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Research Article

COMPARATIVE PHARMACOKINETIC STUDIES OF CETIRIZINE TABLETS IN HEALTHY HUMAN VOLUNTEERS

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ABSTRACT

An Optimized LC-MS condition was proposed for the estimation in human plasma. Cetrizine had been chromatographed on a C_{18} column with a mobile phase Water and Methanol in the ratio of 60:40v/v. the mobile phase was pumped in the flow rate of 0.5ml/min. Erythromycin was used as an internal standard and eluents were monitored at 749 nm. The data presented in this report were validated for the estimation of cetrizine in human plasma over a concentration range of 10.0-600.0 ng/ml. The precision and accuracy are very much within the prescribed limit in this concentration ranges. The values obtained from the system suitability studies demonstrate the suitability of the system for the analysis of Cetrizine in plasma. Limit of Detection of the method was 3.0ng/ml and Limit of Quantization was 10.0ng/ml which shows that the developed method has adequate sensitivity and also more than 50 samples can be processed at a time without affecting the assay values. The method developed was accurate, accordingly to the requirement of LC-MS which can be use4d for the estimation of Cetrizine in plasma samples.

Keywords: Cetrizine, Erythromycin, LC-MS, human plasma, C_{18} column.

INTRODUCTION

Studies to measure Bioavailability and/or establish Bioequivalence (BE) of a product are important elements in support of orally administered drug products in investigational new drug applications (INDs), new drug applications (NDAs), abbreviated new drug applications (ANDAs), and their supplements. Clinical pharmacokinetic studies[1-4] is preferred to examine the absorption, distribution, metabolism and excretion of a drug under investigation in healthy volunteers and/or patients. The systemic exposure profile determined during clinical trials in the IND period can serve as a benchmark for subsequent BE studies.

Mathematical analyses of plasma level vs. time curves permit estimations of half-lives, absorption and excretion rates, extent of absorption (area under the curve), and other constants that are useful in describing the fate of a given drug in an organism. Comparative bioavailability studies permit judgements as to the bioequivalence of drugs. These determinations may, in turn, lead to important decisions related to drug product selection by pharmacists.

Until recently, bioavailability (rate and extent of absorption of medicaments from drug delivery systems) of drugs was not emphasized. It was more or less assumed that if the physical and chemical integrities of a drug product were assured pharmacologic

performance would be observed. It is now recognized that formulation factors can influence the biological availability of a medicament from a dosage unit in mammalian systems. Consequently, it has become common practice to establish bioavailability by measurement of blood levels of drugs following administration of dosage forms.

However, it should be noted that neither bioavailability nor bioequivalence data could be generated without analytic methodology to accurately measure drugs in biological fluids.

Methods of measuring drugs in biological media are increasingly important problems related to bioavailability and bioequivalence, new drug development, drug abuse, clinical pharmacokinetics, and drug research are highly dependent on accurately measured drugs in biological fluids.

For the estimation of the drugs present in the biological fluid, LC-MS method is considered to be more suitable since this is a powerful and rugged method. It is also extremely specific, linear, precise, accurate, sensitive and rapid.

Plan of the present study is as follows

Development and Optimization of chromatographic conditions such as, selection of Mass range, selection of initial separation conditions, nature of the stationary phase, nature of the mobile phase (pH, peak modifier, solvent strength, ratio and flow rate), sensitivity Selection of internal standard.

Validation of developed methods using various validation parameters such as, Accuracy, Precision, Linearity and Range, Selectivity / Specificity, Robustness /Ruggedness,Stability and System suitability.

Estimation of Cetirizine present in the biological fluid .Pharmacokinetic study design and data handling.

The parameters of Bioequivalence study has to be calculated, C_{max} (Maximum Plasma Concentration) t_{max} (T

Literature survey reveals few methods have been reported for the estimation of Cetirizine by HPLC[5] . A liquid chromatographic assay using tandem mass spectrometer detection for the determination of S- Cetirizine and R-Cetirizine in guinea pig[6].The present study, therefore, aims to develop the Bioanalytical method for the estimation of Cetirizine in human plasma and evaluate the pharmacokinetic variables and investigate the bioequivalence of two formulations of Innovator and Test formulations of Cetirizine tablets (10mg) after a single oral dose in 12 healthy male human volunteers in a randomized, two way, two period, complete crossover design.

MATERIALS AND METHODS

MATERIALS USED

Chemicals and reagents used

Acetonitrile, Methanol of HPLC grade, and Formic acid AR grade by Qualigens Fine Chemicals and S.D. Fine chemicals, Water HPLC grade from Milli-Q RO system were used. Working Standards of Cetirizine was purchase from manufactures.

Instruments used

Sartorius single pan digital balance (R200D & 1702)

Systronics - pH meter, μ pH system 361.

Shimadzu 2010A LC- MS system with following configurations

- LC-10 AD-vp solvent delivery system (pump)
- SIL 10 AD-vp Auto injector
- SPD M-10AVP photo diode array detector
- CTO 10 vp column oven
- DGU 14AM degasser
- LC –MS solution data station

Shimadzu 160A UV-VIS spectrophotometer

Perkin-Elmer FT-IR 1600 series

Ultra Sonicator

Solid phase Extraction.

VALIDATION OF THE METHOD

Validation is a process which involves confirmation or establishment by laboratory studies that a method / procedure / system / analyst can give the required accuracy, precision, sensitivity, ruggedness, etc. In the most basic form, validation of an analytical procedure demonstrates that the procedure developed is suitable for its intended purpose. Validation of the method was carried out after the development of the LC-MS method. This section describes the procedure followed for the validation of the methods developed.

