min	0.81	1.44	5.64

Assay of sample:

The results of the assay as described earlier showed better conformity between comparator and our product (batch.230816) (Table 7).

Table 7: Assay of sample

Specification	Batch No.	%Assay
Not less than 96%	Comparator	98
	Batch.230816	102

CONCLUSION

The proposed method was rapid, accurate, precise, reproducible and sensitive for cefotaxime sodium quantification as a raw material for pharmaceutical dosage forms. The method uses a simple working procedure; hence, this method can be routinely employed in quality control for cefotaxime sodium analysis in synthesis process.

REFERENCES

[1] Boscha, M.E., et al., *Recent developments in the analytical determination of cefadroxil.* 亚洲药物制剂科学, 2008. 3: p. 217-232.

[2] Dürckheimer, W., et al., *Recent Developments in the Field of ß-Lactam Antibiotics.* Angewandte Chemie International Edition in English, 1985. 24(3): p. 180-202.

[3] Rodríguez, J.C., et al., *A rapid procedure to prepare cefotaxime.* Il Farmaco, 2000. 55(5): p. 393-396.

[4] Fujimoto, K., et al., *Studies on orally active cephalosporin esters.* J Antibiot (Tokyo), 1987. 40(3): p. 370-84.

[5] Todd, W.M., *Cefpodoxime proxetil: a comprehensive review.* Int J Antimicrob Agents, 1994. 4(1): p. 37-62.

[6] ALOthman, Z.A. and M.A. Abdalla, *Oxidative coupling for the spectrophotometric determination of certain cephalosporins and acetaminophen in drug formulations.* Arabian Journal of Chemistry, 2011. 4(2): p. 239-242.

[7] Ayad, M.M., et al., *Spectrophotometric determination of certain cephalosporins through oxidation with cerium(IV) and 1-chlorobenzotriazole.* J Pharm Biomed Anal, 1999. 20(3): p. 557-64.

[8] Morelli, B., *Derivative spectrophotometry in the analysis of mixtures of cefotaxime sodium and cefadroxil monohydrate.* J Pharm Biomed Anal, 2003. 32(2): p. 257-67.

[9] Omar, M.A., O.H. Abdelmageed, and T.Z. Attia, *Kinetic spectrophotometric determination of certain cephalosporins in pharmaceutical formulations.* Int J Anal Chem, 2009. 2009: p. 596379.

[10] Omar, M.A., O.H. Abdelmageed, and T.Z. Attia, *Kinetic spectrofluorimetric determination of certain cephalosporins in human plasma.* Talanta, 2009. 77(4): p. 1394-404.
[11] Shah, J., et al., *Spectrofluorimetric method for determination and validation of cefixime in pharmaceutical preparations through derivatization with 2-cyanoacetamide.* J Fluoresc, 2011. 21(2): p. 579-85.
[12] Can, N.O., G. Altiokka, and H.Y. Aboul-Enein, *Determination of cefuroxime axetil in tablets and biological fluids using liquid chromatography and flow injection analysis.* Anal Chim Acta, 2006. 576(2): p. 246-52.
[13] Denooz, R. and C. Charlier, *Simultaneous determination of five beta-lactam antibiotics (cefepim, ceftazidim, cefuroxim, meropenem and piperacillin) in human plasma by high-performance liquid chromatography with ultraviolet detection.* J Chromatogr B Analyt Technol Biomed Life Sci, 2008. 864(1-2): p. 161-7.
[14] Lalitha, N. and P.N.S. Pai, *Development and validation of RP-HPLC method for estimation of Cefotaxime sodium in marketed formulations.* Journal of Basic and Clinical Pharmacy, 2009. 1(1): p. 26-28.
[15] Andrási, M., A. Gáspár, and A. Klekner, *Analysis of cephalosporins in bronchial secretions by capillary electrophoresis after simple pretreatment.* Journal of Chromatography B, 2007. 846(1): p. 355-358.
[16] Gáspár, A., M. Andrasi, and S. Kardos, *Application of capillary zone electrophoresis to the analysis and to a stability study of cephalosporins.* Journal of Chromatography B, 2002. 775(2): p. 239-246.
[17] Chen, D., et al., *Chemiluminescence determination of cefotaxime sodium with flow-injection analysis of cerium (IV)–rhodamine 6G system and its application to the binding study of cefotaxime sodium to protein with on-line microdialysis sampling.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2011. 78(1): p. 553-557.
[18] Mervartová, K., M. Polášek, and J.M. Calatayud, *Recent applications of flow-injection and sequential-injection analysis techniques to chemiluminescence determination of pharmaceuticals.* Journal of Pharmaceutical and Biomedical Analysis, 2007. 45(3): p. 367-381.
[19] Aleksić, M.M. and V. Kapetanović, *Voltammetric behavior and square-wave voltammetric determination of cefotaxime in urine.* Journal of Electroanalytical Chemistry, 2006. 593(1): p. 258-266.
[20] Al-ghamdi, A.H., M.A. Al-shadokhy, and A.A. Al-warthan, *Electrochemical determination of Cephalothin antibiotic by adsorptive stripping voltammetric technique.* Journal of pharmaceutical and biomedical analysis, 2004. 35(5): p. 1001-1009.
[21] Jain, R., et al., *Voltammetric determination of cefixime in pharmaceuticals and biological fluids.* Analytical biochemistry, 2010. 407(1): p. 79-88.
[22] Nigam, P., et al., *Trace analysis of cefotaxime at carbon paste electrode modified with novel Schiff base Zn (II) complex.* Talanta, 2009. 77(4): p. 1426-1431.
[23] Xu, M., H. Ma, and J. Song, *Polarographic behavior of cephalexin and its determination in pharmaceuticals and human serum.* Journal of pharmaceutical and biomedical analysis, 2004. 35(5): p. 1075-1081.