Scientific paper

Bioanalytical Method Development and Validation for Simultaneous Estimation of Cefixime and Dicloxacillin by RP-HPLC in Human Plasma

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Abstract

An accurate, rapid and simple reversed-phase high performance liquid chromatography (RP-HPLC) bioanalytical method was developed and validated for simultaneous estimation of cefixime, dicloxacillin in human plasma using ezetimibe as an internal standard. The cefixime, dicloxacillin and internal standard were extracted by liquid–liquid extraction technique. Chromatographic separation is accomplished using CAPCELL PAK C18 (4.6 mm × 250 mm, 5 m) analytical column. The mobile phase consisted of phosphate buffer, acetonitrile and methanol in 42:55:03 proportions. Detection and quantification were performed by UV/Vis detection at 225 nm. The lower limit of quantification was 0.5 μg mL^{-1} for both cefixime and dicloxacillin in human plasma. The calibration curves were linear over the concentration range 0.5 to 40 μg mL^{-1} for both drugs in human plasma. The method was quantitatively evaluated in terms of linearity, precision, accuracy, recovery, selectivity, and stability. The method was found to be simple, convenient and suitable for the analysis of cefixime and dicloxacillin from biological fluids.

Keywords: Bioanalytical method, validation, human plasma, cefixime, dicloxacillin

1. Introduction

Selective and sensitive analytical methods for the quantitative evaluation of drugs and their metabolites (analytes) are critical for the successful evaluation of preclinical, biopharmaceutical and clinical pharmacological studies. Bioanalytical method validation includes all of the procedures which demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, is reliable and reproducible for the intended use.[1]

Cefixime, (6R, 7R)-7-[2-(2-amino-4-thiazolyl) glyoxylamido]-8-oxo3-vinyl-5-thia–1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (**CFX; Fig 1a.**) is a third generation cephalosporin antibiotic. CFX is given orally in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory tract infection and urinary tract infection.[2] Dicloxacillin, (2S,5R,6R)-6-{[3-(2,6-dichlorophenyl)-5-methyl-oxazole-4-carbonyl]amino}-3,3-dimethyl-7-oxo-4-thia–1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**DLX; Fig. 1b**) is used to treat infections caused by susceptible gram-positive bacteria.[12] Both the drugs are available as single and combination therapy for the treatment of various bacterial or viral infections because of their spectrum of activity. This combination penetrates well into body tissue and fluids which makes the combination effective as bactericidal. Moreover, when penicillinase-resistant penicillin (Dicloxacillin) is also used, activity against β lactamase producing strains is enhanced.[3]

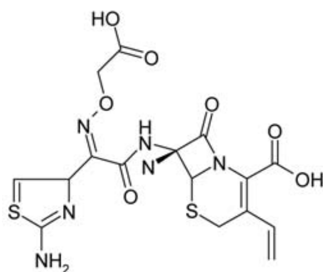

Fig 1a. Structure of Cefixime

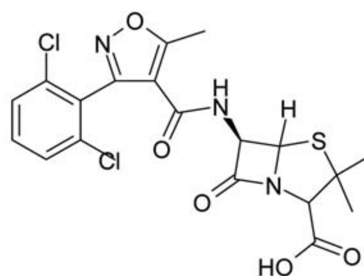

Fig 1b. Structure of Dicloxacillin

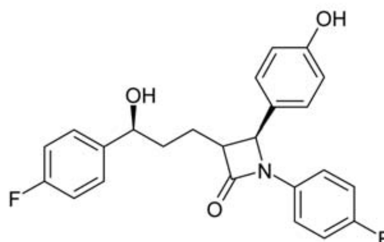

Fig 1c. Structure of Ezetimibe (IS)

Several analytical methods for analysis of CFX and DLX in biological fluid have been reported for single drug analysis. A thorough literature survey revealed reports on several analytical methods such as UV-VIS, HPLC, LC-MS, LC-MS-MS, for the determination of CFX[4–11] and DLX[12–20] alone or in combination with other antibiotics from dosage forms and biological samples.

HPLC method development was reported without the use of internal standard, which was found to be the limitation of method.[21] There are no reports on method developed for analysis of these two antibiotics in combinations from biological fluids like blood. The quantification methods in pharmacokinetic studies of the drug need to be sensitive and specific for simultaneous estimation. In view of above observations, the present investigation was undertaken to establish and validate a simple, versatile isocratic reversed phase HPLC-UV method for simultaneous estimation of CFX and DLX in human plasma using a simple extraction procedure.

