



Pharmacokinetics and Bioequivalence Study of Two Formulations of Cefixime in Healthy Male Volunteers

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Abstract

Cefixime is a significant member of orally active third generation cephalosporin and has excellent activity against many pathogens. The virtual bioavailability of a newly industrial dispersible tablet is compared with a recognized identified formulation. An open-label, single-dose, randomized, 2-way crossover study was conducted in fasted healthy Iranian male volunteers. Eligible participants were randomly assigned in a 1:1 ratio to be given one tablet (400 mg) of the test or reference formulation, followed by a 2-week washout period and administration of the exchange formulation. The study drugs were administered after a 10-hour overnight fast. A simple and available reversed-phase HPLC method with UV detection has been urbanized and validated for cefixime evaluation in human plasma using a C18 analytical column and a mobile phase of tetrabutylammonium hydroxide (pH=6.5)-acetonitril (3:1 v/v). The detection wavelength was 280 nm. The cefixime serum concentration time curves were used to find pharmacokinetic parameters counting AUC₀₋₂₄, AUC_{0-∞}, and C_{max}. In this method major linear response-concentration association all through the cefixime concentration range of 15-100 ng/ml, with the average accuracy within-run and between-run values of 97.29 and 99.27 percent, was observed. The average drug recovery from plasma was 98.2 percent throughout the linear concentration range. The limits of detection (LOD) and quantitation (LOQ) of the method were 5 and 15ng/ml, respectively. The mean (SD) C_{max}, T_{max}, AUC₀₋₂₄, and AUC_{0-∞} values after administration of the test and reference formulations, respectively, were in this manner: 4.68 (0.97) versus 4.72 (0.67) µg/mL, 4.28(0.86) versus 4.25 (0.73) hours, 43.37 (1.13) versus 43.39(1.22) µg/mL/h, and 60.17 (1.98) versus 59.96 (2.13) µg/mL/h. The mean (SD) t_{1/2} was 5.32 (2.01) hours for the test formulation and 5.34 (2.13) hours for the reference formulation. No statistical differences were showed for C_{max} and the area under the

plasma concentration-time curve for test and reference tablets. The calculated 90 % confidence intervals based on the ANOVA analysis for the mean test/reference ratios of C_{max}, AUC_{0-∞} and AUC_{0-24 h} of cefixime were in the bioequivalence range (97 %–119 %). The method is rapid, simple, very stable and specific for the partition, assignment, pharmacokinetic and bioavailability evaluation of cefixime healthy Iranian adult male volunteers.

Keywords: Bioequivalence, Cefixime, Formulations, HPLC, Human plasma, Pharmacokinetics.

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1. Introduction

Cefixime is measured as a significant and energetic member of third generation cephalosporin. The cefixime exists in off white crystals, melts over 220-250 °C and soluble in alcohol [1]. An orally active cefixime has outstanding activity against pathogens such as Anaerobes, Entero bacteria ceae, gram negative species such as Escherichia coli, klebsiella, Haemophilus influenzae, Branhamella Catarrhalis, Neisseria gonorrhoeae, Serratiamarcescens, Providencia, Haemophilus, and Meningococcus including b-lactamase producing strains [1-4]. The absolute oral bioavailability of cefixime is in the range of 22–54 % [5]. Protein binding of cefixime is concentration dependent in human serum only at very high concentrations which are not seen following clinical dosing [4]. The area under the time versus concentration curve is superior

by about 26.4 % and the C_{max} is bigger by around 20.7% compared to the tablet after doses of 400 mg. There are several investigations relating to the bioequivalence study of cefixime alone and in combination with other drugs in pharmaceutical planning and plasma by UV, HPLC, LC-MS, and HPTLC methods [6-12]. These methods engage spectrophotometric methods [13-14], voltammetric method [15], and capillary electrophoresis [16]. Though, HPLC is much more complicated technique as compared to in the past reported methods as it provides gangrenous information for all analytes along with consequent UV/VIS spectra concurrently, which is very useful tool for the analysis of unknown mechanism of a mixture. Based on our pervious works we study on bioavailability, and bioequivalence study of some drugs such as ezetimibe, amlodipine, atorvastatin, enalapril, cellcept by LC-MS and HPLC methods in human plasma [17-23]. This study was intended to evaluate the pharmacokinetics (PK) parameters, bioavailability, and bioequivalence of the cefixime of Exir tablets company versus the cefixime reference tablets Wyeth company as single 400-mg doses to healthy Iranian adult male volunteers. The method has been used effectively during a bioequivalence and PK parameters revise on a generic product of the

drug with the representative results being accessible in the final part of the article by HPLC methods in human plasma.

