Development of HPLC-UV Method for Analysis of Cefixime In Raw Materials and In Capsule

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ABSTRACT

A simple, selective and rapid reversed phase High Performance Liquid Chromatographic (HPLC) Method for the analysis of cefixime in bulk material and capsule has been developed and validated. The chromatographic system consisted of a LC-10 AT VP pump, SPD-10 AVP UV/visible detector. The Separation was achieved from Bondapak C18 column at ambient temperature with a mobile phase consisting of methanol: buffer solution (sodium dihydrogen phosphate) [35: 65 v/v, pH=2.75 adjusted with phosphoric acid] at a flow rate of 1ml/min and the retention time was about 6 minutes. The method is selective to cefixime and able to resolve the drug peak from formulation excipients. The system suitability with retention time was (Mean + %CV) 5.819 + 0.51.The calibration curve was linear over the concentration range of 0.039-20µg/ml (r2 = 0.9998). The proposed method is accurate and precise (Intra day and Inter day variation, RSD were 0.53-1.64) and linear within the desired range. The LOD and LOQ was detected as 0.0195µg/ml and 0.039µg/ml respectively with r2 = 0.9996. The accuracy result of seventy percent drug (70%) was 99.82%, hundred percent (100%) was 99.89%, and one thirty percent (130%) was 100.12%. Therefore, this method could be used as a more convenient and efficient option for the analysis of cefixime in raw material and capsule dosage form.

Keywords: Third generation cephalosporin; Cefixime; Method validation; HPLC method determination; Quantitative analysis.

INTRODUCTION

Cefixime is oral third generation cephalosporin [1, 2]. Several investigations on cefixime have indicated that it is an orally active antibiotic with similar antibacterial spectrum and resistance to β-lactamase as for parenteral third generation cephalosporins [3, 4]. Cefixime has potent antibacterial activity against a wide range of bacteria, highly stable towards β-lactamases and long duration of action [3]. Memon et al. reported cefixime as a safe, effective, and cheaper oral option for the treatment of multidrug-resistance [5].

The chemical structure of cefixime consists of the cephem nucleus, a β-lactam ring fused to a 6-membered dihydrothiazine ring. The cephem nucleus incorporates two important groups: the vinyl group at the 3-position, which is responsible for the intestinal absorption of the intact molecule. Other groups are the aminothiazole ring and the acetic acid oxy-imine group on the side chain at the 7-position, which are associated with the antibacterial activity [4].

Chemical formula:

$C_{16}H_{15}N_5O_7S_2.3H_2O$

A number of methods has been used for the analysis of cefixime in raw material, in pharmaceutical dosage

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forms as well as in biological fluids such as Rathinavel et al., who used RP-HPLC method for the estimation of cefixime in tablets[6], Meng et al., who used liquid chromatographic-tandem mass spectrometric method to determine cefixime in human plasma[7], also, Al-Momani used Spectrophotometric method for the determination of drug in formulations[8], whereas Eric-Jovanovic et al. used HPTLC method for the determination of cefixime in dosage forms[9], and finally Liu et al., used HPLC method for the analysis of cefixime in human plasma and urine[10].

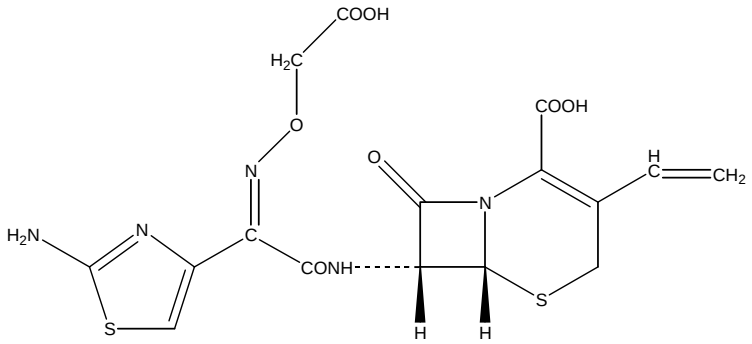


Fig. (1): Cefixime structure

OBJECTIVE OF THE STUDY

The present study was conducted to:

- Describe simple, efficient and reproducible reverse phase HPLC method with UV detection for the determination of cefixime from raw materials and capsules dosage form.
- Develop a cost-effective method in comparison to other mentioned methods.