2. Experimental

2. 1. Instruments

Isocratic high pressure liquid chromatography Cyberlab-chrom-HPLC, V4.0 (Cyberlabs, USA) with LC-P–100 pump, variable wavelength programmable UV/Vis detector LC-UV 100 and operating software cyberstore V4-0512-039 was used. The chromatographic separation was carried out by reverse phase Capcell pak C18 (Shiseido, Japan) DDS5 column (4.6 mm LD × 250 mm i.d. particle size 5 μm). The mobile phase consisted of a mixture 5 mM KH_2PO_4: Acetonitrile: Methanol (42:55:03 v/v/v) with flow rate of 1.0 mL min^{-1} and pH of phosphate buffer was adjusted to 5.4 using 0.1M ortho-phosphoric acid. The UV/Vis detector was set at 225 nm wavelength. An injection volume of 10 μL was used. Ezetimibe was used as an internal standard.

2. 2. Chemicals and Reagents

Cefixime and Dicloxacillin (Blok Pharma Pvt. Ltd, Kolhapur, Maharashtra, India), Ezetimibe (Smruthi Organics, Solapur, Maharashtra, India), acetonitrile (Merck Chemicals, Bangalore, India) and all other chemicals used were of analytical grade. Double distilled water was used for preparation of mobile phase solution. Blank (drug free) plasma was donated by Sangameshwar Teaching Hospital, Gulbarga, Karnataka, India.

2. 3. Preparation of Quality Control Sample

Stock solution containing 1mg mL^{-1} of CFX, DLX and ezetimibe (**IS Fig. 1c**) were prepared in mobile phase. Ezetimibe was further diluted with same solvent to get final concentration of 10 μg mL^{-1}. Further the stock solutions of CFX and DLX were diluted with mobile phase to obtain final concentration of 0.5, 1, 5, 10, 20, and 40 μg mL^{-1}. Stock solutions were stored in freezer maintained at –20 °C.

2. 4. Chromatographic Condition

The mobile phase consisting (5 mM) phosphate buffer: acetonitrile: methanol (42:55:03), (v/v/v) pH 5.4 was degassed and filtered by using Millipore vacuum filter system equipped with 0.45 μm membrane filter. Chroma-

tography was performed at an ambient temperature by pumping the mobile phase with a flow rate of 1.0 mL min^{-1}. The column effluent was monitored at 225 nm.

2. 5. Extraction Procedure

Calibration standards were prepared by adding 10 μL of the appropriate CFX and DLX working solutions (0.5–40 μg mL^{-1}) to 100 μL of blank plasma. Calibration standards, samples and controls were processed by adding 10 μL of internal standard working solution (10 μg mL^{-1}), 100 μL of 0.5 M phosphate buffer (pH 4.5) and 10 μL of 0.5 M HCl. The solution was vortexed for 2 min. Dichloromethane (1 mL) was added as an extracting agent. The solution was again vortex-mixed for 30s and centrifuged at 5000 rpm (2991 × *g*) for 15 min.

The supernatant was transferred to a clean, similarly labeled 10 mL glass conical centrifuge tube. The solvent was evaporated under nitrogen steam at 15 psi in water bath set to temperature of 40 °C. The dried extracts were reconstituted in 100 μL mobile phase. All tubes were vortex-mixed and 10 μL aliquots of the extracted solutions were injected into the HPLC system as per literature.[20]

2. 6. Method Validation

To develop a precise, accurate and reproducible HPLC method for the estimation of CFX and DLX in human plasma, various mobile phases, stationary phases and sample preparation methods were employed. The proposed chromatographic conditions were found to be appropriate for the quantitative determination. After optimization of the analytical conditions, the evaluation of the fundamental parameters, such as system suitability test, linearity, precision, accuracy, recovery selectivity, and stability were performed for the validation of optimized method.[22,23]

2. 6. 1. System Suitability Test

The system suitability test was performed before analysis of every batch of sample to ensure the reproducibility of the chromatographic system.[24] The HPLC system suitability test was performed by running six injections of diluted drug and IS in the linear region of the calibration curve and measuring the percentage relative standard deviation (% RSD).