2. Materials and Methods

2.1. Materials

Cefixime test tablets (Exir), cefixime reference tablets (Wyeth) and cefixime reference standard (99.9% purity) were supplied and identified by Wyeth pharm. Other chemicals and solvents were from chemical lab or HPLC purity grades, whenever needed, and were purchased. Drug-free human plasma was provided by Iranian Blood Transfusion Organization after routine safety evaluations.

2.2. Instrument and HPLC Method

The HPLC system consisted of a double-reciprocating pump (Waters, model 600, MA, USA), a variable wavelength UV detector (Waters, model 2478, MA, USA) was used at a wavelength of 280 nm with the outputs recorded and analyzed using a compatible software (Millennium[®], Waters, MA, USA). The drug separation was determined using a C₁₈ analytical column (250mm×4.6mm, particle size 5µm⁺ Perfectsill, MZ-Analysentechnik, Germany) and arranged by a guard column of the same packing. The mobile phase composed of tetrabutylammonium hydroxide (pH=6.5)-acetonitrile (3:1) with a flow rate of 1 ml/min. Sample injection to system (50µl) was made by a loop injector (Rheodyne[®]7725i, Cotati, CA, USA).

2.3. Standard Preparation

A stock solution of 1000ng/ml cefixime in phosphate buffer (pH=7.4) was geared up, from which the concentrations of 15, 25, 50, 75, and 100ng/ml were prepared by serially diluting this solution with the proper amount of phosphate buffer (PH=7.4). A series of spiked plasma samples with cefixime concentrations of 15, 25, 50, 75 and 100 ng/ml were prepared by 1:10 dilution of the described solutions with drug-free human plasma.

2.4. Assay Procedure

To 150µL calibration standards, QC samples, or plasma samples, 12µL perchloric acid (HClO₄ 75% aqueous solution) and 400 µL methanol and 100 µL acetonitrile were added. The mixtures were vortex mixed for 20 s. After centrifugation at 15 000×g in an eppendorf micro centrifuge tubes for 20 min, 50µL of the supernatant was injected directly onto the analytical column for immediate HPLC analysis. A typical linear regression equation of the method was: $y = 8.5564 x + 0.0654$, with x and y representing cefixime concentration and peak height (in arbitrary units), respectively, and the regression coefficient (r) of 0.9916.

2.5. Estimation of Cefixime in Tablet Dosage Form

Each tablet contains 400 mg of cefixime. Twenty tablets were taken and weighed accurately. The average weight of one tablet was calculated and powdered. Equivalent to 400mg

of cefixime of powder was taken and transferred to a 100 ml volumetric flask and about 75 ml of phosphate buffer at pH 7.2 was added and sonicated to dissolve. The volume was made up to the mark with phosphate buffer. The solution was filtered through a membrane filter (0.22 μm) and sonicated to degas. Then 5 ml of above solution was pipetted out in 50 ml volumetric flask and volume was made up to the mark with phosphate buffer. The prepared solution was injected into the HPLC system and the observation was recorded.

2.6. Dissolution Test

The dissolution test was undertaken using tablet dissolution tester in 6 replicates for each brand. Dissolution media were USP buffer solutions at pH 7.2 (phosphate buffer solution). The medium was maintained at $37 \pm 0.5^\circ\text{C}$. In all the experiments, 5 ml of dissolution sample was withdrawn at 0, 10, 20, 30, 40, 50, and 60 min and replaced with equal volume to maintain sink condition. Samples were filtered and assayed by UV spectroscopic method. The concentration of each sample was determined from a calibration curve obtained from pure samples of Cefixime.