Experimental

Instrumentation

The HPLC system equipped with LC-10 AT VP pump and SPD- 10A VP Shimadzu UV-VIS detector was utilized. Chromatographic system was integrated via Shimadzu model CBM-102 Communication Bus Module to P-III computer. C_{18} Shim–pack CLC–ODS Column (6mmID x 15 cm), protected by octa decyl silane guard column (Pre- column), manual injector was fitted with a Loop-20µl. In addition, electronic balance, microliter syringe, micropipette and micropore filtration assembly were used.

Materials and reagents

Cefixime was a kind gift sample from Hilton Pharma (Private) Limited, Karachi, Pakistan. Six different brands of cefixime capsules were obtained from a retailer of Karachi (Pakistan) markets. All reagents used were of HPLC grade. Methanol, sodium dihydrogen phosphate and ortho-phosphoric acid 85% (Merck) and HPLC grade distilled water was freshly prepared for the mobile phase.

Preparation of standard solution

100 mg of cefixime standard powder was weighed and added into 50 ml volumetric flask and dissolved in few ml of methanol and then used to make up the volume with mobile phase. 5 ml of this standard stock solution was pipette out and transferred into 50 ml volumetric flask. The volume of the solution was made up with the mobile phase. Concentration of working standard was kept equivalent to 0.2mg/ml.

Preparation of mobile phase

12.5mM NaH_2PO_4 buffer solution and methanol (650:350 v/v) was used as the mobile phase. The pH of the final solution was adjusted at 2.75 with 85% ortho-phosphoric acid. Prior to delivery to the system, mobile phase was filtered through 0.45µm HV millipore filter and degassed using a sonicator.

Preparation of sample solution

20 capsules were accurately weighed and the average weight was calculated. The capsule powder was removed from the shell and grinded to a fine powder with the help of mortar and pestle. Then, the amount of powder equivalent to average weight of a capsule was transferred to a volumetric flask, dissolved in mobile phase and shaken for about 10 minutes then filtered through filter paper. The filtered solution was further diluted in the mobile phase to make the final concentration of working sample equivalent to 0.2mg/ml.

Chromatographic condition

The samples were introduced by injector with a 20-µl loop. The analysis was carried out under isocratic conditions using a flow rate 1ml/min at ambient temperature. Chromatograms were recorded at λ = 288nm using a detector SPD- 10A VP Shimadzu UV-VIS.

Validation procedure

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of cefixime. The experiment was carried out according to the official specifications of USP–27, ICH-1996, Global Quality Guidelines-2002, and Centre for Drug Evaluation and Research (CDER) -1994[11, 12, 13, 14]. The method was validated for the parameters like system suitability, range and linearity, limit of detection, limit of quantification, accuracy, precision, ruggedness and robustness.

The system suitability was assessed by six replicate analyses of the drug at a concentration of 20µg/ml. System suitability of the method was evaluated by analyzing the repeatability, peaks symmetry, theoretical plates of the column and retention time.

The selectivity of the chromatographic method was determined to ensure separation of active ingredient in the presence of excipients used in the capsule formulation and also chromatogram was observed and compared with that of the raw material.

A linear relationship may be demonstrated directly on the drug substance by dilution of a standard stock solution and/or separate weighing of synthetic mixtures of the drug product components, using the proposed procedure [15]. The linearity for the present method was determined by analyzing standard solution of cefixime in the concentration range of 0.039-20µg/ml.

To determine accuracy of the method and recovery of cefixime in capsule dosage form, samples of 400mg cefixime, USP were prepared in triplicate at the 70%, 100% and 130% levels of the target cefixime concentration and assayed according to the procedures.

To evaluate the linearity with respect to its limit of detection (LOD) and limit of quantification (LOQ) of the reference drug, serial dilutions were made from the standard stock solution in the range of 0.0195µg/ml-10µg/ml and dilution was made with mobile phase and resolved in a C_{18} column with UV detector at 288nm.

The precision of the method was investigated with respect to repeatability (a minimum of 6 determinations at 100% of the test concentration), intermediate precision (intra-day and inter-day variation), and reproducibility (by means of an inter-laboratory trial).