ACCURACY

Accuracy of the method was determined by relative and absolute recovery experiments. The relative recovery of the drug was calculated by comparing the concentration obtained from the drug supplemented plasma to the actually added concentration. To drug supplemented plasma, standard Cetirizine solution (Three levels) and internal standard solution were added. The preparation of

solution was carried out adopting the procedure used for the preparation of the sample solution. The resulting sample solution was analyzed and the response factor was calculated.

The absolute recovery of Cetirizine was determined by comparing the response factor of the drug obtained from the plasma with response factor obtained by the direct injection of Cetirizine in mobile phase at three different levels. Recovery studies were carried out for three levels at six times and the % recovery, mean, standard deviation and % CV was calculated.

PRECISION

The precision of the method was determined by intraday precision and interday precision. The intraday precision was evaluated by analysis of plasma samples containing Cetirizine at three different concentrations containing internal standard using nine replicate determinations for three occasions. The interday precision was similarly evaluated over two week period. Precision studies were carried out for three levels at nine times and three occasions. The mean concentration, Mean % bias, standard deviation and % CV were calculated.

SELECTIVITY

Method I

The six blank plasma samples obtained from six different volunteers were analyzed and the chromatograms were recorded. These chromatograms were compared with the chromatograms obtained from standard solutions. Each chromatogram was tested for interference.

The combination of the sample preparation procedure and chromatography provided an assay which must be free from significant interfering endogenous plasma components at the retention times of Cetirizine and the internal standard.

Method II

This method involves the peak purity test method using diode array detector. The PDA spectrum, UV spectrum, absorbance ratio curve and diode array first derivative spectrum of the standard and sample peaks were recorded and compared.

LINEARITY AND RANGE

The different concentrations of standard solutions were prepared to contain 10.0 – 600.0 ng/ml of Cetirizine containing 10.0 µg /ml of internal standard. These solutions were analyzed and the peak areas and response factors were calculated. The calibration curve was plotted using response factor Vs concentration of the standard solutions. The calibration curve was constructed on six different days over a two weeks period to determine the variability of the slopes and intercepts.

STABILITY STUDIES

The stability studies of plasma samples spiked with Cetirizine were subjected to three Freeze - thaw cycles, Short term stability at room temperature for 3 hrs and Long term stability at – 70^{o} C over four weeks. In addition, stability of standard solutions was performed at room temperature for 6 hr and freeze condition for two weeks. The stability of triplicate spiked human plasma samples following three freeze thaw cycles was analyzed. The mean concentrations of the stability samples were compared to the theoretical concentrations. The stability of triplicate short term samples spiked with Cetirizine was kept at room temperature for 1.00 to 3.00 hours before extraction. The plasma samples of the long term stability were stored in the freezer at -70 ^{0} C until the time of analysis. The mean concentrations of the stability samples were compared to the theoretical concentrations. The stability of the Cetirizine standard solution at room temperature for 6 Hrs and freezed condition for two weeks were demonstrated by comparing a freshly prepared standard solution. The stability of the internal standard stock solution was also performed by comparing a freshly prepared standard solution containing internal standard.

System suitability

System suitability of the methods was performed by calculating the chromatographic parameters namely, column efficiency, resolution, peak asymmetry factor and capacity factor on the repetitive injection of standard solutions.

LIMIT OF DETECTION

Testing method: By LC-MS In-house method. Based on Signal- to- Noise (3:1) approach

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of Cetirizine with those of blank samples (i.e. mobile phase) and establishing the minimum concentration at which the Cetirizine can be reliably detected.

LIMIT OF QUANTITATION

Testing method: By LC-MS In-house method. Based on Signal to Noise (10: 1) approach.
Determination of the signal- to- noise ratio is performed by comparing measured signals from samples with known low concentrations of Cetirizine with those of blank samples (i.e. mobile phase) and establishing the minimum concentration at which the Cetirizine can be reliably quantified.

RUGGEDNESS OF THE METHOD

The ruggedness of the method was studied by changing the experimental conditions such as, Different operators in the same laboratory, Changing the source of reagents and solvents(different manufacturers like S.D. Fine Chemicals, Ranbaxy, Qualigens Fine Chemicals) and Changing to another column of similar type (Kromasil C_8, Shimpack C_8, Inertsil C_{18}, Lischrospher C_{18}), By estimating the drugs using the assay procedure. The separation factor, resolution time and peak asymmetry factors were then calculated.

For demonstrating the robustness of the method, slight variations in the optimized conditions were made and the standard solution was injected. The variation made were,

- ± 1 % in the ratio of Acetonitrile in the mobile phase,
- ± 0.5 unit in the pH of the buffer,
- ± 0.5 ml volume of the triethyl amine in aqueous phase , and
- ± 0.1 ml of the flow rate.

Then the separation factor, retention times and peak asymmetry were calculated.

ESTIMATION OFCETIRIZINE IN PLASMA

A Shimadzu® 2010A LC – MS system was used for the analysis.

LC Conditions

Stationary phase: PrincetonSPHERC18 (100x4.6mmid, 5µ)

Mobile Phase: A: Water B: Methanol

Elution mode: Isocratic A: B = 60:40% v/v

Flow rate : 0.5ml/min

Injection volume: 20µl using Auto injector

Oven Temperature : 30°C

The mobile phase was filtered through a 0.22µ membrane and degassed using ultrasonicator. The experiments were carried out at 20°C.

