Original Article

Validation Of Novel Hplc Method For Quantitative Determination Of Cefotaxime Sodium In Nanostructure Lipid Carriers

Rajshri Nema[1]*

*[1]Government College, Khurai, Madhya Pradesh, 470117

***Corresponding Author: -** Rajshri Nema

*Government College, Khurai, Madhya Pradesh, 470117, E-Mail: Saurabhnema06@gmail.com
DOI: 10.47750/pnr.2022.13.S10.411

Abstract

Cefotaxime (CFT) is a member of the third generation of the Cephalosporin antibiotics. It used on a wide scale in prescribed antibiotic drugs as anti-infection for gram-positive microorganisms and gram-negative microorganisms. CFT was determined quantitatively in a nanoformulation using the proposed HPLC method. Effective elution was achieved by using a stationary phase of Agilent (Zorbax) C_{18} column (250 mm × 4.6 mm i.d., 5.0µm) and a mobile phase composed of phosphate buffer (pH7.4): methanol (70:30v/v) at a flow rate of 1.2 ml/min. The samples were measured at 234nm using UV detector. The column temperature was kept at 25°C; the run time was 7 min; the injection volume was 20µl. CFT was separated within 6.245±0.04min. The correlation coefficient for the obtained calibration curve was found to be 0.999. The method was validated according to ICH Q2 (R1) guidelines for linearity, sensitivity, robustness, accuracy and precision. The LOD and LOQ were found to be 0.100 and 0.314µg/ml, respectively. The developed and validated method was successfully applied for the quantitative analysis of nanostructure lipid carriers (NLCs). The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of CFT in respective dosage form.

Keywords: RP-HPLC Method, Cefotaxime, Validation, Nanoformulation, Recovery.

INTRODUCTION

Analysis is important in every product but it is vital in medicines as it involves life. The assurance of quality is achieved through analysis of drug product [1]. Now days the drug are very much useful as a antiretroviral, it is mainly used in single therapies, rather than drug used in multiple formulation because of multiple action side effects [2]. Quantification of drug molecule is important task for routine analysis of API in its finished product analysis [3]. Infectious ailments have always been twisted the threat to humanoid and faunas [4]. Therefore, the healing is compulsory using suitable antimicrobial mediators. Even though innumerable antibiotics have been established but cephalosporin group of antibiotics are broadly rummage-sale [5]. CFT is chemically a sodium 7-[2-(2-amino-4-thiazolyl)-2- methoxyimino acetamido]-3-acetoxymethyl-3-cephem-4-carboxylate (Figure 1) with molecular formula $C_{16}H_{16}N_5\,NaO_7\,S_2$ and it is a third-generation cephalosporin antibiotic [6]. It Impedes bacterial cell wall blend by binding to one or more of the penicillin- binding proteins (PBPs), which in turn constrains the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls; thus, inhibiting cell wall biosynthesis[7]. CFT indicated for the treatment of patients infected with Septicaemia (Bacterial infection of blood), Bacterial Meningitis, Bacterial Endocarditis, Bacterial infections of lungs and respiratory tract, Bacterial infections of bones and joints [8]. CFT has been quantitatively analyzed in bulk material and different pharmaceutical dosage forms by infrared spectroscopy [9], spectrophotometric determination [10-14], Voltammetric determination [15], HPLC-MS [16]. Mass spectrometric methods may have the highest sensitivity, but the determination process is complicated to use and very expensive. Chromatographic separation technique is one of the most convenient, essential, easiest and powerful in most qualitative and quantitative analysis. HPLC is currently the most satisfying tool for an excellent and optimum separation [17-19]. This paper describes the development and validation of reliable, simple, robust, time and money saving reversed phase HPLC method, using PDA detection, for the estimation of CFT in bulk and in-house formulation. The developed method validated according to ICH guidelines [20].

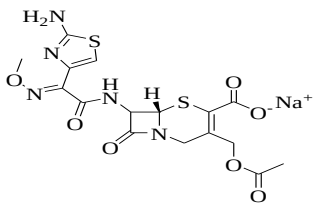


Figure 1 Chemical structure of cefotaxime sodium

MATERIALS AND METHODS

Instrumentation

Liquid chromatographic system from AGILET (1100) comprising of manual injector, water G1310A Iso pump for constant flow and constant pressure delivery and UV-Visible detector connected to software chemstation for controlling the instrumentation as well as processing the generated data. Weighing was done on a Digital Micro Balance (CX-265) manufactured by Citizen Scale (I) Pvt. Ltd.

