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Research Article

Cefixime and Management Protocols for COVID-19 Second Wave. Does its Accurate Detection in Human Plasma Make Sense?

Sara AS[2], Mohamed Raslan[1,2], Eslam MS[2] and Nagwa A Sabri[1]*

[1]*Department of Clinical Pharmacy, Faculty of Pharmacy- Ain Shams University, Cairo, Egypt*

[2]*Drug Research Centre, Cairo, Egypt*

***Corresponding Author:** Nagwa A Sabri, Department of Clinical Pharmacy, Faculty of Pharmacy- Ain Shams University, Cairo, Egypt.

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Abstract

Background: Cefixime is a cephalosporin antibiotic used for treatment of gonorrhoea and infections of the respiratory and urinary tracts.

Aim: Establishment of a bio-analytical method in order to quantify cefixime in plasma and investigate cefixime pharmacokinetics in human plasma and its application in clinical studies including comparative bioavailability studies.

Methods: After extraction of cefixime from human plasma, it was chromatographed with mobile phase consisting of methanol: 10mM ammonium acetate: acetonitrile (4/16/80) v/v/vat flow rate 0.63ml/min, ESI positive mode, and m/z 454→285, 396.1→277.2 for cefixime and cefdinir as an internal standard respectively. Clinical application as a bioequivalence study involving 26 volunteers in a crossover pattern and pharmacokinetic parameters AUC_{0-t}, $AUC_{0-inf,}$ C_{max}, and T_{max} were used for assessment of bioequivalence of generic and reference products

Results: The developed bioanalytical method showed that the average recovery of Cefixime from human plasma was 92.259%. The limit of quantitation was 0.1ug/ml, and the correlation coefficient (r^2) was 0.9994. Analysis of variance showed that there was no significant difference between generic and reference products.

Conclusion: The LC/MS/MS method presented is direct, simple, reproducible, sensitive, and linear for determination of cefixime in human plasma, and is suitable for clinical pharmacokinetic studies, clinical trials, and monitoring drug levels in plasma to ensure clinical efficacy and safety and to avoid therapeutic failure or incidence of adverse events. Moreover, generic product was found to provide the same rate and extent of drug absorption as the reference product.

Keywords: Cefixime; Plasma; COVID-19

Introduction

Cefixime is an orally active 'third generation' cephalosporin and has a longer duration of action than the other cephalosporins which are active by mouth and is licensed for acute infections [1]. It is given orally to treat infections due to susceptible gram-positive and gram negative bacteria, including gonorrhoea and infections of the respiratory and urinary tracts [2].

Cefixime is marketed in the form of hard gelatin capsules and tablets as 200mg and 400mg, under brand name Suprax® 200 mg and 400mg hard gelatin capsules and tablets [1,3], it is indicated for the treatment of the following conditions: uncomplicated urinary tract infections, otitis media, pharyngitis and tonsillitis, acute exacerbations of chronic bronchitis, and uncomplicated gonorrhea (cervical/urethral) [4].

Cefixime is recommended dosing for acute infections due to sensitive gram-positive and gram-negative bacteria is 200 to 400mg per day as a single dose or 2 divided doses, and a single 400-mg dose is given for uncomplicated gonorrhea [1,2].

A study was designed to assess the clinical efficacy, bacteriological eradication rates and tolerability of cefixime in children with community-acquired upper respiratory tract infection (URTI), lower RTI (LRTI) and uncomplicated urinary tract infections (UTI). Treatment with cefixime was successful in (100%) of patients suffering from acute otitis media (AOM), in (83.3%) with acute sinusitis, in (100%) of patients with pneumonia, in (88.57%) with uncomplicated UTI. The antibiotic was well tolerated, and considerd as a good choice for a successful clinical response [5].

Another study conducted in an African country showed that cefixime was found to be superior to ciprofloxacin in terms of efficacy in the treatment of community-acquired pneumonia in adults. The study included 73 patients were 39 (53.4%) patients in the cefixime group and 34(46.6%) in ciprofloxacin group. On day 7, patients on cefixime had a statistically significant lower temperature than patients on ciprofloxacin ($P < 0.01$). Bacteria cure was obtained in 96% of the patients in the cefixime group and 83% in the ciprofloxacin group [6].

Preliminary studies have shown excellent clinical outcomes with switch therapy to cefixime after 2–3 days for a variety of serious infections. Switch therapy, or step-down therapy, is the concept of switching from an intravenous antibiotic to an oral preparation after a few days, once the condition of the patient has improved and the pathogen and its susceptibility have been determined. Importantly, dramatic cost benefits have also been found, particularly with respect to reduced length of hospital stays [7].