MS Conditions

Interface	: APCI
Operation mode	: SIM
Polarity	: Positive
Probe temperature	: Ambient
CDL Temperature	: 250°C
Block Temperature	: 200°C
Detector Voltage	: 1.3Kv
Nebulizer Gas Flow	: 2.5 L/min
Drying Gas	: 10 L/min
Detection	: Cetirizine – 389.00-nm
Data Station	: LC MS Solution data station
Internal Standard	: Erythromycin-749.00-nm

PREPARATION OF SOLUTIONS FOR ESTIMATION

Preparation of Cetirizine standard stock solution.

Accurately transferred 100mg of Cetirizine working standard into 100ml volumetric flask and dissolved in Acetonitrile and made the final volume with Acetonitrile to give 1.0mg/ml solution of Cetirizine. Labeled and stored the solution in a refrigerator below 8°C

Preparation of Cetirizine standard solution

Prepare 10ml, each of 200.0, 400.0, 1000.0, 3000.0, 5000.0, 8000.0, 10,000.0, 12,000.0 ng/ml of Cetirizine standard solution using Cetirizine standard stock solution and mobile phase. Labeled and stored at -20 ± 2°C until analysis.

Standard solution for CC

Prepare 10 ml each of 200.0, 400.0, 1000.0, 3000.0, 5000.0, 8000.0, 10,000.0 and 12,000ng/ml of Cetirizine standard stock and mobile phase. Labeled and stored at -20 ± 2°C until analysis.

Standard solution for QC

Prepare 10 ml each of 1000.0, 5000.0 and 12,000 ng/ml of Cetirizine standard stock and mobile phase. Labeled and stored at -20 ± 2°C until analysis.

Preparation of Calibration curve samples (CC)

Prepare 10ml each of 10.0, 20.0, 50.0, 150.0, 250.0, 400.0, 500.0 and 600ng/ml of Cetirizine

calibration curve samples using 0.5 ml of Cetirizine standard stock solution and make up the
volume with blank plasma and store at -70 ± 2^{0}C until processing.

Preparation of Quality control (QC) Samples

Prepared 10ml of each 50.0, 250.0 and 600.0 ng/ml of Cetirizine Quality curve samples using 0.5 ml of Cetirizine standard stock solution and make up the volume with blank plasma and store at -70 ± 2^{0}C until processing

Preparation of plasma samples

At the time of analysis the sample were removed from the deep freeze and kept in the room temperature and allowed to thaw.Samper solid phase extraction column C18 (50µm, 70A) 100mg/1ml solid phase extraction cartridge was conditioned with methanol, water sequentially. To this 0.5ml of plasma samples and 0.5ml of 10.0µg/ml of internal standard was added. The cartridge was with 2.0ml of water. The drug and internal standard was eluted from the cartridge using mobile phase. The resulting solution used for the analysis.

METHOD OF ANALYSIS

Analytical Batch Organization

Inject 50 µl of each sample in the following order:

Cetirizine - aqueous solution

Calibration curve samples.

Plasma blank

Zero samples

Plasma samples

Quality control samples Validation and in study validation

The standard solutions, CC samples, QC samples and Plasma sample solutions are injected with the above chromatographic conditions and the chromatograms are recorded. The quantification of the chromatogram is performed using peak area ratios (response factor) of the drug to internal standard. The calibration curves are constructed routinely for spiked plasma containing Cetirizine and internal standard during the process of prestudy validation and in study validation

PHARMACOKINETIC STUDY DESIGN AND DATA HANDLING

STUDY DESIGN

A randomized, two-treatment, two-period, two-sequence, single dose, crossover bioequivalence study for ZYRTEC 10mg containing 10mg of Cetirizine hydrochloride of UCB FARCHIM SA BULLE-SWITERLAND LTD. with Cetirizine tablet 10mg containing 10 mg of Cetirizine hydrochloride Neo pharma, Abu Dhabi of in 12 healthy, adult, male, human subjects under fasting conditions.

PRODUCTS FOR EVALUATION

Test Product (T): Cetirizine tablets 10mg (containing 10mg of Cetirizine hydrochloride)

Mfg. By : Neo pharma Abu Dhabi.
Batch No. : CHT1-22
Mfg. Date : 02\2007
Exp Dt. : 01\2009

Reference Product (R): ZYRTEC 10mg tablet (containing 10mg of cetirizine hydrochloride)

Mfg. By : UCB FARCHIM SA BULLE-SWITERLAND LTD
Batch No. : 03J14A
Mfg. Date : 14/10/2003
Exp Date : 14/10/2008

DRUG ASSIGNMENT

The subject was assigned a code number. Each subject was randomly assigned to one of the following dosing sequences. They were dosed in order of code number.

	Period I	Period II
6 subjects, sequence I	T	R
6 subjects, sequence II	R	T

The dosing sequences were randomized in blocks of two. Therefore, each group of two subjects should be the same gender, to the extent possible.

Volunteer Details

Code	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
Phase I	T	R	T	R	T	R	T	R	T	R	T	R
Phase II	R	T	R	T	R	T	R	T	R	T	R	T

(T -Test Product; R - Reference Product)

Volunteers received either of the study formulations according to their code number along with 240 ml of water.

ETHICS REVIEW PROCEDURE

The detail of the study was submitted to the Ethical Committee in advance of the study commencement and the approval was obtained.