2. 6. 2. Linearity

The linearity was studied using six concentrations as 0.5, 1, 5, 10, 20 and 40 μg mL^{-1} of CFX and DLX. Linearity experiment was performed six times to check the detector response to the drug to be linear in function with various concentrations (0.5 to 40 μg mL^{-1}). The working standards were prepared by adding different concentrations of CFX, DLX and fixed concentrations of IS (10 μg mL^{-1}) solution spiked in plasma to obtain the required concentration range. Samples were extracted and injected into the HPLC system. The drug/IS peak area ratio was plotted against the concentration of the drug and expressed in terms of coefficient of determination (r^2).

2. 6. 3. LLOQ (Sensitivity)

The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision. The LLOQ is considered being the lowest calibration standard. In addition, the analyte signal of the LLOQ sample should be at least 5 times the signal of a blank sample.[1,29]

2. 6. 4. Precision and Accuracy

Precision and accuracy of the developed method was determined by analysis of quality control (QC) samples at three different concentrations covering the low, medium and higher ranges of the calibrations curve. Intra-day variation of the assay was done by injecting six samples for each concentration on the same day. Inter-days variation was assessed by injecting nine sample of each concentration (on 15 days) over a period of two weeks. The precision of the method is expressed in terms of percent relative standard deviation (% RSD), and accuracy was expressed as a percentage of the theoretical concentration (observed concentration × 100 / theoretical concentration).[25]

2. 6. 5. Recovery

The recoveries for the CFX, DLX and IS were determined by spiking known amount of CFX, DLX and IS into drug-free human plasma to obtain three different concentration covering the low, medium and higher ranges of the calibration curves. Recoveries were determined by comparing the peak area of extracted QC samples with the peak area of recovery standards at the same nominal concentrations.[25]

2. 6. 6. Specificity

The specificity was verified by checking the interference of endogenous compound in human plasma at the retention time of the CFX, DLX and IS by evaluating six lots of plasma.

2. 6. 7. Stability

Short-term stability study of the analytes was evaluated in the following three different ways;

1) Human plasma samples were stored over-night at room temperature on the bench at 15–20 °C after the first injection cycle and then it was re-injected on the consecutive next day;

2) Human plasma samples were stored over-night in the freezer at –20 °C followed by unassisted thawing at room temperature.
3) Standard stock solutions were stored for 1 week at –20 °C in freezer, brought to room temperature and injected within 1 h after thawing.[22,26,27]

3. Results and Discussion

In the present study, dichloromethane was the solvent of choice, in order to obtain satisfactory values for recovery of CFX and DLX which showed good resolutions with no interferences peak. Hence, extraction with dichloromethane was optimized as the sample treatment procedure.[20] The mobile phase was optimized to provide sufficient selectivity towards the drugs. Phosphate buffer contributed high sensitivity and selectivity when compared with other buffers. Methanol and Acetonitrile as organic components resulted in better sensitivity. However, resolution and runtime of peak was affected by variation in the volume of organic component. Variation of buffer pH resulted in bad peak shape and increased interference from the plasma. Hence pH of buffer was adjusted at 5.4. The optimized mobile phase consisted 5 mM phosphate buffer

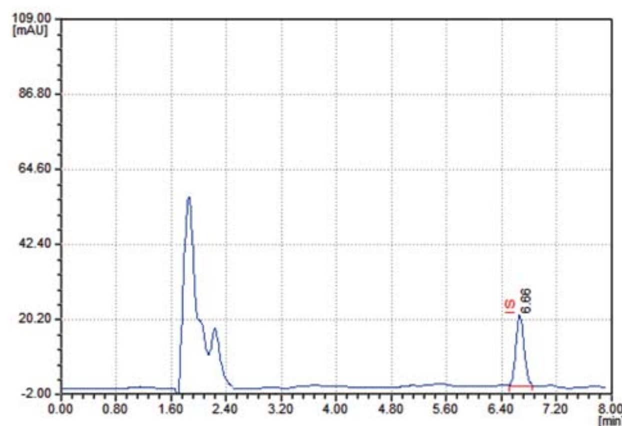

Fig 2. Typical chromatogram of human plasma and IS (ezetimibe)