Robustness studies were performed on method precision sample concentration 20µg/ml by making slight variations in flow rate, volume of methanol and change in pH of mobile phase.

Ruggedness tests were performed on method precision sample concentration 5µg/ml-0.625µg/ml by checking inter-day variation, which was performed by different analysts.

Assay for cefixime capsule

20 capsules were accurately weighed and the average weight was calculated. The capsule powder was removed from the shell and grind to a fine powder with the help of mortar and pestle and the amount of powder equivalent to average weight of capsule was transferred to volumetric flask and dissolved in mobile phase and shaken for about 10 minutes. Then, the solution was filtered through 0.45µm HV millipore filter paper. The filtered sample solution was further diluted in a mobile phase. The final concentration of working solution was kept equivalent to 0.2mg/ml.

RESULT AND DISCUSSION

Chromatographic methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids. The use of HPLC methods for the quantification of drug has now become a routine consideration in quality control of drug and drug products.

The objective of this study was to develop and validate a simple, selective, and cheap HPLC method for the estimation of cefixime in capsule as most of Pakistani Pharmaceutical companies are engaged in manufacturing cefixime in capsule form.

According to the Global Quality guideline (2002), the meaning of analytical validation is the process that confirms that an analytical procedure does what it is purported to do, that is, to document through laboratories' that the measurement procedure can reliably assess the identity strength and quality of a bulk drug substance, excipients or finished pharmaceutical products[13]. The United States Pharmacopoeia (USP-27), International Conference on Harmonization (ICH-1995), and the Food and Drug Administration (FDA-1987)[11, 12, 16] provide a framework for performing such validations. In general, for the validation of pharmaceutical analytical method, the following parameters must be considered i.e. linearity, accuracy, precision, range, detection limit, quantification limit, robustness and ruggedness.

System suitability

The system suitability results are summarized in Table (1). System suitability test is used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done [11]. In the present method, the percentage of coefficient of variation (%CV) of the retention time and peak area were 0.51 and 3.66, respectively. The mean theoretical plate count and the tailing factor were 4439.333 and 1.12, respectively.

Table: (1): Summary of system suitability results for Cefixime

Injection Number	Retention Time of Cefixime (min.)	Peak Area of Cefixime	USP Tailing Factor	USP Tangent
1	5.80	729068	1.15	4478
2	5.821	723185	1.11	4439
3	5.811	719796	1.13	4384
4	5.801	726310	1.11	4463
5	5.803	723547	1.12	4476
6	5.878	789885	1.11	4396
Mean	5.819	735298.5	1.12	4439.333
% CV	0.51	3.66	1.4	0.9

In this study, there was no internal standard used for system suitability because CDER (1994) dose not specify whether the method must use an internal or external standard for quantification. This internal standard method is compulsory where the sample preparation procedures are complicated or complex and low concentration sample is required for pharmacokinetics studies [17]. The present method proposed a very simple procedure for the preparation of a sample.

Linearity test

A linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration [17]. The linearity of response for the present method was determined by analyzing standard

solution of cefixime in the concentration range of 0.039-20µg/ml. The results showed that the peak area responses are linear within the concentration range of the analysis. The correlation coefficient was $r^2 = 0.9998$ (Fig. 1).