No concomitant medication (other than the study drug) was allowed during the study phase and two weeks prior to the study. The volunteers were also instructed to refrain from consuming alcohol, smoking or other stimulant drinks during this period.

INFORMED CONSENT

Prior to the commencement of the study, each subject was provided with a information sheet giving details of the investigational drugs, procedure and potential risk involved. They were instructed that they are free to withdraw their consent and to discontinue their participation in the study at any time without prejudice. All subjects names were filed confidentially in the investigation files and all data were handled confidentially. The subjects were asked to sign the consent form after discussion with the investigator.

BLOOD COLLECTION

Volunteers were given code numbers and were allocated to the treatment R / T (Reference and Test products) in accordance with the randomization code. All the volunteers were assembled in Bioequivalence Centre, Centre for Advanced Drug Research and Testing, J.S.S. College of Pharmacy, Ootacamund at 7.00 p.m. on the prior day of each phase. After overnight fasting of 12 hrs, their pulse rates, BP were recorded and an sterile intravenous catheter was introduced with strict aseptic precautions for blood collection.

They received either of the study formulations according to their code no with 240 ml of water. A total of 13 blood samples were collected at 0 hr (before drug administration) 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0 12.0, 18.0, and 24.0 h post dosing. Through I.V. Cannula, blood samples (5ml) were collected via disposable syringes in pre-citrated centrifugal tubes. The withdrawn samples were centrifuged at 3500 rpm for 10 minutes to separate plasma. They were transferred into airtight containers and stored at deep freeze condition.

EVALUTION OF PHARMACOKINETIC PARAMETERS

- Plasma concentrations and time points
- Subject, period, sequence, treatment
- AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, and $t_{½}$
- Intersubject, intrasubject, and / or total variability, if available
- C_{min} (concentration at the end of a dosing interval)
- C_{av} (average concentration during a dosing interval)
- degree of fluctuation $[(C_{max}-C_{min}) / C_{av}]$ and
- In addition, the following statistical information should be provided for
- AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}
- Means and Ratio of means
- Confidence intervals.

RESULTS AND DISCUSSION

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Optimization of chromatographic conditions are intended to take into account the various goals of the method development and to weigh each goal (resolutions, run time, sensitivity, peak symmetry, etc) accurately, according to the requirements of LC-MS can be used for the estimation of Cetirizine in plasma samples.

The optimized LC-MS conditions for the estimation of Cetirizine was,

A Shimadzu® 2010A LC – MS system was used for the analysis.

LC Conditions

Stationary phase : PrincetonSPHERC18 (100x4.6mmid, 5µ)

Mobile Phase : A: Water B: Methanol

Elution mode : Isocratic A: B = 60:40% v / v

Flow rate : 0.5ml / min

Injection volum : 20µl using Auto injector

Oven Temperature : 30°C

The mobile phase was filtered through a 0.22µ membrane and degassed using ultrasonicator. The experiments were carried out at 20°C.

MS Conditions

Interface : APCI

Operation mode : SIM

Polarity : Positive

Probe temperature : Ambient

CDL Temperature : 250°C
Block Temperature : 200°C
Detector Voltage : 1.3Kv
Nebulizer Gas Flow : 2.5 L / min
Drying Gas : 10 L / min
Detection : Cetirizine – 389.00-nm
Data Station : LC MS Solution data station
Internal Standard : Erythromycin-749.00-nm

With the optimised condition, blank plasma sample, standard and sample solutions were injected and the chromatograms were recorded. The optimised condition used for estimation provided a well defined separation between the drug, internal standard and endogenous components. The blank plasma samples showed no interference at retention time of the drugs and their internal standards.

VALIDATION OF THE DEVELOPED METHODS

Accuracy (Table 1)

Accuracy of the method was determined by relative and absolute recovery experiments. The absolute recovery of Cetirizine was determined by comparing the response factor of the drug obtained from the plasma with response factor obtained by the direct injection of Cetirizine in mobile phase at three different levels. Recovery studies were carried out for there levels at six times and the % recovery, mean, standard deviation and % CV was calculated and is presented in Table 1.

An analysis of the results shows that the % CV of absolute and the relative recovery values are less than 5.00% thus establishing that the developed method is accurate and reliable.

Precision (Table 2)

The precision of the method was determined by intraday precision & interday precision studies. The intraday precision was evaluated by analysis of blank plasma sample containing Cetirizine at three different concentrations of LQC, MQC and HQC using nine replicate determinations for three occasions. The interday precision was similarly evaluated over two week period. The mean concentration, Mean % bias, standard deviation and % CV were calculated and is presented in Table2.

The results of the precision studies reveal that the developed method is precise.

Selectivity

Method I

The six blank plasma samples obtained from six different volunteers were analyzed & the chromatograms were recorded. These chromatograms were compared with the chromatograms obtained from standard solutions. Each chromatogram was tested for interference. The combination of the sample preparation procedure and chromatography provided an assay which is free from significant interfering endogenous plasma components at the retention times of Cetirizine and the internal standard. Endogenous interferences were not detected at the retention time of Cetirizine and internal standard.

Method II

This method involves the peak purity test method using diode array detector. The PDA spectrum, UV spectrum, absorbance ratio curve and diode array first derivative spectrum of the standard and sample peaks were recorded and

not interfere with the Cetirizine and internal standard peaks.