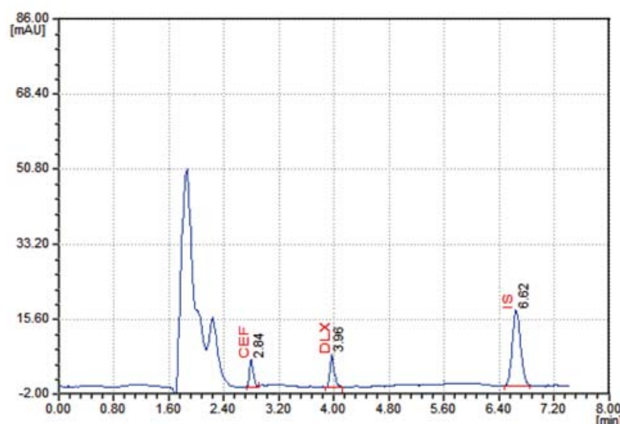

Fig 3. Typical chromatogram obtained for human plasma spiked with 1 μg mL^{-1} CFX and DLX and ezetimibe as IS

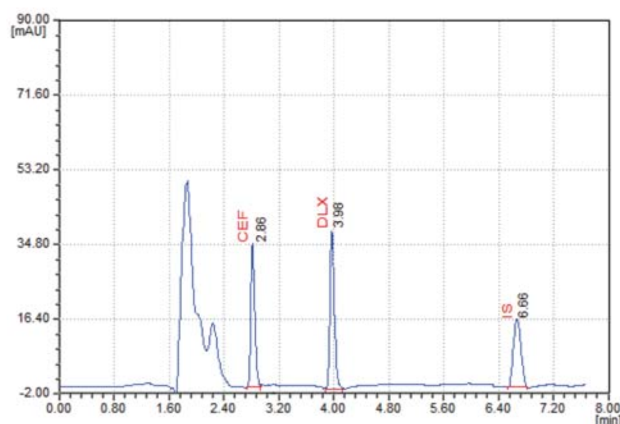

Fig 4. Chromatogram obtained for human plasma spiked with 10 μg mL^{-1} CFX and DLX and ezetimibe as IS

(pH 5.4): acetonitrile: methanol (42:55:03 v/v/v). Injection volume was optimized to 10 μL. The column temperature was maintained at 25 °C (ambient). Retention times were 2.88 ± 0.05 min for CFX, 3.96 ± 0.06 min for DLX and 6.64 ± 0.04 min for IS. The representative chromatogram human plasma with IS (ezetimibe) is depicted in **Fig 2**. Drug free human plasma was screened and interference

Table 1. Result from determination of system suitability test for CFX and DLX

Ob. No.	Concentration (μg mL^{-1})	Peak Area CFX	Peak Area DLX	Peak Area IS	Area Ratio CFX	Area Ratio DLX	Retention Time CFX	Retention Time DLX	Theoretical Plate CFX	Theoretical Plate DLX	Tailing Factor CFX	Tailing Factor DLX
1	10	192457	238834	163401	1.18	1.46	2.83	3.94	5134	4167	1.56	1.74
2	10	193723	239167	169843	1.14	1.41	2.84	3.94	4998	3955	1.54	1.72
3	10	189957	231598	161043	1.18	1.44	2.85	3.95	5004	4027	1.60	1.71
4	10	191709	240045	159854	1.20	1.50	2.83	3.93	5162	3956	1.60	1.74
5	10	190098	241673	159980	1.19	1.51	2.83	3.95	4956	4040	1.59	1.76
6	10	195564	241054	160012	1.22	1.51	2.83	3.95	5162	4014	1.54	1.75
		Mean			1.184	1.471	2.835	3.947	5069.333	4026.500	1.572	1.737
		S.D (±)			0.027	0.042	0.008	0.008	93.335	77.668	0.029	0.020
		RSD (%)			2.278	2.865	0.290	0.197	1.841	1.929	1.822	1.152

Table 2. Linear regression analysis of calibration curves (n = 6)

Drug	Linearity Range (μg mL^{-1})	Intercept (Mean ± S.D)	Slope (Mean ± S.D)	Coefficient of Determination (r^2) Mean ± S.D)
CFX	0.5–40	–116.09 ± 313455.73	19246.0723 ± 127.38	0.9999 ± 1.7238E–05
DLX	0.5–40	–326.49 ± 759.03	24131.1475 ± 109.22	0.9999 ± 2.5388E–05

of endogenous substances was not observed at retention time of CFX, DLX and IS which represented the selectivity of the method. **Fig 3** illustrates chromatogram of human blood plasma spiked with 1 μg mL^{-1} CFX, DLX and ezetimibe as IS. Chromatograms for 10 μg mL^{-1} of drugs and IS spiked blood plasma, are shown in **Fig 4**.