2.7. Method validation

This method was validated for selectivity, linearity, accuracy, precision, recovery, stability, repeatability of method, reproducibility, detection limit and quantitation limit according

to the principles of the FDA industry guidance in our pervious study for cefixime [24].

2.8. Pharmacokinetic study

As a fraction to bioequivalence revise, enrolling the twelve male subjects in a randomized, two-treatment, two-period, single-dose crossover study with washout between the first dosing in period I and the first dosing of period II. Single dose assign Subjects fasted from the night before dosing until 2 h after dosing for each assembly, The 400 mg cefixime formulation was administered and blood samples were obtained prior to dose administration (time 0) and at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 24.0 and 48.0 h after the dose for cefixime reference group. The blood samples were instantly centrifuged at $1600 \times g$ for 10 min. The plasma was detached and stored at -20°C until analysis was done. The pharmacokinetic parameters for cefixime were designed using standard non-compartmental methods. The peak serum concentration (C_{max}) and the time to reach it (T_{max}) were evaluated from visual examination of the data and used as criteria of the rate of absorption. The area under plasma concentration time (AUC_{0-t}) curve was determined by the linear trapezoidal rule from the measured serum concentrations from zero to time of the last quantifiable concentration (C_t). The apparent elimination rate constant (β) was determined by linear regression of log-transformed data in the terminal phase of the serum concentration-time profile [25]. The

elimination half-life ($t_{1/2}$) was considered by the quotient of $0.693/\beta$. The $AUC_{0-\infty}$, the area under the serum concentration-time curve extrapolated to perpetuity, was designed according to the following equation [25]: $AUC_{0-\infty} = AUC_{0-t} + Ct/Kel$. The pharmacokinetic profile of cefixime from two tablet formulations were compared and the comparative bioavailability of test/reference product was calculated using the ratio of $AUC_{0-\infty}$ (test)/ $AUC_{0-\infty}$ (reference). The pharmacokinetic parameters were statistically compared by analysis of variance (ANOVA) to evaluate the consequence of formulations, periods, sequences, and subjects. The 90 % confidence intervals were constructed for the ratio of the income of the test and reference products and were compared to the reference intervals (0.8–1.25) as recommended by the FDA [25].

3. Results and Discussion

3.1. Method Development

In response to lack of an accessible, consistent, and simple to use analysis method for cefixime assay in plasma as an vital part of pharmacokinetic and bioequivalence estimate projects on the drug we urbanized a simple and offered HPLC method with UV detection based on the available equipments found in most pharmaceutical laboratories. To this end, initially a series of isocratic as well as gradient conditions using different usual mobile phase compositions, polarities, ionic strengths, and pH values were tested in order to determine the best

condition for the analyte separation. Protein precipitation was necessary and important because this technique can not only purify but also concentrate the sample. Methanol, perchloric acid and acetonitrile were all attempted and acetonitrile: perchloric acid: methanol (75:5:20 v/v/v) was finally adopted because of its high extraction efficiency and less interference. Precipitation with and without adding 0.1M NaOH (100 μ l) were both tried, and obvious differences were not observed, so the precipitation using acetonitrile without adding 0.1M NaOH was used at last.

3.2. System Suitability Tests

The number of theoretical plates (N), peak symmetry, and retain ability (K') of the method for cefixime were 1296, 1.143, and 2.75, respectively. These data show that the developed method is of appropriate separation efficiency and peak shape, both of which are important factors in estimation of the chromatographic method outputs. Typical chromatograms produced from the developed method are shown in figure 1. Figure 1.A shows a representative chromatogram of a plasma sample obtained at 7 h from a subject who received a single oral dose (400 mg), no interferences of the analyte were observed and the retention times of cefixime was 6.67 min and the total HPLC analysis time was 7.21 min per sample. The HPLC chromatogram for a blank plasma sample indicating that no endogenous peaks at the retention positions of cefixime was shown in figure 1. B and the total

HPLC analysis time was 10.55 min per sample for blank.

3.2. Linearity

The method produced linear responses throughout the cefixime concentration range of 15-100ng/ml, which is suitable for intended

respectively, and the regression coefficient (r) of 0.9916.