Linearity and range (Table 3)

The different concentrations of standard solutions were prepared to contain 10.0 – 600.0 ng / ml of Cetirizine and 10.00 µg / ml of internal standard. These solutions were analyzed and the peak areas and response factors were calculated. The calibration curve was plotted using response factor Vs concentration of the standard solutions. The Standard curve fitting is determined by applying the simplest model that adequately describes the concentration-response relationship using appropriate weighting and statistical tests for goodness of fit. The calibration curve was constructed on six different days over a two weeks period to determine the variability of the slopes and intercepts. The lowest standard on the calibration curve was 10.00 ng / ml.

The results indicated little interday variability of slopes and intercepts, as well as good linearity (r^2 >0.99) over the concentration range studied, which indicates good precision and linearity of the method.

Stability studies (Table 4)

The stability studies of plasma samples spiked with Cetirizine were subjected to three Freeze - thaw cycles, Short term stability at room temperature for 3 hrs and Long term stability at – 70° C over eight weeks. In addition, stability of standard solutions was performed at room temperature for 6 hr and freeze

condition for four weeks. The stability of triplicate spiked human plasma samples following three freeze thaw cycle were analyzed. The mean concentrations of the stability samples were compared to the theoretical concentrations. The stability of triplicate short term samples spiked with Cetirizine was kept at room temperature for 1.00 to 3.00 hours before extraction. The plasma samples of the long term stability were stored in the freezer at -70^0 C until the time of analysis. The mean concentrations of the stability samples were compared to the theoretical concentrations. The stability of the Cetirizine standard solution at room temperature for 6 h and freezer condition for four weeks were demonstrated by comparing a freshly prepared standard solution. The stability of the internal standard stock solution was also performed by comparing a freshly prepared standard solution containing internal standard.

The results show that Cetirizine containing plasma samples can be stored for two months without degradation of Cetirizine in frozen state. The results of short term storage at room temperature stability and freeze thaw cycles indicated no degradation of Cetirizine in plasma as well as in sample solution therefore plasma samples in light protected containers could be handled without special precautions (Table 4).

System suitability studies (Table 5)

The parameters namely column efficiency, resolution, peak asymmetry factor and capacity factor for the standard solutions was calculated. On repetitive injection of standard solutions, the parameters of system suitability were calculated (Table 5).

The values obtained demonstrated the suitability of the system for the analysis of the Cetirizine in plasma.

Limit of detection

Testing method

By LCMS In-house method Based on Signal-to- Noise (3:1) approach.

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of Cetirizine with those of blank samples (i.e. mobile phase) and establishing the minimum concentration at which the Cetirizine can be reliably detected.

Limit of detection is 3.0 ng/ml.

2.8. Limit of quantitation

Testing method: By LCMS In-house method Based on Signal to Noise (10: 1) approach

Determination of the signal- to- noise ratio is performed by comparing measured signals from samples with known low concentrations of Cetirizine with those of blank samples (i.e. mobile phase) and establishing the minimum concentration at which the Cetirizine can be reliably quantified.

Limit of quantitation is 10.0 ng/ml.

Ruggedness and Robustness of the method

The ruggedness of the method was studied by changing the experimental conditions such as, Different operators in the same laboratory, Different instruments in the same laboratory (using Shimadzu HPLC system, Waters HPLC system and Agilent HPLC system),Changing the source of reagents and solvents (different manufacturers like S.D. Fine Chemicals, Ranbaxy, Qualigens Fine Chemicals) and Changing to another column of similar type (Kromasil C_8, Shimpack C_8, Inertsil C_{18}, Lischrospher C_{18}), and estimating the drugs using the assay procedure. The separation factor, resolution time and peak asymmetry factors were then calculated. For demonstrating the robustness of the method, slight variations in the optimized conditions were made and the standard solution was injected. The variation made were, $\pm$ 1 % in the ratio of Acetonitrile in the mobile phase, 0.05 ml of the flow rate. Then the separation factor, retention times and peak asymmetry were calculated.

CONCLUSION

Based on the data presented in this report, it can be concluded that the present method is validated for the estimation of Cetirizine in human plasma over concentration range of 10.0 – 600.0 ng/ml. The precision and accuracy are very much within the prescribed limits in this concentration range. Expected recoveries were observed in the present processing technique for LQC, MQC and HQC. The drug is found to be very stable to the effect of three freeze-thaw cycles and up to 3 hours delay on the bench-top. The values obtained from system suitability studies demonstrated the suitability of the system for the analysis of the Cetirizine in plasma. Limit of detection of the methods is 3.0 ng/ml and Limit of quantitation is 10.0

ng/ml which shows that the developed method has adequate sensitivity and also more than 50 samples can be processed at a time without affecting the assay values. The long-term stability is established for these molecules for the required period of subject samples analysis.

ESTIMATION OF CETIRIZINE BY LC-MS METHOD

Estimation of plasma samples from the volunteers was carried out using the optimized chromatographic conditions. The standard and sample solutions were injected and chromatograms were recorded. The typical chromatograms of the standard and sample solutions are given in fig. 1 to 4.

The calibration curves were constructed routinely for spiked plasma containing Cetirizine and internal standard during process of pre-study validation and in-study validation. The mobile phase used for the estimation provided a well defined separation between the drug, internal standard and endogenous components. The zero hours (pre-dose) samples of all subjects showed no interference at the retention time of both the Cetirizine and internal standard.The response factor of the standard and sample solutions was calculated. The concentration of Cetirizine present in plasma samples were calculated and presented in Table 6 - 8.