After single dose administration of cefixime 400mg tablet, mean cefixime C_{max}, $AUC_{o\text{-}inf}$, $T_{1/2}$ was 4408.2150 ± 1021ng/ml, 39727.7618 ± 8405ng.hr/ml, 3.9582 ± 0.78266hr respectively. Mean T_{max} was equal to 4.3208 ± 1.26345 hour [8]. In a public assessment report of cefixime 400mg tablet, the following data for reference product was obtained, as follows: mean C_{max}, $AUC_{o\text{-}inf}$, $T_{1/2}$ was 6.11 ± 1.35ug/ml, 57.5 ± 12.9ug.hr/ml, and 4.3 ± 0.5hr respectively, and median T_{max} was 4.5hr [9]. In a comparative bioavailability study of cefixime 400mg tablet, the mean values of C_{max}, $AUC_{0\text{-}t}$, $AUC_{o\text{-}inf}$, $T_{1/2}$, and T_{max} were 4.72 ± 0.67ug/ml, 43.39 ± 1.22ug.hr/ml, 59.96 ± 1.67ug.hr/ml, 5.34 ± 2.13hr, and 4.25 ± 0.73hr respectively [10]. In a bioequivalence study of cefixime 400mg tablet versus 400mg capsule and 400mg solution, the capsule formulation results showed mean C_{max}, $AUC_{0\text{-}t}$, $AUC_{o\text{-}inf}$, $T_{1/2}$, and T_{max} of 3.39 ± 1.08 ug/ml, 23.7 ± 7.12 ug.hr/ml, 23.9 ± 7.22 ug.hr/ml, 3.0 ± 0.46 hr, and 4.2 ± 0.80hr respectively [11].

Different analytical methods are investigated and developed for evaluation of cefixime in pharmaceutical dosage form and human plasma; those methods include, HPLC, HPTLC, LC-MS [12-17], spectrophotometric [18], voltametric [19], and capillary electrophoresis [20] methods.

An analytical method used for determination of cefixime in biological samples using HPLC-UV method and sample extraction with protein precipitation procedure using 6 % trichloroacetic acid (TCA), shows a lower quantitation limits LLOQ of 83ng/ml [21]. Another analytical method used for determination of cefixime in biological samples using HPLC-UV method, and the same sample extraction procedure shows a lower quantitation limits LLOQ of 100ng/ml [22].

To obtain a better sample clean up, an analytical method used a liquid extraction for sample preparation procedure, and LC/MS/MS for detection shows a lower quantitation limits LLOQ of 114.5ng/ml [23].

For a sensitive determination of cefixime in biological samples, an analytical method using LC/MS/MS is used, after protein precipitation of human plasma with acetonitrile, the compounds were chromatographed on a reversed phase zorbax eclipse XBD C18 column with a mobile phase of acetonitrile, methanol and 0.5% formic acid in a ratio of 23:10:67% V/V/V. Mass parameters were set on single ion monitoring mode (SIM) and positive ESI, using the respective mass to charge ratios, m/z 453.8 for cefixime, and m/z 402.0 for moxifloxacin as an internal standard with a quantitation limit (LLOQ) of 40 ng/ml and a linear dynamic range of 40 ng/ml to 6000ng/ml [24].

For more linear dynamic range a sensitive LC/MS/MS assay developed in which drug extracted by protein precipitation from human plasma, where, chromatographic separations were achieved on a C_8 column using mobile phase of acetonitrile–water–formic acid (40:60:0.5 v/v/v). The method was fully validated. The multiple reaction monitoring was based on m/z 454→285 for cefixime, m/z 398→241 for cefetamet. The lower limit of quantitation LLOQ was 0.05 ug/mL and linear over a concentration range from 0.05 to 8 ug/mL [25].

A conducted comparative bioavailability of cefixime 400mg hard gelatin proved that the method is accurate, precise, selective and fully validated. The study was conducted on 26 healthy volunteers as per protocol. The subjects received one hard gelatin capsule of generic product and one hard gelatin capsule of reference product, in a randomized fashion with a washout period of one week. Analysis of plasma would be done through developing and validating an LC/MS/MS method in compliance with the international guidelines [27]. WinNonlin program was used to perform pharmacokinetics calculaions, and SAS software was used to perform statistical analysis. The 90% C.I. for AUC_{0-t}, AUC_{0-inf}, and C_{max} were calculated for the ratio between treatments and results showed to be in the limit of 80% to 125% confidence limits [28].