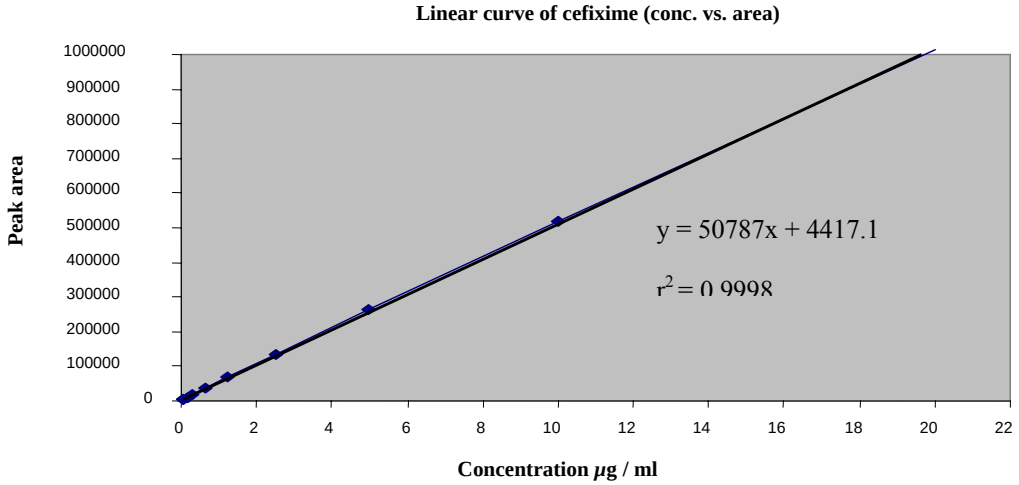


Fig. (1): Calibration curve shows linearity over the concentration range 0.039-20µg/ml

Accuracy test

The accuracy of the method is the closeness of the measured value to the true value for the sample [13]. For assay method, samples were prepared and spiked in triplicate at three levels over a range of 70-130% of the target concentration. The analyte levels in the spiked samples were determined using the same quantitative procedure as will be used in the final method procedure. The percent recovery was then being calculated.

Accuracy was determined by preparing samples of CEF-1 400mg cefixime capsule in triplicate at the 70%, 100% and 130% levels of the target cefixime concentration and assayed according to the procedure. The percentage recovery ranged from 99.62% to 100.37% of the label claim of cefixime at all levels of the recovery analysis, and percentage of CV values for each level ranged from 0.2% to 0.3%. The overall mean percent recovery was 99.94% of the label claim of cefixime with an overall CV of 0.26% (Table (2)).

Table (2): Accuracy results for the assay of cefixime capsule

Level	Sample Number	Sample Amount (mg)	Cefixime		% Recovery	% Mean Recovery	(%CV)
			Theoretical mg	Measured mg			
70%	1	366.10	279.67	280.13	100.16	99.82	0.3
	2	369.60	282.35	281.29	99.62		
	3	366.80	280.21	279.37	99.70		
100%	1	524.0	400.31	401.12	100.2	99.89	0.3
	2	528.0	403.36	402.63	99.81		

Level	Sample Number	Sample Amount (mg)	Cefixime Theoretical mg	Cefixime Measured mg	% Recovery	% Mean Recovery	(%CV)
	3	527.0	402.59	401.29	99.67		
130%	**1**	687.70	525.36	527.32	100.37	100.12	0.2
	2	658.10	523.37	522.94	99.91		
	3	690.30	527.12	528.12	100.1		
Mean						99.94	0.26

Limit of detection and Limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were evaluated based on cefixime standard solution in which the peak responses were three times and nine times the base line noise, respectively. Cefixime standard solutions were prepared and analyzed for linearity from the quantification limit (LOQ = 0.039µg/ml) as well as analyzed up to detection limit (LOD = 0.0195µg/ml). The obtained results showed that the peak area responses are linear up to LOQ that ranged from 0.039µg/ml-10 µg/ml and the curve values for cefixime was depicted in Table-3 with $r^2 = 0.9996$, slope, intercept and detection limit.

Table (3): Estimation of LOD and LOQ of cefixime

Standard Number	Concentration µg/ml	Peak Area, AU
1	10	296736
2	5	146389
3	2.5	75847
4	1.25	42853
5	0.625	21665
6	0.313	11255
7	0.156	5957
8	0.078	3483
9	0.039	1736
Slope	9384	
Intercept	2155	
r^2	0.9996	
LOD	0.0195µg/ml	
LOQ	0.039 µg/ml	

Precision test

Precision study was performed to determine the reproducibility of the method. Six samples were prepared at the 100% level and assayed according to the procedure described. The results of the precision study are summarized in Table (4). The mean percent recovery of cefixime was 99.85% of the label claim with percentage of CV of 0.3%. The results obtained from intermediate precision (inter-day and intra-day) also indicated a good method of precision (Tables 7, 8).

