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Fully Investigation of RP- HPLC Analytical Method Validation Parameters for Determination of Cefixime Traces in The Different Pharmaceutical Dosage Forms and Urine Analysis

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ABSTRACT

Cefixime (Cfx) is a member of the third generation of Cephalosporin antibiotics. It used on a wide scale in prescribed antibiotic drugs as anti-infection for *Gram-positive* and *Gram-negative* microorganisms. The present study aimed to develop an HPLC method of Cfx analysis enjoyed highly linearity, repeatability, robustness, ruggedness, selectivity, rapidly, and economical to use. The chromatographic system depends on the RP- BDS column (250 mm x 4.6 mm x 5 μm). The mobile phase was prepared by mixing Methanol: Phosphate buffer (3:7, v/v) at flow rate 1.0 ml/min with wavelength detection at 254 nm, the temperature at 30° C with injection volume 20 μL. The method revealed that satisfied linearity regression R^2 (0.9996) with repeatability (0.94%) with DL and QL; 59.3 ng/ml and 179.8 ng/ml respectively. The method showed successful and satisfying results for Cfx in bulk and pharmaceutical formulations and urine samples at low levels.

Keywords: Validation, Pharmaceuticals, Cefixime, Detection limit, Quantitation limit

INTRODUCTION

The IUPAC name of Cfx is (6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2(carboxymethoxyimino)acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azabicyc-

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lo[4.2.0]oct-2ene-2-carboxylic acid [1]. Cfx is a member of the third generation of the Cephalosporin antibiotics. It was derived semi-synthetically from the marine fungus Cephalosporium acremonium. Cfx contains the Cephalosporins β-lactam core ring as shown in Figure 1 A, B.

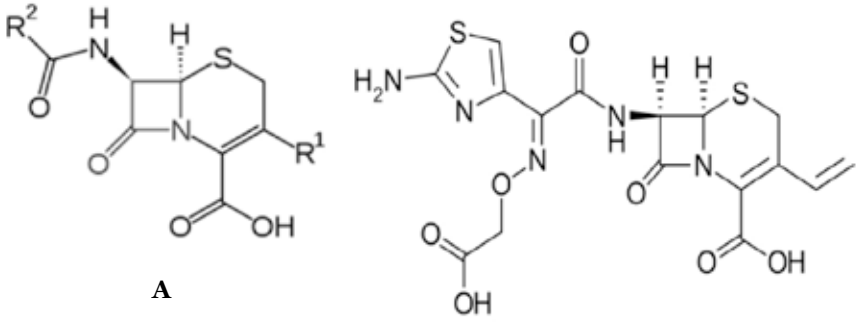

Figure 1. Structure of Cephalosporins *β*-lactam core ring (A) and Cefixime (B)

It used to treat many and various bacterial infections and it has excellent activity against many pathogens as, Enterobacteriaceae, Anaerobes, *Gram-negative* class such as *Haemophilus influenzae*, *Branhamella Catarrhalis*, *Escherichia coli*, *Neisseria gonorrhoeae, klebsiella, Serratia marcescens, Haemophilus, Providencia,* and *Meningococcus* including strains of β -lactamase producing. It is the best oral antibiotic for switch therapy due to its safety profile, high efficacy. Additionally, it has an inexpensive nature [2]. It works by killing bacteria and it has an analytically and clinically significant due to its broad spectrum as stability and antimicrobial activity [3]. Cfx is used for the reduction of the development of drug-resistant bacteria. It is introducing under different finished products; a powder for oral suspension, capsules, and tablets [4].

Several analysis methods have been developed to determine Cfx in different pharmaceutical dosage forms. These methods include different analysis techniques as microbiological methods and high-performance liquid chromatography (HPLC) [5].

Cfx has been quantitatively analyzed in bulk materials and different pharmaceutical dosage forms by Spectrofluorimetric [6-8], Spectrophotometric determination [9-11] Colourimetry, HPLC by capillary electrophoresis [12], Voltammetric determination [13-15], HPLC-MS; mass spectrometric methods may have the highest sensitivity, but the determination process is complicated to use and very expensive [2, 16].

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 excellent and optimum separation [5, 17-19].