Reagents and chemicals

Analytically pure sample of CFT was a generous gift from Hetero Lab, Hyderabad, along with their analytical reports. HPLC grade methanol, acetonitrile and OPA were obtained from Merck (India) limited. All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house.

Chromatographic conditions

The isocratic mobile phase consisted of phosphate buffer (pH7.4): methanol (70:30v/v), flowing through the column at a constant flow rate of 1.2 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Zorbax (C-18) column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 234 nm was selected as the detection wavelength for UV-Visible detector.

STANDARD PREPARATION

Preparation of stock solution: Accurately weighed 10 mg of CFT was transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of methanol and sonicate for 10 min., then volume was made up to 50 ml with methanol and vortex it to get complete dissolution and then filtered by whatman filter paper (no.41). Concentration of CFT in methanol was 200µg/ml (stock- A).

Preparation of sub stock solution: 5 ml of solution was taken from stock-A of CFT and transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent to give concentration of 100µg/ml (Stock-B).

Preparation of different solution

0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with methanol. This gives the solutions of, 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of CFT.

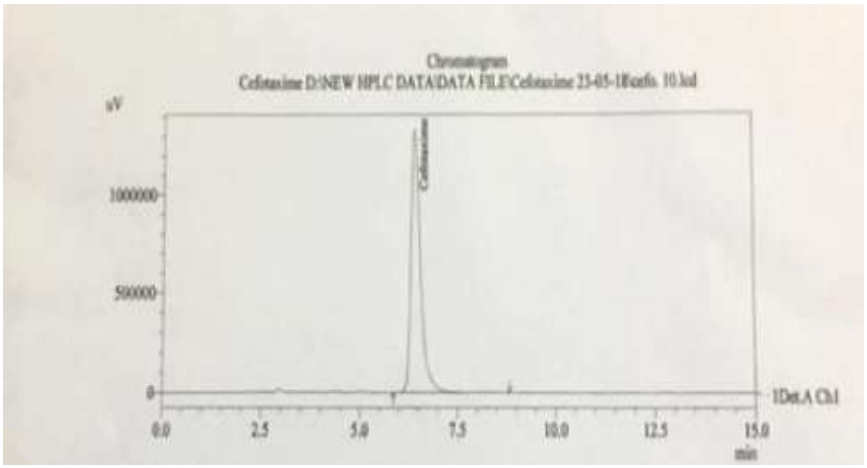
Analysis of CFT loaded nanostructure lipid carriers (C-NLCs)

Cefotaxime loaded nanostructure lipid carriers were prepared using high shear homogenization followed by probe sonication method. Components of E-NLCs are stearic acid-a solid lipid, oleic acid- a liquid lipid, tween 80- a surfactant, cryoprotectant-trelaholse dehydrate. A central composite rotatable design-response surface methodology (CCRD-RSM) was used for the optimization of formulation. In this method, stearic acid was melted and kept in water bath at 80 ^{0}C and oleic acid was added to it. Then to the molten lipid phase, cefotaxime was added. The aqueous phase comprised of tween 80 dissolved in water. Both the phases were maintained at 80^{o}C. At this temperature, the hot surfactant phase was then dispersed in hot lipid phase, obtaining a pre-emulsion under mechanical stirring at 10,000 rpm at 80^{o}C for 15 minutes. Then A-NLCs dispersion so formed was probe-sonicated with 40 % vibration amplitude, 5" on/5" off for 30 sec" with 10 sec" rest between 5-10" cycles according to sample turbidity or conc., for 10 minutes which was then allowed to cool and used for further characterization. An equivalent amount to 200mg of cefotaxime was taken in 50 ml volumetric flask. This was dissolve in 25 ml of methanol by sonication for about 10 minutes. The volume was made up to the mark by methanol as per the UV spectrophotometry method. The solutions were filtered (whatman filter paper no.41) and Then different concentration of solution were prepared by serial dilution technique, as per standard and each dilution was analyzed.