BIOEQUIVALENCE STUDY OF CETIRIZINE

SUBJECTS

Twelve subjects were enrolled. Data on 12 completed subjects have been presented in the results of the clinical studies. The mean age of human volunteers participated in the study was 22.67 ± 2.27 years. Their height and weights were 170.67 ± 5.79 cm and 61.67± 6.89 Kg, respectively.

STUDY DESIGN

A randomized, two-treatment, two-period, two-sequence, single dose, crossover bioequivalence study for Cetirizine tablets containing 10mg of Cetirizine hydrochloride (Reference) with Cetirizine tablet 10mg containing 10 mg of Cetirizine hydrochloride (test) of in 12 healthy, adult, male, human subjects under fasting conditions.

PRODUCTS FOR EVALUATION

Test Product (T): Cetirizine tablets 10mg (containing 10mg of Cetirizine hydrochloride

Batch No. : 22
Mfg. Date : 02\2007
Exp Data. : 01\2009

Reference Product (R): Cetirizine tablets (containing 10mg of Cetirizine hydrochloride)

Batch No. : 03J14A
Mfg. Dat : 14/10/2003
Exp Date : 14/10/2008

PHARMACOKINETICS DATA

- Pharmacokinetic parameters such as Peak plasma concentration (C_{max}), Time to peak Concentration (t_{max}), Area under the plasma concentration - time curve (AUC_{0-t} & $AUC_{0-\infty}$), elimination rate constant (k_{eli}), Elimination half-life ($t_{1/2}$) were calculated separately and the blood level data of the Reference product and the Test product were compared. The observations are given in Table 1 to 10.
- On oral administration of the Reference product and the Test product in the fasting state exhibited Measurable Cetirizine blood levels in all the volunteers from 0.50 hr onwards.
- Measurable Cetirizine blood levels were noticed in all subjects up to 18.00h for both Reference and Test Products.
- The mean peak plasma concentration i.e. C_{max} for Cetirizine after administration of the Test and Reference product was 95.76 ± 8.01 and 98.51 ± 7.32 ng /ml, respectively (Table 4).
- The time to peak concentration i.e. T_{max} for Cetirizine after administration of the Test and Reference product was 3.69 ± 0.72 and 3.71 ± 0.86 hrs, respectively (Table 5).
- The area under the plasma concentration-time curve i.e. AUC_{0-24} for Cetirizine after administration of the Test and Reference product was 1320.63 ± 180.86 and 1389.87 ± 171.26 ng.h/ml, respectively (Table 6).
- The elimination rate constant (k_{eli}) for Cetirizine after administration of the Test and Reference product was 0.05 ±0.01and0.049± 0.01 h^{-1}, respectively (Table 7).

- The elimination half-life ($t_{1/2}$) for Cetirizine after administration of the Test and Reference product was 13.52 ± 1.68 and 14.30 ± 1.83 hrs, respectively (Table 8).
- The area under the plasma concentration-time curve for infinitive time ($AUC_{0-\infty}$) for Cetirizine after administration of the Test and Reference product was 1934.60 ± 312.04 and 2104.34 ± 345.44 ng.h/ml, respectively (Table 9).

Table 1: Recovery Studies

Level	Concentration of drug added ng/ml	Amount of drug recovered (ng/ml) in plasma sample	Recovery (%)	Amount of Drug recovered (%) in Mobile phase	Relative Recovery (%)
Level-I	50.0	47.22 ± 2.93	Mean : 94.44 CV : 1.47 N : 6	Mean : 98.96 CV : 1.64 N : 6	95.43
Level-II	250	247.17 ± 1.64	Mean : 98.86 CV : 1.32 N : 6	Mean : 97.98 CV : 1.89 N : 6	100.89
Level-III	600	593.01 ± 4.16	Mean : 98.83 CV : 3.72 N : 6	Mean : 99.01 CV : 1.34 N : 6	99.81

Table 2: Precision Studies

Nominal concentration(ng/ml)			
S.N	LQC	MOQ	HQC
	50.00	250.00	600.00
1	47.327	243.116	586.937
2	46.025	248.976	588.649
3	48.109	242.229	591.463
4	44.910	247.697	595.337
5	43.864	246.326	598.467
MEAN	46.047	245.669	592.171
S.D (+/-)	1.728	2.908	4.742
C.V (%)	4.05	1.18	0.80
%NOMINAL	92.09	98.27	98.70
n	5	5	5
Nominal concentration(ng/ml)			
S.N	LQC	MOQ	HQC
1	46.229	249.337	547.349
2	47.632	247.805	548.337
3	48.976	246.198	549.789
4	49.165	247.114	534.778
5	47.398	248.667	541.967
MEAN	47.880	247.824	544.444
S.D (+/-)	1.212	1.240	6.159
C.V (%)	2.53	0.05	1.13
%NOMINAL	95.76	99.13	90.74
n	5	5	5
Nominal concentration(ng/ml)			
S.N	LQC	MOQ	HQC
1	47.468	241.924	589.334
2	46.229	244.695	592.771
3	48.164	246.393	563.005
4	49.165	247.748	574.981
5	48.794	247.966	568.117
MEAN	47.964	245.746	577.642
S.D (+/-)	1.165	2.502	13.016
C.V (%)	2.43	1.02	2.25
%NOMINAL	95.93	98.30	96.27
n	5	5	5