In the present study, an HPLC method with a photodiode array detector (PDA) was developed for the determination of the lower concentration of Cfx in different pharmaceutical dosage forms. The proposed analytical method of Cfx was found to be precise, repeatable, linear, accurate, rugged, robust, specific, selective, and economic.

METHODOLOGY

Cfx standard (99.7%) was supplied by Covalent laboratories PVT.LTD (India) as a gift sample from Smart pharma (Assuit, Egypt). Methanol HPLC-grade, Sodium dihydrogen phosphate, Hydrochloric acid, Phosphoric acid 85%, Sodium hydroxide, and Hydrogen peroxide (Scharlau, Spain). Deionized water used in the analysis was prepared by reverse osmosis and passed through a 0.45 μm Millipore filter (Millipore Company, USA) before use. Phosphate buffer was prepared by weighing about 16.8 g of sodium dihydrogen phosphate and 0.5 ml of phosphoric acid 85% in 700 ml deionized water.

Chromatographic system configuration

Cfx was measured using the LC-20A HPLC instrument with the PDA (Shimadzu, Japan). The method was conducted using the RP BDS column (250 mm x 4.6 mm x 5 μm) (Thermo Scientific, USA). The mobile phase was prepared at the ratio "Methanol: Phosphate buffer" (3:7, v/v) at flow rate 1.0 ml/min with wavelength detection at 254 nm with column oven 30° C and injection volume 20 μL.

Parameters of method validation

The validation of the HPLC method was carried out according to International Conference on Harmonization (ICH), Food and Drug Administration (FDA), United States of American Pharmacopoeia (USP) and European Pharmacopoeia (EP) guidelines concerning parameters including tuning system and suitability of the system, Range linearity, detection limit, quantification limit, repeatability, recovery and accuracy, robustness, ruggedness, the stability of the solution, specificity and selectivity [20-25].

Tuning and suitability of the system

The performance of the chromatographic system comes first. So, the instrument performance was checked at a standard tuning solution was prepared in the mobile phase at a concentration of 2.0 μg/ml.

Range & linearity

It was said the method is linear if there is a good proportion between the response and working concentration starting from the lowest point in the tested range and the highest point and the R^2 should be ≥ 0.999 [20-24].

Regression linearity equation:

Y = a X+b (1)

Where, Y= Peak area, X= Concentration (%), a is the slope and b is the intercept.

Linearity was conducted using different five concentrations (50%-150%) of the Cfx standard. The working concentrations were prepared as, 1.0, 1.6, 2, 2.4, and 3.0 µg/ml using the mobile phase as a solvent. The later solutions were injected in triplicates.

Detection limit (DL)

It was defined as the minimum concentration of the analyte in the matrix that can be distinguished using the instrument detector. Additionally, it should not be represented in the precision and linearity range [20-24].

Quantitation limit (QL)

It was defined as the minimum concentration of the analyte in the matrix that can be distinguished using the instrument detector. On the contrary to the detection limit, it should be represented in the precision and linearity range [20-24].

DL and QL were calculated according to the linearity of the calibration curve and its standard error according to the following equations:

DL = 3.3 σ / S (2)

QL = 10 σ / S (3)

Where σ: is the standard error and S: is a slope of the linearity calibration curve.

Accuracy and recovery

Recovery and accuracy, each of them are used interchangeably. The accuracy of the measurement is defined as the closeness of the actual concentration (measured value) to the theoretical concentration (true value) [20-24].

Accuracy and recovery were conducted using the addition of three sets of Cfx standard to the in-active ingredient of the drug to give concentration at (1.6 µg/ml), (2.0 µg/ml), and (2.4 µg/ml). recovery estimation was linearity equation dependent:

Recovery %= Act. Conc.% / Th. Conc.% x 100 (4)

Repeatability and precision

Repeatability was conducted using 6 different preparations of the concentration (2.0 µg/ml) of Cfx by the same analyst on the same day using the same equipment [20-24].

Robustness

Robustness was investigated using conscious small changes including the slight diversity in the temperature, composition of the mobile phase, etc [20-24].

Changes were involved in a different organic solvent ratio (Methanol) at (± 10%) and different temperature ± 2^0 C.