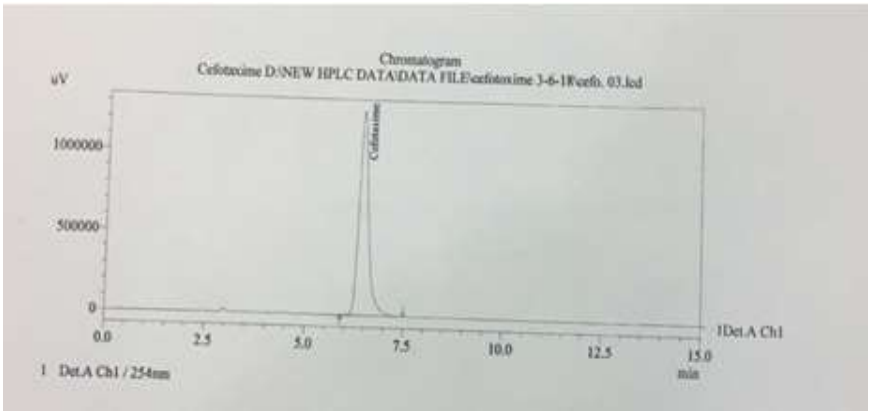
RESULTS AND DISCUSSION

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of phosphate buffer (pH7.4): methanol (70:30v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 min were studied. A flow rate of 1.2 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using a reversed-phase C_{18} column, the retention times for CFT was observed to be 6.245±0.04min. Total time of analysis was less than 7 min. The maximum absorption of CFT was detected at 234nm and this wavelength was chosen for the analysis Figure 2.



(A)



(B)

Figure 2 Chromatograms of (A) Cefotaxime (15µg/ml) as reference substances (C) C-NLCs (15µg/ml)

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for CFT was 2143±61.34.

Table 1 Results of system suitability parameters

Parameters	Cefotaxime
AUC*	771.4593
No. of Theoretical Plates	2143±61.34
Tailing Factor*	0.865±0.072
Retention time*	5.347± 0.04
Calibration range (µg/ml)	5-25

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 5-25µg/ml for CFT. The linearity was represented by a linear regression equation as follows:

Y (Cefotaxime**)** = 28.76conc+23.67 (r^2 = 0.999)

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to pre-analysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed. The value of percentage RSD was found less than 2 show good recovery at all three level 80, 100 and 120% respectively. Each level was made in triplicate Table 2.

Table 2 Results of recovery study

% Level	% Mean ± SD*
	Cefotaxime
80%	99.716±0.149
100%	98.857±1.575
120%	100.236±0.544

***** Value of three replicate and three concentrations.

PRECISION

Repeatability

Five dilutions in three replicates were analyzed in the same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in Table 3.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variations and results were found within acceptable limits (RSD < 2) as shown in Table 3.

ROBUSTNESS

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, phosphate buffer (pH7.4): methanol (70:30v/v), to (80: 20% V/V) and method is found robust as RSD is again found < 2.0 table 3.

Table 3 Statistical data for precision and robustness

Statistical parameter	Cefotaxime		
	Mean*	**S.D***	**R.S.D***
Repeatability	99.188	0.506	0.510
Intermediate Precision (I) (A day to day)	99.074	0.667	0.673
(II) Analyst to Analyst	99.22	0.166	0.167
Robustness	99.074	0.667	0.673

*Mean of 15 determinations (three replicates at five concentration level)

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve Table 4.

Table 4 LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Cefotaxime	0.100	0.314

Analysis of C-NLCs

The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipients in the estimation of drug Table 5.

Table 5 Assay of A-NLCs

	Cefotaxime
Label Claim (mg)	**200mg**
% Found (mg)	199.32
% Assay	99.66
% RSD	0.312

*Average of three determination

CONCLUSION

The present study was undertaken to develop a sensitive, robust, accurate and precise method for the quantitative determination of cefotaxime in polymeric nano-formulations. The developed HPLC method was validated according to ICH Q2 (R1) guidelines and was confirmed to be sensitive, precise, accurate and robust. The method proved to be sensitive as evidenced in the form of least LOD and LOQ values. The proposed HPLC method will be beneficial for researchers as routine analysis of cefotaxime in NLCs.