Table 3: LINEARITY AND RANGE

Drug Concentration (ng/ml)	Internal Standard Concentration (ng/ml)	Response Factor (RSD)
10.0	10.0	0.0007
20.0	10.0	0.0038
50.0	10.0	0.0132
100.0	10.0	0.0247
250.0	10.0	0.0563
400.0	10.0	0.0927
500.0	10.0	0.1135
600.0	10.0	0.1364

Table 4: Stability of Cetirizine in Plasma During Storage and Sample Handling

Nominal Concentration (ng /mL)			
Freeze and Thaw	LQC	MQC	HQC
	50.00	250.00	600.00
Cycle 1	46.318	246.789	547.123
Cycle 2	47.961	234.166	597.468
Cycle 3	45.497	245.978	596.277
MEAN	46.592	242.311	580.289
S.D (+/-)	1.255	7.065	28.729
C.V (%)	2.69	2.92	4.95
% NOMINAL	93.18	96.92	96.71
N	3	3	3
Nominal Concentration (ng /mL)			
Short term plasma at room temperature	LQC	MQC	HQC
	50.00	250.00	600.00
After 1 hr	47.221	246.794	555.671
After 2 hr	47.061	245.685	559.462
After 3 hr	48.967	240.961	558.493
MEAN	47.750	244.480	557.875
S.D (+/-)	1.057	3.098	1.970
C.V (%)	2.21	1.27	0.35
% NOMINAL	95.50	97.79	92.98
N	3	3	3
Nominal concentration (ng/ml)			
Long Term Plasma at Room Temperature	LQC	MQC	HQC
	50.00	250.00	600.00
After 1 hr	46.921	241.679	578.964
After 2 hr	47.309	240.881	580.395
After 6 hr	48.333	246.909	584.033
MEAN	47.521	243.156	581.131
S.D (+/-)	0.729	3.274	2.613
C.V (%)	1.54	1.35	0.45
% NOMINAL	95.04	97.26	96.86
N	3	3	3
Nominal Concentration (ng /mL)			
Standard Stock solutions	LQC	MQC	HQC
	50.00	250.00	600.00
After 3 hr	49.225	249.348	596.487
After 6 hr	50.021	248.167	598.871
After 6 hr	49.987	249.364	599.335
MEAN	49.741	248.960	598.231
S.D (+/-)	0.447	0.687	1.528
C.V (%)	0.90	0.28	0.26
% NOMINAL	99.48	99.58	99.71
N	3	3	3

Table 5: System Suitability Studies

S.No	Parameters	INT STD	DRUG
1	Theoretical Plate	46315	32487
2	Resolution factor	0.95	
3	Asymmetric factor	1.16	1.04
4	LOD(ng/ml)	1.0	3.0
5	LOQ(ng/ml)	3.0	10.0

Table 6: Plasma Concentration (Ng/Ml) Data of Test Product

HOURS	v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12
0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	252.995	323.365	255.27	318.485	261.577	287.038	342.868	353.598	338.486	351.337	219.092	342.05
1	369.313	420.077	420.795	355.416	368.408	403.896	424.462	472.186	480.05	475.609	372.87	441.232
1.5	464.8	475.263	491.208	433.313	466.316	445.942	473.33	486.801	553.459	497.261	480.628	490.3
2	471.577	453.391	482.539	456.06	443.563	493.592	495.879	467.041	475.737	483.451	454.056	484.884
2.5	385.895	372.991	427.04	402.172	396.807	337.461	442.303	425.075	465.948	476.098	433.899	449.294
3	360.011	301.765	343.699	382.651	336.888	349.71	338.037	385.981	419.865	386.846	415.23	390.555
4	322.195	235.14	313.674	364.526	244.657	281.712	267.752	320.304	363.265	364.754	373.284	285.82
5	238.041	228.417	276.835	217.496	229.074	222.043	201.164	262.905	280.947	296.207	326.248	247.485
6	157.216	156.98	164.731	159.918	184.522	116.267	162.942	228.036	256.474	157.159	253.00	186.115
8	122.727	127.534	102.728	126.462	117.372	90.834	143.95	135.057	181.642	132.024	171.00	140.973
10	81.364	82.588	81.46	77.755	89.4	86.413	126.272	107.852	125.678	106.135	121.647	78.959
12	53.032	60.077	54.955	63.276	37.875	35.197	86.445	67.881	51.363	83.753	59.94	34.777
18	24.122	23.497	27.805	20.776	17.639	14.86	14.553	16.904	23.634	73.701	30.89	98.974
24	0	0	0	0	0	0	0	0	0	0	0	0