Ruggedness

Ruggedness was investigated using conscious and major observable changes including analyst- analyst, column- column, and day- day with maintaining on the rest of experimental parameters and conditions at a constant rate.

Stability of solution

This test was conducted *via* performing the test at the target concentration of (2.0 µg/ml). It was measured over 12 working hours to assess the stability of the solution.

Specificity and selectivity

It can be defined as the measuring of the analyte in the presence of its degradants or interferences interpreted the connotation of specificity [20-24].

- Acid hydrolysis: It was conducted using HCl 0.1 N for 5 minutes.

- Oxidation hydrolysis: It was conducted using H_2O_2 3% *wt/v* for 5 minutes.

Test of the validated method

Cfx analysis in the different commercial dosage forms in the Egyptian local market

Suprax 200 mg capsules, Suprax 100 mg/5ml powder for oral suspension, and Cefipharmart 400 mg dispersible tablet for oral suspension were be tested using the validated method of Cfx.

Cfx traces analysis in the different urine samples

The method was tested for identification and quantitative analysis for 4 different urine samples.

RESULTS AND DISCUSSION

Tuning and suitability of the system

Cefixime peak was stated about at 7.8 minutes as revealed in **Figure 2**. **Table 1** showed a good performance for the selected method parameters where the RSD % < 2.0 % [20-24].

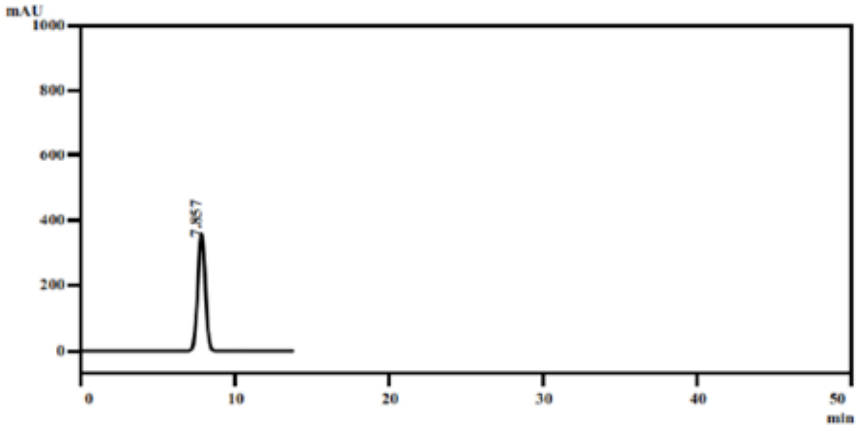

Figure 2. Cfx chromatogram

Table 1. Tuning and suitability of the system

Replicate #	P. A	Tailing	Plates
1	4651	1.221	12521
2	4655	1.223	12565
3	4658	1.218	12476
4	4630	1.216	12515
5	4684	1.218	12542
6	4628	1.218	12548
RSD%	0.44%	0.21%	0.25%

Range and linearity

The results revealed high linearity "R^2 = 0.9996" in between the working concentration range (50 %-150 %) as we can see in Figure 3 and Table 2.

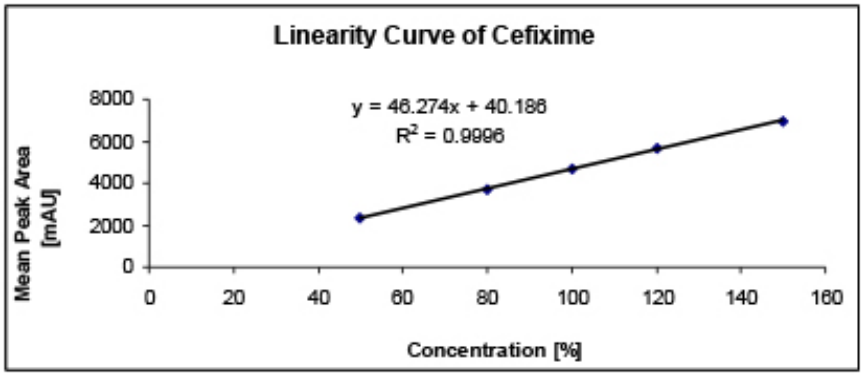

Figure 3. Linearity calibration curve of Cfx

Table 2. Range and linearity

Conc %	Average P. As	Statistical data	
50%	2351	R^2	0.9996
80%	3705	Slope	46.274
100%	4702	Intercept	40.186
120%	5632	Standard error	41.60657
150%	6948		

DL and QL

DL and QL limits were determined simply using the linearity calibration data of Cfx. DL was found to be 59.3 ng/ml where QL was 179.8 ng/ml.