Materials

Chemicals and reagents

Purified Water for LC/MS/MS grade, Human plasma (Vacsera Blood Bank), Methanol (SIGMA Aldrich, Germany), Acetonitrile (Scharlab, Spain), Ammonium Acetate (Scharlab, Sapin).

Equipment

Adjustable pippettes (P200, and P1000), disposable plastic pipettes tips, labtip yellow (range 5 - 200 µL) and labtip blue (range 200 1000 µL), disposable glass test tubes 120 x 12 mm, vortex mixer (Boeco, Germany), vacuum pump (Boeco, Germany), PH-meters (Boeco, Germany), water purifier (Purelab option- R7ELGA, U. K.), sonicator (Crest, U.S.A.), analytical balance (Sartorius, U.S.A.), concentrator plus/vacufuge® plus (Eppendorf, Germany), LC-MS/MS Agilent 6410B Triple Quad, USA.

Methods

The bioanalytical method prameters

Chromatographic conditions

In house developed chromatographic conditions was used, and mobile phase composition is methanol: 10mM ammonium acetate: acetonitrile (4/16/80) v/v/v. The flow rate was set at 0.63ml/min. Injection volume was set at 2ul and MS/MS 6410B detector was operated at ESI positive mode, m/z was 454→285, 396.1→277.2 for cefixime and cefdinir as an internal standard respectively.

Fragmentor energy was set at 120 for cefixime and cefdinir and collision energy was set at 10 for cefixime and cefdinir.

Preparation of solutions

Cefixime master standard solution

Accurately weighed 11.19mg of cefixime trihydrate working standard (equivalent to 10mg cefixime base) were transferred to a 100 ml volumetric flask, about 80 ml methanol was added and sonication was done for 10 minutes, and volume was completed with methanol in order to obtain a solution containing 100ug/ml cefixime "Solution A".

From "Solution A" the following were prepared: Working Solutions

Solution used	Volume taken	Conc. obtained	Final volume (ml)
"Solution A"	100ul	1ug/ml	10
"Solution A"	250ul	2.5 ug/ml	10
"Solution A"	500ul	5 ug/ml	10
"Solution A"	1ml	10 ug/ml	10
"Solution A"	2ml	20 ug/ml	10
"Solution A"	4ml	40 ug/ml	10
"Solution A"	6ml	60 ug/ml	10
"Solution A"	8ml	80 ug/ml	10
"Solution A"	10ml	100 ug/ml	10

Table a

Cefdinir standard solution

Accurately weighed 10mg of cefdinir standard were transferred to a 100 ml volumetric flask and about 80 ml of methanol were added, sonication was done for 10 minutes and the volume was completed with methanol to obtain a solution contains 100ug/ml cefdinir solution (A), from which 50 ml was transferred to a 100ml volumetric flask and volume was completed with methanol to obtain 50 ug/ml cefdinir solution (B).

Preparation Serial Dilutions of Standard Cefixime in human plasma

The standard samples in plasma were prepared by transferring a 20 ul aliquot of prepared working solutions of cefixime at concentrations ranging from 1 to 10 ug/ml to a centrifuge tubes containing 200 ul of blank plasma.

Sample preparation

Volunteers human plasma samples (200 ul) were transferred into appropriate centrifuge test tubes 20 ul of the internal stan-

dard (cefdinir working solution 50 ug/ml) was added, then vortex-mixed for approximately 30 seconds, 1ml of methanol was added and vortex-mix for approximately 1 to 2 minutes. Centrifugation of samples was done at 4000rpm for 5 minutes at 4°C and the clear supernatant layer was transferred to vials for injection and quantitation on LC/MS/MS device.

Quantitation

Unknown drug concentrations of plasma samples withdrawn were calculated using the following equation: y = ax + b, where; Y: response ratio, X: unknown concentration of drug in plasma samples, a: calibration curve slope, b: Y- Intercept.

Bioequivalence study

Study ethics

This study was conducted in accordance with the ICH and GCP guidelines adopted by the european agency for the evaluation of medicinal products (EMEA) and after Ethics Committee approval on the bioequivalence study protocol of cefixime 400mg capsule (Study Code: AER-RIVP-BES-0417/247). Essential documents and records were all archived according to drug research center (DRC) internal procedures for authorized direct access.