3.3. Estimation of Cefixime in Tablet Dosage Form

The percent content of cefixime in tablet dosage form was found to be 100.65%. The USP

Figure 1.a

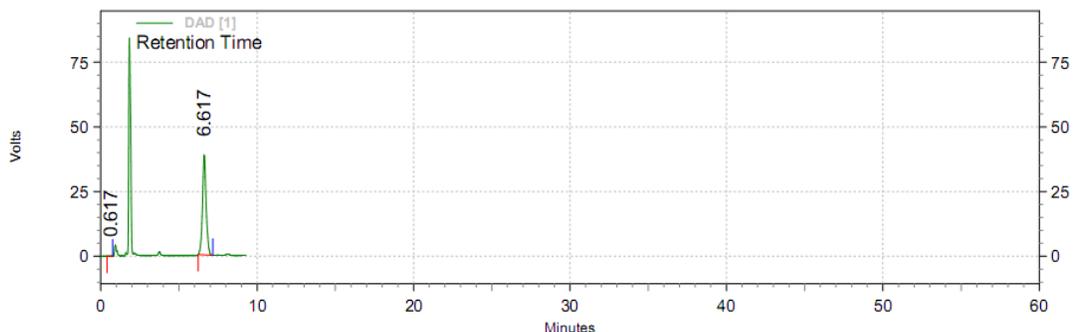


Figure 1.b

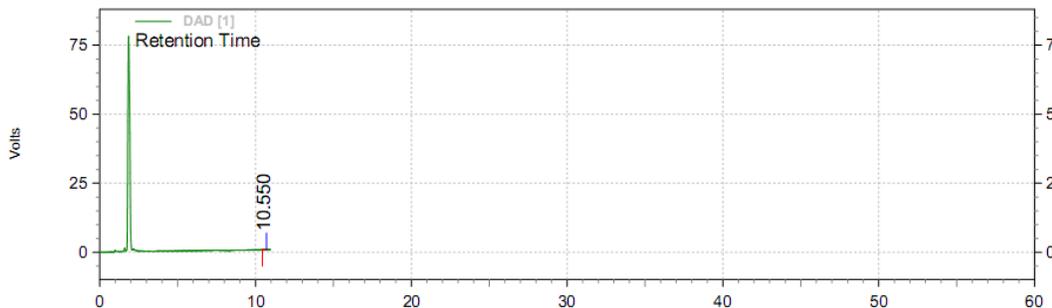


Figure 1. Typical chromatograms of the HPLC method developed for cefixime assay in human plasma: Fig. 2 A) chromatogram of a plasma sample obtained at 7 h from a subject who received a single oral dose (400 mg); Fig. 2.B the HPLC chromatogram for a blank plasma.

purposes. A typical linear regression equation of the method was: $y = 8.5564 x + 0.0654$, with x and y representing cefixime concentration (in mcg/ml) and peak height (in arbitrary units),

specifications for assay are that the cefixime content should be less than 90 % and not more than 110 %. Therefore, the assay results ascertain the presence and compendia quality of cefixime in all these products.

Table 1. The release date of test cefixime tablets with dissolution tester.

Time (min)	1	2	3	4	5	6	Mean	SD	RSD
5	63.8 5	56.89	82.09	45.98	76.55	72.87	66.37	13.4 5	20.2 7
10	90.7 3	87.61	108.7 4	89.66	91.49	92.18	93.40	7.68	8.23
15	92.1 7	87.85	96.50	91.49	96.78	100.4 6	94.21	4.54	4.82
20	96.5 0	90.97	98.42	92.18	97.01	102.0 7	96.19	4.09	4.25
25	98.4 2	105.6 2	99.14	95.86	98.85	100.9 2	99.80	3.28	3.29
30	98.6 6	109.7 0	99.86	95.86	99.08	99.54	100.4 5	4.75	4.73
45	102. 98	112.8 2	108.2 6	96.55	100.0 0	97.24	102.9 8	6.45	6.26
60	108. 98	103.2 2	108.0 2	100.6 9	102.9 9	97.24	103.5 2	4.42	4.27

Table 2. The release date of reference cefixime tablets with dissolution tester.