Table (4): Results for Cefixime Capsules method Precision

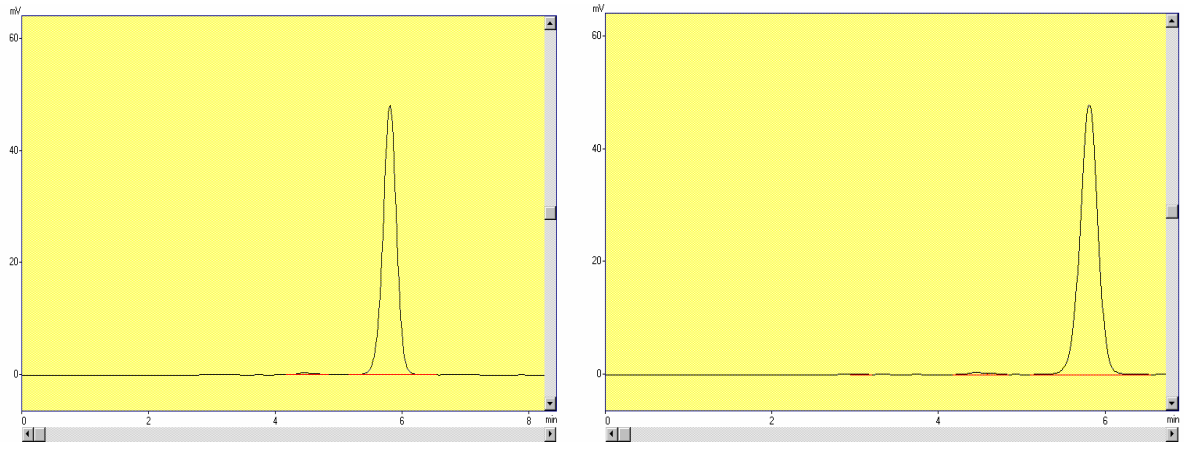
Sample Number	Sample Amount (mg)	Cefixime		% Recovery
		Theoretical (mg)	Measured (mg)	
1	522	398.78	399.1.3	100.0
2	527	401.59	400.25	99.66
3	521	398.01	399.12	100.2
4	526	401.83	400.16	99.58
5	520	397.24	398.21	100.2
6	528	403.36	401.25	99.47
Mean	524.33	400.13	99.68	99.85
%CV				0.3

Robustness test

The robustness of a method is its ability to remain unaffected by small changes. Robustness study was performed on method precision sample # 2 by making slight variations in flow rate, amount of methanol, and buffer pH changes one at a time (Table 5). The results of robustness in the present method showed no significant changes occurring over changes in flow rate from 0.9 ml/min. to 1.1ml/min, showing a recovery of 99.6% and 99.8%, respectively, and in the case of the change in pH of buffer from 2.75 to 3.30, the recovery was 100.4% and 101.5%, respectively. Methanol variation from 30% to 35% showed a recovery of 101.5% and 99.8% (Fig.2).

Table (5): Results for Cefixime robustness test

Parameter	Changes	% Recovery	% of Target
Target Conditions		99.66	100.0
Flow rate	0.9 ml/min	99.6	100.2
	1.1 ml/min	99.8	101.5
Buffer pH	2.75	100.4	101.7
	3.30	101.5	102.1
Methanol Variation	30 %	101.5	99.9
	35 %	99.8	101.3



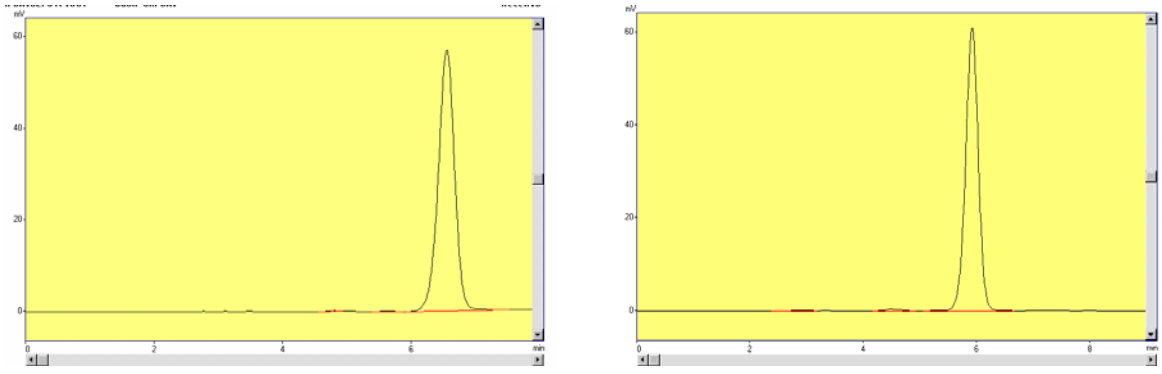
A B

A = Chromatogram of Cefixime raw material B = Chromatogram of Cefixime capsule dosage form