Accuracy and recovery

Table 3. Revealed satisfaction results for recovery and accuracy within the tested range (80-120 % from the target concentration).

Table 3. Accuracy and recovery

Theoretical conc%	Average P. As	Actual conc%	Recovery %
80%	3798	81.2%	101.5%
100%	4715	101.0%	101.0%
120%	5539	118.8%	99.0%

Repeatability and precision

The RSD% of Peak areas was used for judgment on the repeatability of the analyte using six different preparations at the same concentration as in Table 4. It was found to be 0.94 % as it demanded in repeatability requirements [20-24].

Table 4. Repeatability and precision

#	Sample P. A	Statistical data	
1	4605	Average P. As	4622.333
2	4670	STDEV	43.509
3	4574	RSD%	0.94%
4	4590		
5	4614		
6	4681		

Robustness

The results of conscious small changes included temperature ± 2^0 C and organic (±10 %) were determined using RDS %. The RSD% was found to be < 2 % in all cases as shown in Tables 5 and 6.

Table 5. Change in temperature results

Replicate #	Set # 1 30° C	Set # 2 (32° C)	Set # 3 (28° C)
1	4651	4698	4616
2	4655	4691	4605
3	4658	4686	4594
4	4630	4712	4587
5	4644	4674	4621
6	4628	4695	4600
Pooled mean		4646.944	
Pooled RSD%		0.84%	

Table 6. Change in organic ratio results

Replicate #	Set # 1 300 ml	Set # 2 330 ml	Set # 3 270 ml
1	4651	4777	4574
2	4655	4779	4545
3	4658	4786	4596
4	4630	4751	4550
5	4644	4758	4558
6	4628	4735	4555
Pooled mean		4657.222	
Pooled RSD%		1.86%	

Ruggedness

The results of conscious major and observable changes including analyst-analyst, column-column, and day-day. Data were be presented as shown in Tables 7-9. RSD % found to be < 2 % in all cases [20-24].

Table 7. Day-to-day precision results

Replicate #	Set # 1 First day	Set # 2 Second day	Set # 3 Third day
1	4651	4718	4798
2	4655	4733	4718
3	4658	4735	4742
4	4630	4728	4757
5	4684	4749	4811
6	4628	4725	4774
Pooled mean		4716.333	
Pooled RSD%		1.16%	

Table 8. Analyst-to-Analyst precision results

Replicate #	Analyst 1	Analyst 2	Analyst 3
1	4651	4581	4611
2	4655	4580	4625
3	4658	4572	4687
4	4630	4588	4628
5	4684	4510	4601
6	4628	4529	4681
Pooled mean		4616.611	
Pooled RSD%		1.09%	

Table 9. Column-to-Column precision results

Replicate #	Column #1	Column #2
1	4651	4752
2	4655	4758
3	4658	4747
4	4630	4792
5	4684	4772
6	4628	4764
Pooled mean	4707.583	
Pooled RSD%	1.31%	

Stability of solution

The experimental results guided us that the tested solution of Cfx can be given repeatable and precise data over 12 hours at room temperature as in Table 10.

Table 10. Stability of solution

#	0 hour	3 hours	6 hours	12 hours	Average P. As	STDEV	RSD%
Test P. A	4703	4568	4661	4575	4626.8	66.123	1.43%

Specificity and selectivity

The current method supplied us with highly specific information about the resolution and separation performance of the nearest co-eluted peaks with a resolution parameter at least 5.2 as in Figure 4 A, B.