Written informed consents were signed by the participant, clinical investigator, and other responsible persons. All study aspects where discussed with participants before starting of screening without any obligations on volunteers to continue the study if they didn't want to.

Clinical Investigator and study director (principal investigator) were responsible for supervising of all study procedures, licensed physicians were responsible for physical examination and following-up of the subjects for the appearance of any side or adverse effects, measurement of vital signs throughout the study including blood pressure, pulse rate, body temperature, respiratory rate before and all over the study and registered nurses were responsible for blood sampling.

Inclusion criteria

Volunteers age should be within 18 to 55 years and calculated body mass index should lie within normal acceptable limits, no history of contribution in any pharmacokinetics study, normal physiological examination, and laboratory data were within normal limits. Subjects should not be alcoholic or drug abusers and shouldn't have any known history for both. It is preferred to select non-smoker subjects and if subjects are smokers, they should not smoke more than 8 cigarettes per day.

Exclusion criteria

A known drug hypersensitivity, GIT problems, auto-immune diseases, kidney diseases or kidney dysfunction, CVS diseases, diabetics, hepatic disease, hematological abnormalities, respiratory diseases, alcohol intake or drug abuse history, positive HIV-I, (smoking and if including they should be identified), abnormal laboratory values, subject administered any medication less than two weeks of the study starting date, subjects who have donated blood or who participated in clinical studies that requires more than 500 ml of blood to be withdrawn within month and half preceding study starting date.

Subjects

Twenty-six healthy adult volunteers participated in the comparative bioavailability study after being subjected to complete medical and laboratory assessment, and ensuring that they were in compliance with the required inclusion/exclusion criteria. Concurrent medications were not allowed during the study time course, no food intake was allowed for four hours after study dose administration. At 11:30 they received a standard meal and at 15:30 a second standardized meal was introduced.

Study design

The design of this study was a randomized two-way crossover design comparing the bioavailability of generic versus reference cefixime 400 mg capsules in 26 healthy adult volunteers under fasting state with a washout period of two weeks. The number of required blood samples and their disposition after collection, besides, the required wash out period were designed according to cefixime pharmacokinetics.

Sample collection

The number of blood collections for drug analysis was 16 X 5ml blood samples in each study period at the following intervals; 0 (directly prior to dosing), 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 10, 12, and 24 hours after drug administration, where, the total amount of blood withdrawn during the whole study did not exceed 160 ml.

Blood sample collection was performed into a tubes containing anticoagulant EDTA disodium and centrifuged at approximately 4000 r.p.m. for 10 minutes, plasma samples were separated in a plastic wassermann tubes and were kept at -80°C until analysis.

Analysis of plasma samples

The withdrawn volunteers' samples were analyzed by using LC-MS/MS technique for the quantitation of cefixime in human plasma.

Pharmacokinetic calculations

The following pharmacokinetic parameters (variables) of cefixime were assessed; C_{max}, t_{max}, $t_{1/2e}$, K_e, and AUC_{0-t}, and AUC_{0-inf}.

Statistical analysis

Analysis of variance (ANOVA) were performed by using SAS software, where, the bioequivalence could be demonstrated for cefixime within the prescribed 90% confidence interval of 80.00% to 125.00% for AUC_{0-t}, AUC_{0-inf} and C_{max} with respect to the parametric method on Ln-transformed data.

Results

Validation of the bio analytical method

- **Chromatograms of Cefixime:** Cefixime were well separated and their retention time was 1 minute, sharp, and symmetrical peaks were obtained with good baseline resolution and minimum tailing, thus facilitating the accurate measurement of the peak responses.
- **Linearity, Accuracy and Precision**: Peak area ratios of varying amounts of cefixime in human plasma from 0.1 to 10 ug/ml was highly linear with correlation coefficient (r^2) of 0.9994. The results of intraday precision C.V. % was 0.501% which is in accordance with the FDA Guidelines [27]. Accuracy and precision was assessed at three different concentrations in the range of predicted drug concentrations on within and between-day basis. Intra-day and inter-day accuracy results showed an average recovery percentage of 98.329% and 97.871% with an average C.V. % of 0.501%. Results of stability study showed an average stability percentage greater than 95% ensuring that cefixime was stable in the studied conditions.