Fig. (2): HPLC Chromatogram shows no interference between active drug and excipients

Selectivity

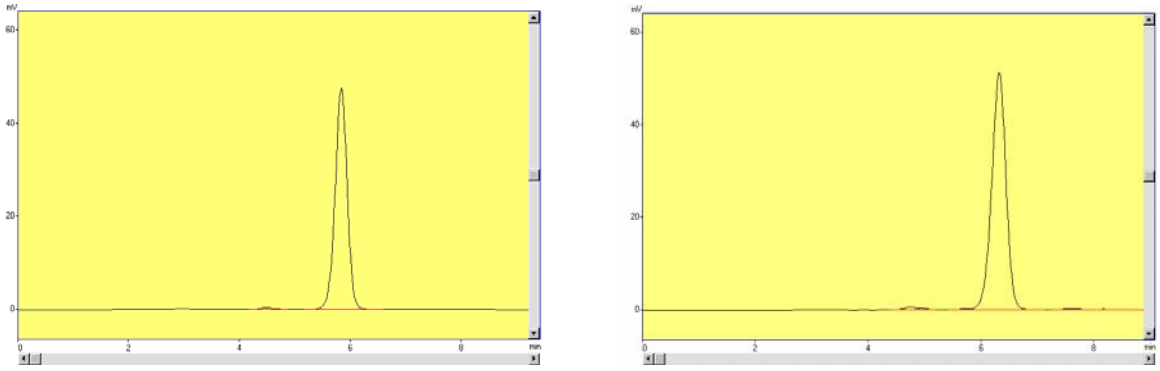
For demonstrating the selectivity of the method for capsule, the drug was spiked, and so, it was observed that the excipients used in different brands of cefixime capsule did not interfere with the active drug peak (Fig.3). In Fig. (3) Chromatogram A shows the separation of cefixime from raw material and B from capsule dosage forms. In between zero to 6 minutes (retention time), there appeared no any peak of any excipients which had been used in the manufacturing of capsule formulation. So that the chromatogram shows the method selectivity due to that the active ingredient, cefixime, would easily be separated without any interference.



A B

A= Chromatogram of cefixime whereas methanol = 30%

B = Chromatogram of cefixime whereas methanol = 35%



A B

A = Chromatogram of cefixime whereas buffer pH = 2.75

B = Chromatogram of cefixime whereas buffer pH = 3.30

Fig. (3): HPLC Chromatograms that shows robustness (no significant changes occur in peak area response)

Table (6) shows the results of the assay of cefixime capsules. The active content recovery of the six (6) brands were between 95% and 105%. It indicates that inspite of the difference in the excipients used by manufacturers, the proposed method was able to recover the active drug as per BP/USP specifications and this represents the good selectivity of the method.

Table (6): Content assays of Cefixime capsules by HPLC

ASSAY	CEF-1	CEF-2	CEF-3	CEF-4	CEF-5	CEF-6
Assay-1	99.1%	102.33%	98.45%	101.6%	101.29%	99.22%
Assay-2	99.36%	101.82%	98.12%	104.08%	101.16%	99.03%
Assay-3	99.76%	102.27%	99.75%	101.03%	100.59%	98.44%
SUM	298.22	306.42	296.32	306.71	296.69	296.69
MEAN	**99.40667**	**102.14**	**98.77333**	**102.237**	**101.0133**	**98.89667**
± S.D	0.332466	0.278747	0.861762	1.62161	0.372335	0.406735
± SEM	0.135	0.113	0.351	0.662	0.152	0.166

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the same samples under a verity of conditions, such as different laboratories, different analysts, different instruments and different days [17]. Results of three different days (inter-day variation) performed by different analysts proved the ruggedness of the developed method (Table 7).