3. 1. System Suitability Test

Number of area ratio, retention time, theoretical plates, and tailing factor were also determined as a means of validation parameter. The values obtained are listed in **Table 1**. The % RSD calculated for the method was found to be less than 2%, which revealed the suitability of the developed method and the optimized chromatographic conditions. These values met the requirements of USP24/ NF19[28] and were therefore found to be satisfactory.

3. 2. Linearity

Calibration curves of CFX and DLX in human plasma demonstrated linearity in the concentration range from 0.5–40 μg mL^{-1}. The coefficients of determination were 0.9999 for CFX and 0.9999 for DLX. The results of the linearity experiment are listed in **Table 2**.

3. 3. LLOQ (Sensitivity)

The LLOQ was experimentally determined by diluting known concentrations of CFX and DLX in human plasma for six replicate determinations. The present assay method offered an LLOQ of 0.5 μg mL^{-1} for CFX and DLX in human plasma. Intra-day precision of the method was found to be 2.4863 and 2.4640% RSD and accuracy of the method was found to be 97.95% and 98.72% for CFX and DLX, respectively. Inter-days precision of the method was found to be 2.6091 and 2.8091% RSD and accuracy of the method was found to be 96.89% and 97.90% for CFX and DLX, respectively.

3. 4. Specificity

The absence of peak at migration time of CFX, DLX and IS indicates specificity of the developed method as shown in **Fig. 5**.

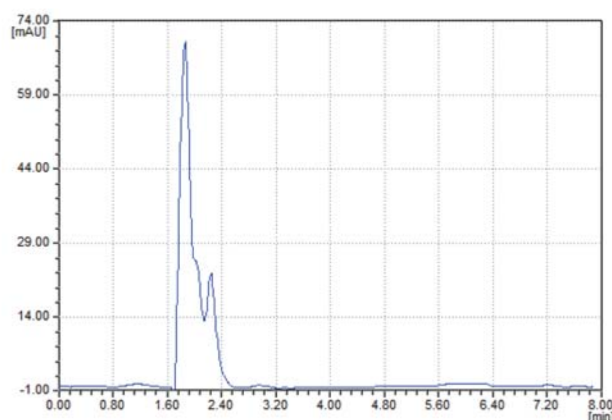

Fig 5. Typical chromatogram of human blank plasma

Table 3. Result from study of intraday and interdays precision and accuracy for CFX and DLX

Drugs	Concentration Added (μg mL^{-1})	Intra-day[a] Measured Concentration (mean ± S.D.)	Intra-day[a] RSD (%)	Intra-day[a] Accuracy (%)	Inter-days[b] Measured Concentration (mean ± S.D.)	Inter-days[b] RSD (%)	Inter-days[b] Accuracy (%)
	1	1.005 ± 0.019	1.935	100.50	1.007 ± 0.021	2.056	100.73
CFX	5	5.002 ± 0.072	1.432	100.03	4.994 ± 0.038	0.771	99.87
	10	9.998 ± 0.086	0.859	99.98	9.995 ± 0.044	0.443	99.95
	1	1.007 ± 0.020	1.974	100.74	1.004 ± 0.021	2.089	100.45
DLX	5	5.073 ± 0.083	1.633	101.46	4.991 ± 0.076	1.530	99.82
	10	10.076 ± 0.071	0.706	100.75	9.982 ± 0.056	0.559	99.82

[a] Mean values represent six different plasma samples for each concentration. [b] Interday was determined from nine different runs over two-week period. The concentration of each run was determined from a single calibration curve run on the first day of the study.

3. 5. Precision and Accuracy

The precision of the method was measured by the percentage relative standard deviation (% RSD) over the concentration range of high, middle and low QC samples respectively of drug during course of validation. Intra-day precision of the method ranged from 0.7058 to 1.9742% RSD. Inter-days precision of the method was found to be 0.4434 to 2.0895% RSD. Nominal values (%) for recovery of CFX and DLX from QC samples were tested of intra-day and inter-days. Intra-day accuracy ranges from 99.98 to 101.46% whereas Inter-days accuracy ranges from 99.82 to 100.73%. Result from determination of intra-day and inter-day, accuracy and precision are given in **Table 3**. Reproducibility of developed assay method was observed on same day and at different days. Relative standard deviation (% RSD) was found to be less than 15% for both samples over the concentration range assayed.