Time (min)	1	2	3	4	5	6	Mean	SD	RSD
5	52.57	48.97	93.86	46.67	51.03	55.63	58.12	17.77	30.58
10	85.45	96.50	94.34	91.72	87.36	89.66	90.84	4.19	4.61
15	91.69	97.94	95.30	93.33	93.79	95.86	94.65	2.19	2.31
20	96.50	100.34	95.78	97.47	98.39	99.54	98.00	1.76	1.79
25	98.42	107.30	96.50	97.93	98.85	99.54	99.76	3.83	3.84
30	99.62	98.42	101.30	99.31	105.29	101.61	100.93	2.46	2.44
45	112.82	110.42	113.30	97.93	98.85	101.84	105.86	7.11	6.72
60	115.22	113.30	113.30	102.07	103.91	101.15	108.16	6.43	5.95

3.4. In Vitro Drug Release Study

The release profiles of different brands of cefixime tablets are shown in figure 2 and table 1, 2. All dissolution data are based on the actual drug content of 10 min and almost 100% drug was released within 60 min from all the brands in phosphate buffer.

3.4.1. Applicability Test

The mean serum concentration-time profiles after single oral dose administration of reference and test formulations are shown in figure 3. As it is shown in figure 3, A. Pharmacokinetic profile of cefixime following oral administration of 400 mg test to healthy volunteers; Fig. 3. B. Pharmacokinetic profile of cefixime following oral administration of 400 mg reference to healthy volunteers; Fig. 3. C.

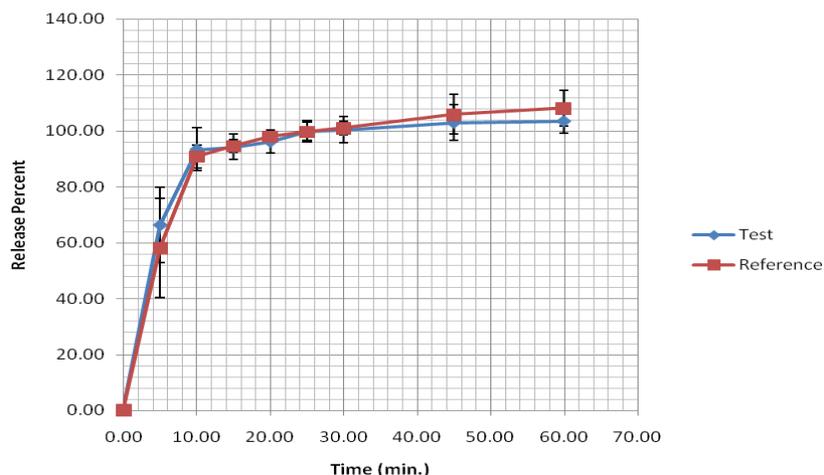


Figure 2. The release profiles of cefixime tablets.

Table 3. Pharmacokinetic parameters of cefixime after single oral dose of the test and reference product (Mean ± SD).

Pharmacokinetics Parameters	Test	Reference
C_{max} (µg/ml)	4.68± 0.97	4.72±0.67
t_{max}	4.28±0.86	4.25± 0.73
AUC_{0-24} (µg/L.hr)	43.37 ± 1.13	43.39± 1.22
$AUC_{0-\infty}$ (µg/L.hr)	60.17 ±1.98	59.96±1.67
MRT (hr.)	14.49 ± 1.21	13.97 ± 0.98
Cl (L/hr)	6.15 ± 0.43	6.23 ± 0.76
V_d (L)	101.32 ± 1.34	98.87 ± 1.43
$T_{1/2}$ (h)	5.32 ± 2.01	5.34 ±2.13