Bioequivalence study

- **Clinical Observation (Safety and Tolerability):** The drug was well tolerated by all participating subjects and blood sampling was obtained during the two periods completely at the proper time. No side effects or adverse events were reported during the study time course. Moreover, the recorded measurement of subjects' vital signs during the two periods of the study were in normal ranges without any reported abnormality.
- **Pharmacokinetic Data and Assessment of Bioequivalence:** The mean values of C_{max}, t_{max}, $t_{1/2e}$, AUC_{0-t}, and $AUC_{0-\infty}$ were 4.519 ± 0.914ug/ml and 4.510 ± 0.869ug/ml, 3.904 ± 0.693hr and 3.923 ± 0.659hr, 3.864 ± 0.413 hr and 3.873 ± 0.552hr, 24.659 ± 7.504 ug.hr/ml and 24.919 ± 8.016 ug.hr/ml,25.374 ± 7.554ug.hr/ml and 25.626 ± 7.981 ug.hr/ml for generic and reference products respectively.
- **Statistical Analysis:** The results of 2-way ANOVA on C_{max}, AUC_{0-t}, and AUC_{0-inf} for cefixime showed that there was no significant difference between generic and reference product. The point estimate (%) results for C_{max}, AUC_{0-t}, AUC_{0-inf} were 99.992%, 99.139%, and 99.131% respectively. The 90% C.I. of parametric means of C_{max}, AUC_{0-t}, and AUC_{0-inf} were 90.544% to 110.426%, 84.647% to 116.112%, and 84.272% to 116.610% respectively.

Discussion

The LC/MS/MS method used in this study was simple, of excellent sensitivity and specificity, precision and accuracy. The method was linear over the range of 0.1 to 10 ug/ml and r^2 was 0.9994 which is in accordance with FDA Guidelines [27], moreover, the developed bioanalytical method could be applied in different clinical applications, including; pharmacokinetics and bioavailability studies, clinical trials, and therapeutic monitoring of cefixime to insure achievement of therapeutic goals.

The developed in-house bioanalytical method provides a linear dynamic range more than those reported in the literature [23-25]. Besides, it is nearly in accordance with them [23-25] after modification in extaction and chromatographic conditions method. The used extraction method in our developed method was simple step protein precipitation.

The results of cefixime pharmacokinetic parameters obtained was nearly in accordance with reported literature which stated that T_{max} were found to be 4.2 hours in average (range from 3 to 5.5 hours), C_{max} 4.4ug/ml in average (ranged from 3.39 to 6.11 ug/ml), $T_{1/2}$ 4.3 hours in average (ranged from 3 to 5.3 hrs) [8-11].

The importance of therapeutic monitoring of cefixime was being emerged from being cephalosporin antibiotic that only licensed for use in acute infections [1]. Additionally, therapeutic monitoring ensures that patients' drug levels are within the required therapeutic range and absence of subtherapeutic levels or toxic levels which increases the incidence of adverse events.

In the current bioequivalence study, the 90% confidence interval of 80% to 125% for AUC_{0-t}, AUC_{0-inf} and C_{max} on Ln-transformed data should be fulfilled. In this study the point estimate (%) results for C_{max}, AUC_{0-t}, AUC_{0-inf} were 99.992%, 99.139%, and 99.131% respectively. The 90% confidence intervals of parametric means of C_{max}, AUC_{0-t}, and AUC_{0-inf} were 90.544% to 110.426%, 84.647% to 116.112%, and 84.272% to 116.610% respectively, thus providing a 90% C.I. limits lying within FDA acceptance limits (80 % to 125%) [28].

Subject	T_{max} (hr)	C_{max} (ug/ml)	AUC_{0-t} (ug.hr/ml)	AUC_{0-inf} (ug.hr/ml)	K_{el} (hr^{-1})	$T_{1/2}$ (hr)	MRT_{inf} (hr)
Mean	3.923 ± 0.659	4.510 ± 0.869	24.919 ± 8.016	25.626 ± 7.981	0.182 ± 0.022	3.873 ± 0.552	7.104 ± 0.428
C.V.%	16.790	19.261	32.170	31.143	12.278	14.255	6.027
Range	3.00- 5.500	3.280-6.522	15.938-47.000	16.586-47.504	0.116-0.229	3.020-5.975	6.203-7.811
(Median)	(4.000)	(4.209)	(23.021)	(23.729)	(0.177)	(3.921)	(7.114)

Table 1: Pharmacokinetics of Cefixime Reference Product following administration of single oral dose given to 26 Volunteers.