3. 6. Recovery

The recovery for the CFX, DLX and IS were determined by spiking known quantitative of CFX, DLX and IS into drug free human plasma to obtain three different concentration covering the low, medium and higher ranges of the calibration curve. The samples were then extracted and analyzed as described earlier. The recovery was calculated by comparing the peak areas of the drugs with those obtained from pure standards in mobile phase and IS in mobile phase at the same concentration.[25] The recovery of CFX and DLX ranges from 92.23 ± 1.4422 to 97.89 ± 1.4311%, while the absolute recovery for IS was 91.06 ± 0.6859% (**Table 4**).

3. 7. Stability

Low value of percentage difference (< 15) between area ratio for stability test samples and fresh QC samples confirm the stability of drug on the bench top for 2 h, in an auto sampler for 12 h and inside the freezer for 120 h. Results of stability are given in **Table 5.**

4. Conclusion

The developed RP-HPLC bioanalytical method is an accurate, specific and simple method for simultaneous determination of cefixime and dicloxacillin. The method involves simple extraction procedure, separation on a reversed phase column with an internal standard and UV/Vis detector. The validation data demonstrated good precision and accuracy, which proves the reliability of proposed method. Thus the method suits for routine therapeutic drug monitoring (TDM), specializes in the measurement of medication concentrations in blood for cefixime and dicloxacilline. It is also helpful in pharmacogenetic, demographic and clinical information, and/or on the a posteriori measurement of blood concentrations of drugs (pharmacokinetic monitoring) of cefixime and dicloxacilline in human plasma. The present developed method could be adapted for the determination of bioavailability and bioequivalence required for filing NDA and ANDA.

Table 4. Recovery study of CFX, DLX and IS

Drugs	Concentration Added (μg mL^{-1})	Recovery[a] (%mean ± S.D.)
	1	92.23 ± 1.442
CFX	5	95.03 ± 1.227
	10	96.99 ± 1.040
	1	95.11 ± 2.095
DLX	5	97.37 ± 1.585
	10	97.89 ± 1.431
IS		91.06 ± 0.686

[a] Mean values represent six different plasma samples for each concentration

5. Acknowledgment

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6. References

1. Guidance for Industry-Bioanalytical Method Validation, U.S. Department of Health and Human Services, USFDA, **2001.**

Table 5. Result from stability study for CFX and DLX

Statistical Properties	Bench Top Stability				Freeze Thaw Stability				Autosampler Stability			
	CFX		DLX		CFX		DLX		CFX		DLX	
	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC	LQC	HQC
Mean	0.9893	10.0419	1.0059	9.9979	0.9861	9.9760	0.9872	10.0132	0.9836	9.9716	0.9961	9.9893
S D ()	0.0174	0.0653	0.018	0.0585	0.0204	0.0239	0.0172	0.0444	0.0152	0.0243	0.0188	0.0294
C V ()	1.7617	0.6509	1.7894	0.5853	2.0757	0.2396	1.7462	0.4436	1.5524	0.2444	1.8963	0.2950
Nominal(%)	98.93	100.41	100.59	99.97	98.615	99.76	98.72	100.13	98.36	99.71	99.61	99.89