REFERENCES

1. Willard H.H, Merritt L.L, Dean J.A, Settle Jr.F.A, Instrumental Methods Of analysis. CBS Publishers and distribution, Delhi, 2001; 7th Ed: 118-19
2. Christan G.D, Analytical chemistry. John Wiley and sons. 2003; 6th Edn: 1-3
3. Jeffery G.H, Bassat J, Mendham J, Denny R.C, Vogel's textbook Of Quantitative Chemical Analysis. Elbs with Longman Publication Harlow, 1989; 5th edn: 1-6.
4. Fauci A S, Touchette N A, Folkers G K. Emerging Infectious Diseases: a 10-Year Perspective from, National Institute of Allergy and Infectious Diseases. 2005;11(4): 519-25. doi: 10.3201/eid1104.041167.
5. Kumar S A, Shetty Y R, Ahmed M, Sridhar B K, Vijaya Kumar M L. Development and Validation of RP-HPLC Method for Quantitative Estimation of Cefoperazone in Bulk and Pharmaceutical dosage forms, International Journal of Chem Tech Research, 2011; 3(3); 1075-79.
6. Indian Pharmacopoeia, Government of India, The Controller of Publications, Delhi, Vol. I (1996) p. 148.
7. Rohan S. Badgujar, Saurabh Kumar Banerjee, G. Venkateshwarlu. Development and validation of rp-hplc method for simultaneous determination of cefotaxime sodium and sulbactum sodium in bulk and injection. International Journal of Chemical Sciences. 2010; 8(3): 1502-1510.
8. Vandana P. Patil, Suwarna H. Kale, Rushikesh H. Patil. Validated rp-hplc for simultaneous estimation of cefotaxime and sulbactum in reconstituted solids. Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry. 2016; 4(2):47-57.
9. Consortti L, Salgado H. Green method for quantification of sodium cefotaxime in lyophilized powder by infrared spectroscopy. Journal of Pharmaceutical Sciences Emerging Drugs. 2017; 5: 2-6.

10. CH AK, Gurupadayya B, Sloka N. Determination and validation of cefadroxil, ceftriaxone and cefotaxime by using n-bromosuccinamide in human plasma and pharmaceutical dosage form. International Journal of Research in Pharmaceutical Sciences. 2011; 2: 206-212.
11. Sayed RA, Hassan WS, El-Mammli MY, et al. A new extractive spectrophotometric method for the determination of gatifloxacin and cefotaxime sodium in pure and pharmaceutical dosage forms. Oriental journal of chemistry. 2012; 28: 639.
12. Rageh AH, El-Shaboury SR, Saleh GA, et al. Spectophotometric method for determination of certain cephalosporins using 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl). Natural Science. 2010; 2: 828.
13. Sayed RA, Hassan WS, El-Mammli M, et al. Spectrophotometric method for the determination of cefotaxime sodium and cefoperazone sodium in pure and pharmaceutical dosage forms. American Chemical Science Journal. 2013; 3: 514-525.
14. Gh AB, Rezvani M, Roshanzamir S. Spectrophotometric complexation of cephalosporins with palladium (II) chloride in aqueous and non-aqueous solvents. Spectrochimica Acta Part A: Molecular Biomolecular Spectroscopy. 2012; 89: 317-321.
15. Lalitha N. Development and validation of RP-HPLC method for estimation of Cefotaxime sodium in marketed formulations. Journal of basic clinical pharmacy. 2010; 1: 26-28.
16. Saranya CL, Thejaswini J, Gurupadayya B, et al. Simultaneous Determination of Cefotaxime Sodium and Paracetamol by LC-MS. IOSR J. Pharm. 2014; 4: 12-18.
17. Iqbal MS, Bahari MB, Darwis Y, et al. An RP-HPLC-UV Method with SPE for Cefotaxime in All-in-One Total Parenteral Nutritional Admixtures: Application to Stability Studies. Journal of AOAC International. 2013; 96: 290- 294.
18. Wang P, Yuan T, Hu J, et al. Determination of cephalosporin antibiotics in water samples by optimised solid phase extraction and high performance liquid chromatography with ultraviolet detector. International journal of environmental analytical chemistry. 2011; 91: 1267-1281.
19. Samanidou VF, Tsochatzis ED, Papadoyannis IN. HPLC determination of cefotaxime and cephalexine residues in milk and cephalexine in veterinary formulation. Microchimica Acta. 2008; 160: 471-475.
20. Code Q2 (R1) -Text on Validation of Analytical Procedures: Text and Methodology Current Step 4 version, 2005, ICH Harmonised Tripartite Guideline.