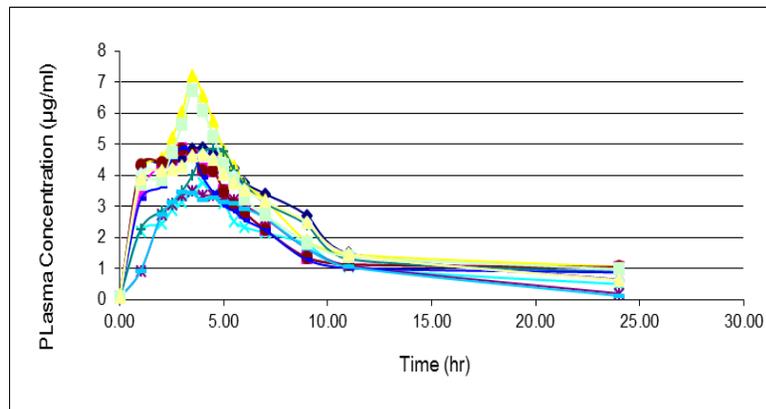
Comparative pharmacokinetic profile of cefixime following oral administration of 400 mg test and reference products to healthy volunteers (Linear scale). Figure 3. D. Comparative pharmacokinetic profile of cefixime following oral administration of 400 mg test and reference products to healthy volunteers (Log-Linear scale). Figure 3. C. shows linear scale the mean serum concentration-time profiles after single oral dose administration of reference and test formulations

of cefixime. As it is shown the mean serum concentration-time curves from the two tests and reference products are about super imposable. Furthermore, there was no important distinction between cefixime serum concentrations at each time point subsequent oral administration of the two formulations. Figure 3. D. shows Log-Linear scale of the mean serum concentration-time profiles after single oral dose administration of reference and test formulations of cefixime. As it is

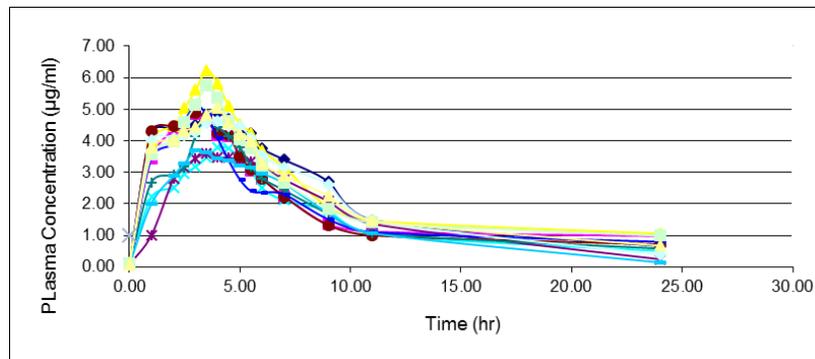
shown, the mean serum concentration-time curves from the two tests and reference products are about super imposable. Furthermore, there was no important distinction between cefixime serum concentrations at each time point subsequent oral administration of the two formulations. At the first case time (0.5 h), the drug was computable in all subjects following the administration of both arrangements.

The resulting pharmacokinetic parameters are shown in Table 3. Mean maximum serum concentrations of $4.68 \pm 0.97 \mu\text{g/ml}$ and $4.28 \pm 0.67 \mu\text{g/ml}$ were obtained for the test and reference formulations, respectively. Tmax, the

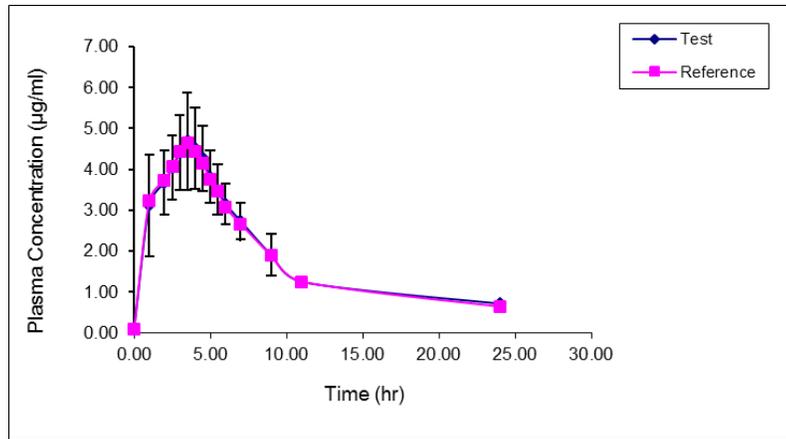
time required to reach the maximum serum concentration, was $4.28 \pm 0.86 \text{ h}$ and $4.25 \pm 0.73 \text{ h}$, respectively. Additionally to Cmax and Tmax, the ratio of $C_{\text{max}}/\text{AUC}_{0-\infty}$ also can be used as a parameter for determination the absorption rates in bioequivalence studies[26-27]. These calculated ratios were 7.77 % and 7.87 % for the test and reference formulations. The parameters used as procedures of the amount of absorption are AUC_{0-24} , $\text{AUC}_{0-\infty}$. The AUC_{0-24} and $\text{AUC}_{0-\infty}$ for the test formulation were $43.37 \pm 1.13 \mu\text{g}\cdot\text{h/ml}$ and $60.17 \pm 1.98 \mu\text{g}\cdot\text{h/ml}$, respectively. The considered values for the reference formulation were $43.39 \pm$