Subject	T_{max} (hr)	C_{max} (ug/ml)	AUC_{0-t} (ug.hr/ml)	AUC_{0-inf} (ug.hr/ml)	K_{el} (hr^{-1})	$T_{1/2}$ (hr)	MRT_{inf} (hr)
Mean	3.904 ± 0.693	4.519 ± 0.914	24.659 ± 7.504	25.374 ± 7.554	0.181 ± 0.018	3.864 ± 0.413	7.102 ± 0.595
C.V.%	17.754	20.229	30.430	29.769	9.773	10.695	8.372
Range	3.00-5.500	3.209-6.475	14.098-47.024	15.006-47.559	0.135-0.220	3.144-5.144	6.170-8.613
(Median)	(4.000)	(4.247)	(22.362)	(23.038)	(0.183)	(3.795)	(7.093)

Table 2: Pharmacokinetics of Cefixime Generic Product following administration of single oral dose given to 26 Volunteers.

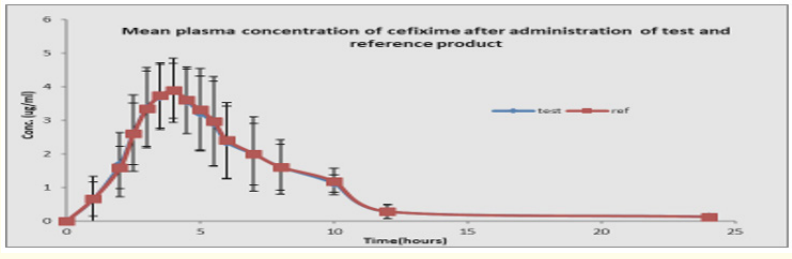

Figure 1: Mean plasma concentration following single dose administration of generic (test) and reference products of cefixime 400mg hard gelatin capsules.

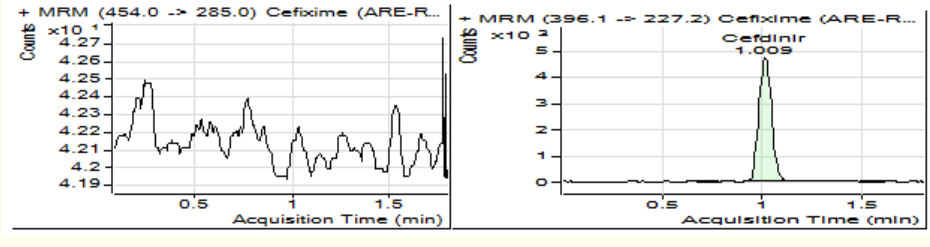

Figure 2: Chromatogram representing an MRM data of blank plasma sample spiked with internal standard cefdinir.

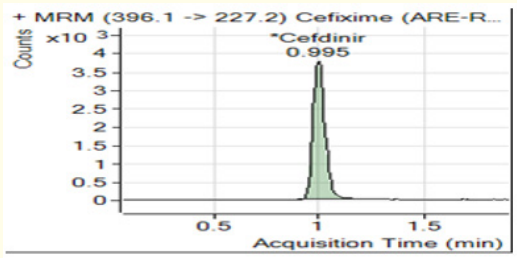

Figure 3: Chromatogram representing an MRM data of blank plasma sample spiked with 0.1ug/ml cefixime and internal standard Cefdinir.

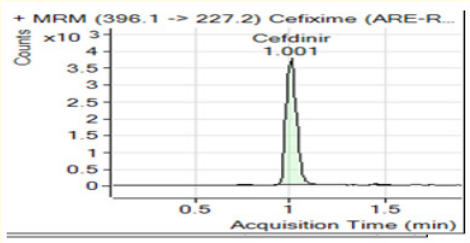

Figure 4: Chromatogram representing an MRM data of blank plasma sample spiked with 4ug/ml Cefixime and internal standard Cefdinir.

Pharmacokinetic Parameter	90% Confidence intervals of parametric means		
	Point estimate (%)	Lower limit (%)	Upper limit (%)
C_{max}	99.992	90.544	110.426
AUC_{0-t}	99.139	84.647	116.112
AUC_{0-inf}	99.131	84.272	116.610

Table 3: The 90% Confidence Interval for Generic and Reference Products of Cefixime.

Conclusion

It can be concluded that the developed bioanalytical method for the determination of cefixime in human plasma was valid, sensitive, specific, precise, accurate and could be used for the determination of drug pharmacokinetic parameters and other clinical applications. Besides, the results of the bioequivalence study of cefixime 400mg hard gelatin capsule showed that both generic and reference product were bioequivalent since they deliver equivalent amounts to systemic circulation at the same rate.

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