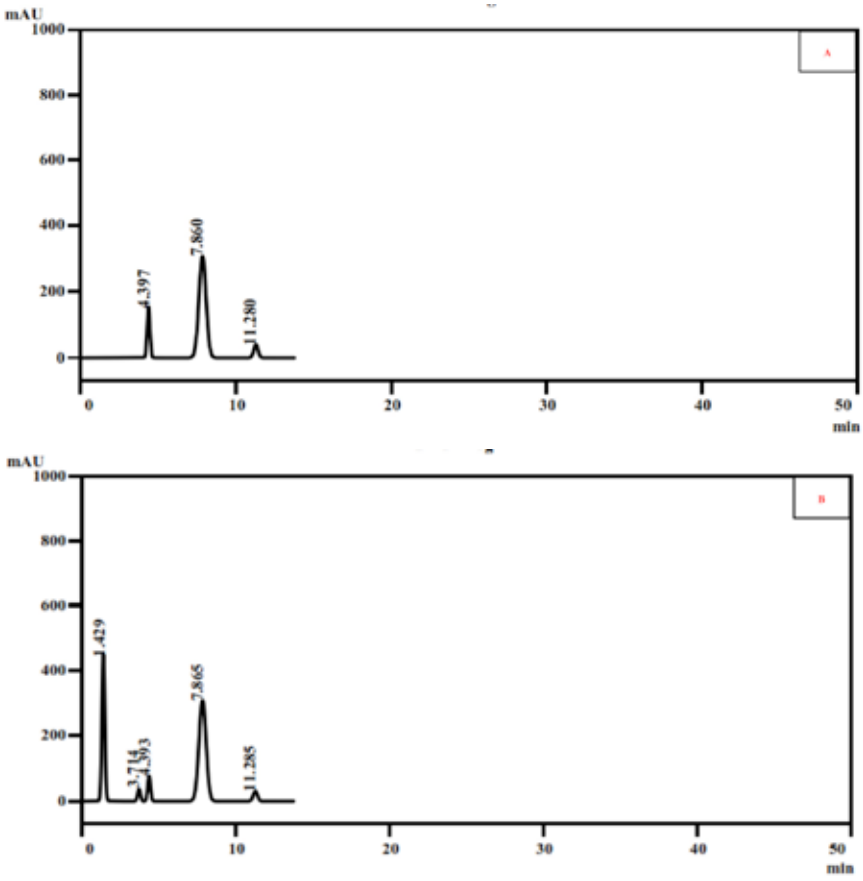

Figure 4. Effect of acid degradation (A) and H_2O_2 degradation (B) in specificity test

Test of the validated method

Cfx analysis in the different commercial dosage forms in the Egyptian local market

The Cfx average assay results of Suprax 200 mg capsules, Suprax 100 mg/5ml powder for oral suspension, and Cefipharmart 400 mg dispersible tablet for oral suspension revealed good results; 103.5 %, 101.8 %, and 104.4 % respectively.

Cfx traces analysis in the different urine samples

The method was succeeded in Cfx traces analysis at low concentrations reached 77.6, 98.1, 199.5, 260.7 ng/ml.

The current method introduces a sensitive, rapid, easy, economical, and accurate method of Cfx analysis. The method revealed a good behavior as linear, precise

(repeatable), robust, rugged, selective, and specific as the resolution factor between Cfx peak and any adjacent peak at least anyway > 1.5. DL and QL also, evaluated and showed an appreciated and satisfying value as 59.3 ng/ ml and 179.8 µg/ ml respectively. So, the analysis method is valid to use for Cfx determination at the minimum level of concentrations with convenient tools of analysis. The validated method gave satisfying results for the practical application of Suprax and Cefipharmart assay determination for three different dosage forms as revealed in the results. Also, the method showed a good result to investigate and quantitative analysis against urine samples at low concentration levels.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication and dissemination of the information provided here.

ABBREVIATIONS

Cfx	Cefixime
HPLC	High- performance liquid chromatography.
PDA	Photodiode array detector
UV	Ultraviolet
EP	European Pharmacopeia
USP	United States Pharmacopeia
DL	Detection limit
QL	Quantitation limit
Conc	Concentration
P. A	Peak area
P. As	Peak areas
STDEV	Standard deviation
RSD	Relative standard deviation
Th.	Theoretical

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