2. S. D. Bhinge, S. M. Malipatil, L. V. Sonawane, *Thai J. Pharma. Sci.*, **2012,** *36*, 63–71.
3. http://dmpharma.co.in/Cefixime,Dicloxacillin(ER).html dated 08/02/2013.
4. G. Rathinavel, P. B. Mukherjee, J. Valarmathy, L. Samueljoshua, M. Ganesh, T. Sivakumar, T. Saravanan, *E J. chem.*, **2008**, *5*, 648–651.
5. M. Fang, C. Xiaoyan, Z. Yalin, Z. Dafang, *J. Chromatogr. B*, **2005**, *819*, 277–282.
6. L. O. White, D. S. Reeves, A. M. Lovering, A. P. MacGowan, *J. Antimicrob. Chemother*., **1993**, *31*, 450–451.
7. C. M. Nahata, *J. Liq. Chromatogr*., **1991**, *14*, 3755–3759.
8. G. U. Liu, and R. G. Sha, *Yao Xue Xue Bao*, **1993**, *28*, 216–221.
9. J. A. McAteer, M. F. Hiltke, S. B. Michael, D. R. Faulkner, *Clini. Chem*., **1987**, *33*, 1788–1790.
10. A. Khan, I. Zafar, M. I. Khan, J. Khalid, A. Khan, L. A. Y. Shah, N. Fazli, *J. Chromatogr. B*, **2011,** *879*, 2423–2429.
11. E. Nemutlu, S. Kir, D. Katlan, M. S. Beksac, *Talanta*, **2009**, *80*, 117–126.
12. T. G. Barot, P. Kanhaiyalal, K. Nayan, N. Vyas, *E J. Chem*., **2009**, *6*, 955–964.
13. M. A. Ezzat, A. A. Mohammad, A. G. El-Rasheed, A K. Nashaat, *Talanta*, **1993**, *40*, 811–817.
14. M. A. Ezzat, *J. Pharma. Biomed. Anal*., **1991**, *9*, 187–190.
15. D. V. C. Awang, G. Lauriault, D. Kindack, *J. Chromatogr*., **1984,** *283*, 449–452.
16. W. J. Blanchflower, S. A. Hewitt, D. G. Kennedy, *Analyst*, **1994**, *119*, 2595–2601.
17. L. K. Sorensen, B. M. Rasmussen, J. O. Boison, L. Keng, *J. Chromatogr. B*, **1997,** *694*, 383–391.
18. L. K. Sorensen, L. K. Snor, T. Elkaer, H. Hansen, *J. Chromatogr. B*, **1999**, *734*, 307–318.
19. P. S. Rao, T. J. SunderRaj, C. H. Bhrti, K. RangaRao, G. K. A. Narayan, K. Parikh, *J. Pharma. Biomed. Anal*., **2007,** *43*, 1470–1475.
20. A. Oscar, F. G. Dinora, L. M. D. Rivero, C. T. Nelly, *J. Chromatogr. B*, **2004**, *805*, 353–356.
21. K. Kathiresan, R. Murugan, M. S. Hameed, K. Gokula, T. kanyadhara, *Rasayan J. Chem*., **2009,** *2*, 588–592.
22. Quality assurance of pharmaceuticals – a compendium of guideline and related materials, volume 1, *Universal Publishing*, India, **1992**.
23. L. R. Snyder, J. J. Kirkland, and J. L. Glajch, Practical HPLC method development, *John Weley and Sons*, USA, **1997**.
24. M. M. Baing, V. V. Vaidya, R. T. Sane, S. N. Menon, K. Dalvi, *Chromatogr*., **2006,** *64*, 293–296.
25. K. M. Patil, S. L. Bodhankar, *J. Pharma. Biomed. Anal*., **2005**, *39*, 181–186.
26. R. T. Sane, S. Menon, A. Y. Deshpande, A. Jain, *Chromatogr*., **2005,** *61*, 137–142.
27. M. T., Zzaman, S. A., Khan, A., Arora, O. Ahmad, *J. Biomed. Anal*., **2009**, *3*, 41–47.
28. United States Pharmacopoeia (USP 24/NF19), US Pharmacopoeial Convention, USA, **2000**.
29. EMA: Guideline on bioanalytical method validation, EMEA/CHMP/EWP/192217/2009, Committee for Medicinal Products for Human Use, **2011**.

Povzetek

Razvili in validirali smo točno, hitro in enostavno bioanalizno metodo z visokoločljivostno tekočinsko kromatografijo na reverzni fazi (RP-HPLC) za hkratno določanje cefiksima in dikloksacilina v človeški plazmi ob uporabi ezetimiba kot internega standarda. Cefiksim, dikloksacilin in interni standard smo ekstrahirali z ekstrakcijsko tehniko tekoče-tekoče. Kromatografsko ločbo smo izvedli z analizno kolono CAPCELL PAK C18 (4,6 mm × 250 mm, 5 m). Mobilna faza je bila sestavljena iz fosfatnega pufra, acetonitrila in metanola v razmerju 42:55:03. Detekcija in kvantifikacija je potekala z UV/Vis detektorjem pri 225 nm. Spodnja meja kvantifikacije je bila 0,5 μg mL^{-1} tako za cefiksim kot za dikloksacilin v človeški plazmi. Umeritveni krivulji za obe učinkovini v človeški plazmi sta bili linearni v koncentracijskem območju 0,5 to 40 μg mL^{-1}. Metodo smo kvantitativno ovrednotili s stališča linearnosti, natančnosti, točnosti, izkoristka, selektivnosti in stabilnosti. Ugotovili smo, da je metoda enostavna, prikladna in ustrezna za analizo cefiksima in dikloksacilina v bioloških tekočinah.