Table (7): Intra-day variation in the analysis of Cefixime

Conc. (µg/ml)	9.00 am	12.0 am	3.00 am	6.00 am	Mean	±SD	RSD
5	265203	264413	262653	261224	263373	1546.5	0.58
	264323	264321	262754	261354	263188	1237.3	0.47
Mean	264763	264367	262703.5	261289	263281	1385.4	0.53
2.5	134978	134876	134452	132357	134166	1062.7	0.79
	134769	134884	132252	132162	133517	1310.8	0.98
Mean	134873.5	134880	133352	132259.5	133841	1105.2	0.83
1.25	68328	68176	67554	67471	67882	374.8	0.55
	68283	68268	67662	67442	67914	370.06	0.54
Mean	68305.5	68222	67608	67456.5	67898	370.8	0.55
0.625	35428	34915	34232	33924	34625	586.3	1.69
	35281	34863	34185	33876	34551	552.2	1.6
Mean	35354.5	34889	34208.5	33900	34588	569.1	1.64

Table (8): Inter-day variation in the analysis of cefixime

Concentration (µg/ml)	1st day	2nd day	3rd day
5	265203	264237	261592
	264323	264351	261665
Mean	26476.3	264294	261628.5
2.5	134978	133364	131767
	132769	132421	131467
Mean	133873.5	132892.5	131617
1.25	68328	67826	65421
	68283	67964	65153
Mean	68.05.5	67895	65287
0.625	35428	34174	32949
	35281	34926	33014
Mean	35354.5	34550	32982

CONCLUSION

The proposed method is rapid, precise, accurate, selective, cost-effective and least time consuming HPLC method for the qualitative and quantitative analysis of cefixime from raw materials, in bulk drugs and in capsule dosage form.

Most of the existing methods were used for the determination of cefixime in biological fluids or in tablet dosage form and were also found costly by means of equipments [7, 9, 10], there was no known method for the determination of cefixime in capsule dosage form. The proposed method is not only suitable for assay of cefixime in capsules but it is easy to perform, and the equipment used was user-convenient and usually available.

Some of the known methods for determining cefixime were having LOD around 0.1µg/ml and calibration curve to be 5µg/ml-250µg/ml, while the present method has the LOD equal to 0.0195µg/ml and calibration curve is 0.039µg/ml-20µg/ml, which makes the present method suitable for the determination of cefixime even in very low concentrations.

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تطوير طريقة لتحليل سيفكسيم (Cefixime) في المواد الأولية وفي الكبسولات باستخدام الكروماتوغرافيا (HPLC)

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[2] قسم الصيدلانيات، كلية الصيدلة، جامعة كراتشي، كراتشي، باكستان.

ملخـص

لقد تم تطوير طريقة بسيطة وسريعة ونوعية باستخدام الكروماتوغرافيا السائلة، وذلك لتحليل السيفوكسيم في المواد الأولية العكسية، وتلك الموجودة في المحافظ. تكونت الكروماتوغرافيا المستعملة من مضخة نوع LC-10AT VP ومن كاشف (Detector) تحت بنفسجي/ ضوئي، كما تم الفصل باستخدام عمود فصل نوع Bondpak-C18 وطور سائل مكون من ميثانول: ماء (صوديوم دي هيدروجين فوسفات) بنسبة (65:35، ودرجة حموضة 2.75) وسيلان بمقدار 1مل/دقيقة وزمن انحباس مقداره 6 دقائق.
كانت الطريقة اختيارية للسيفوكسيم وقادرة على تحديد الدواء وفصله بشكل واضح عن المواد المضافة الأخرى وبزمن انحباس 5.819±0.51 كان المنحنى المعياري خطي عند تركيز 0.039-20كلغ/مل (ز=509996).
إن الطريقة أعلاه المقترحة دقيقة ونوعية وخطية ضمن التركيز المفحوص. أما LOD وLOQ فقد تم تحديدها بـ 5.5195 كلغ/مل و0.039كلغ/مل على التوالي، وقيمة ر= 0.9996 وإن دقة النتيجة المحصول عليها لـ 70% من الأدوية المضمونة كانت بحدود 99.82%، 100% كانت 99.89% ولـ 130% كانت 100.12%. إن هذه الطريقة يمكن استخدامها وبسهولة لتحديد سسفيكسين في المواد الأولية والمحافظ.

الكلمـات الدالـة: سيفالوسبورين الجيل الثالث، سيفوكسيم، طريقة الإثبات، تحديد طريقة الكروماتوغرافيا السائلة، التحليل الكمي.

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