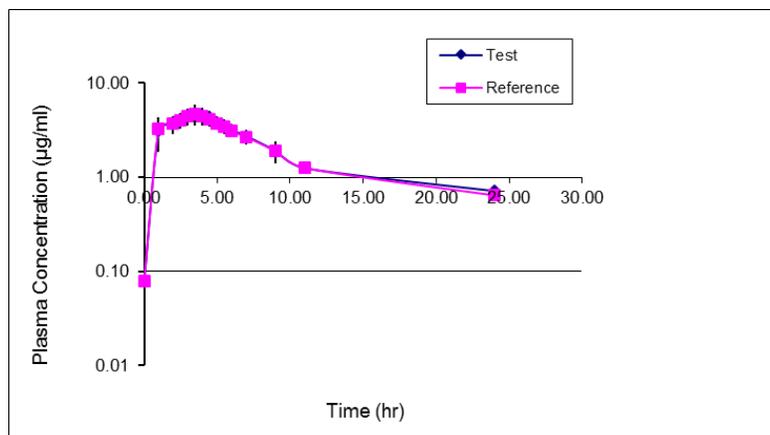
A



B



C



D

Figure 3. A Pharmacokinetic profile of cefixime following oral administration of 400 mg test to healthy volunteers; Figure 3. B. Pharmacokinetic profile of cefixime following oral administration of 400 mg reference to healthy volunteers; Figure 3. C. Comparative pharmacokinetic profile of cefixime following oral administration of 400 mg test and reference products to healthy volunteers (Linear scale). Figure 3. D. Comparative pharmacokinetic profile of cefixime following oral administration of 400 mg test and reference products to healthy volunteers (Log-Linear scale).

1.22 µg·h/ml and 59.98 ± 1.67 µg· h/ml in the order mentioned.

The confidence limits shown in Table 4 reveal that these values are completely within the bioequivalence acceptable range of 80–125 % planned by the FDA and European

Medicines Evaluation Agency (EMA) [25]. The multivariate analysis proficient through analysis of variance (ANOVA) indicated that there were no statically differences between the two formulations with any of the pharmacokinetic parameters. Furthermore,

Table 4. Analysis of variance (ANOVA) results.

Pharmacokinetics Parameters	ANOVA(P-value)	90% CI
C_{max}	1.01	97-105
t_{max}	1.15	96-112
AUC_{0-24}	1.01	98-105
$AUC_{0-\infty}$	1.09	99-119

periods and sequence property did not influence the outcome of the statistical analysis.

4. Conclusion

An easy HPLC method was urbanized and validated for cefixime evaluation in plasma. The plasma training for analysis consisted of a solid-phase extraction using a commercially available cartridge and the analyte detection was made by a fixed-wavelength UV detector. The validation tests on the developed method indicated acceptable degree of linearity, sensitivity, precision, accuracy, and recovery for the method. The method was used successfully for quantization of cefixime in plasma samples of healthy volunteers throughout a bioequivalence study. System appropriateness tests showed that the residential method is of suitable separation competence and peak shape. In the light of the obtained results of the studies reported here it can be concluded that the cefixime test and reference formulations are bioequivalent in terms of rate and extent of absorption.

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