Table 7: Plasma Concentration (ng/ml) data of the reference Product

hours	v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12
0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	247.346	269.844	212.373	384.134	270.494	395.593	342.284	243.097	327.843	341.217	321.475	336.632
1	409.401	338.104	337.163	447.882	334.792	410	403.799	449.381	447.116	446.329	425.647	375.451
1.5	461.483	484.853	43.283	465.681	445.592	484.737	464.346	478.722	525.071	502.083	485.895	493.918
2	426.692	469.606	480.997	512.69	398.994	460.168	413.795	453.614	459.93	549.663	423.491	438.224
2.5	312.729	348.613	445.349	441.752	377.754	454.26	345.758	421.234	409.96	468.758	351.7443	358.137
3	253.824	324.572	400.626	342.879	341.386	312.868	280.698	319.424	370.593	436.14	335.51	305.611
4	231.905	256.905	343.503	310.121	290.836	268.822	255.158	632.764	317.832	443.739	252.289	231.484
5	221.765	228.327	299.27	299.614	173.258	239.543	210.544	255.276	257.651	297.316	203.349	181.1
6	134.821	138.577	249.344	225.468	134.173	222.201	175.906	155.907	235.823	243.874	146.618	129.487
8	113.831	109.611	199.143	174.601	107.866	128.928	127.053	124.733	166.085	195.354	83.642	101.104
10	80.175	82.814	82.634	132.183	62.07	41.899	76.983	65.755	119.155	127.841	75.816	69.094
12	42.003	51.224	40.942	86.647	46.139	22.584	35.428	45.288	57.579	58.785	35.87	53.45
18	25.387	24.183	21.76	37.286	32.515	19.662	17.138	18.877	14.33	31.223	13.767	30.854
24	0	0	0	0	0	0	0	0	0	0	0	0

Table 8: Mean plasma concentrations of Reference and test product (ng/ml)

Time (h)	Test Product		Reference Product	
	Mean	S.D	Mean	S.D
0.0	0.0000	0.0000	0.0000	0.0000
0.5	303.8468	46.4230	307.6943	58.0283
1.0	417.0262	44.3842	402.0888	45.2669
1.5	479.8851	29.8438	444.6387	128.0940
2.0	471.8142	17.0932	457.3220	42.7219
2.5	417.9153	40.3074	394.6707	51.7475
3.0	367.6032	35.1132	335.3443	49.6788
4.0	311.4236	48.7113	319.6132	115.0578
5.0	252.2385	36.9388	238.9178	44.1962
6.0	181.9467	42.7010	182.6833	48.5113
8.0	132.6919	25.4314	135.9959	38.2581
10.0	97.1269	19.1813	84.7016	27.6606
12.0	57.3809	16.9050	47.9949	15.9246
18.0	32.2796	26.2938	23.9156	7.6594
24.0	0.0000	0.0000	0.0000	0.0000

Table 9: Demographic Data of Volunteers

V.NO	Name of the volunteer	Subject code	Phase I	Phase II
1	Deepan	A	T	R
2	Ananda	B	R	T
3	Raj	C	T	R
4	Sabeersh	D	R	T
5	Babu	E	T	R
6	Arun	F	R	T
7	Mohan	G	T	R
8	Kiran	H	R	T
9	Krishna	I	T	R
10	Manju	J	R	T
11	Magesh	K	T	R
12	Vikram	L	R	T

Table 10: Mode of Treatment

V.NO	Phase I	Phase II
1	T	R
2	R	T
3	T	R
4	R	T
5	T	R
6	R	T
7	T	R
8	R	T
9	T	R
10	R	T
11	T	R
12	R	T

Table 11: Peak Plasma Concentrations - C_{max} (ng/ml)

Vol. Code	Test Product	Reference Product
A	471.5770	461.4830
B	475.2630	484.8530
C	491.2080	480.9970
D	456.0600	512.6900
E	466.3160	445.5920
F	493.5920	484.7370
G	495.8790	464.3460
H	486.8010	632.7640
I	553.4590	525.0710
J	497.2610	549.6630
K	480.6280	485.8950
L	490.3000	493.9180
MEAN	488.1953	501.8341
SD	24.2285	50.1057

Table 12: Time To Peak Concentration, T_{max} (H)

Vol. Code	Test Product	Reference Product
A	2.00	1.50
B	1.50	1.50
C	1.50	2.00
D	2.00	2.00
E	1.50	1.50
F	2.00	1.50
G	2.00	1.50
H	1.50	4.00
I	1.50	1.50
J	1.50	2.00
K	1.50	1.50
L	1.50	1.50
MEAN	1.6667	1.8333
SD	0.2462	0.7177

Table 13: Area Under Curve The Plasma Concentration-Time Curve, Auc_{0-24} (Ng.H/Ml)

Vol. Code	Test Product	Reference Product	T/R	T- R
A	2803.4003	2483.4410	0.8859	-319.9593
B	2749.4283	2626.8805	0.9554	-122.5478
C	2888.8318	2934.7230	1.0159	45.8913
D	2877.8043	3515.0378	1.2214	637.2335
E	2642.3935	2554.7240	0.9668	-87.6695
F	2508.8445	2728.6585	1.0876	219.8140
G	3029.8198	2577.6875	0.8508	-452.1323
H	3175.8653	3059.8825	0.9635	-115.9828
I	3462.5578	3178.9283	0.9181	-283.6295
J	3582.2480	3656.8640	1.0208	74.6160
K	3381.1210	2476.3217	0.7324	-904.7994
L	3382.0898	2576.5238	0.7618	-805.5660
MEAN	3040.3670	2864.1394		
SD	350.8854	407.3157		

Table 14: Elimination Rate Constant (k_{eli} (h^{-1}))

Vol. Code	Test Product	Reference Product
A	0.1908	0.1757
B	0.1827	0.1871
C	0.1882	0.1590
D	0.1966	0.1588
E	0.2069	0.1771
F	0.2170	0.2264
G	0.1969	0.2031
H	0.2018	0.2146
I	0.1965	0.2100
J	0.1367	0.1841
K	0.1785	0.2203
L	0.1416	0.1745
MEAN	0.1862	0.1909
SD	0.0243	0.0234

Table 15: Half-Life, $t_{½}$ (hrs)

Vol. Code	Test Product	Reference Product
A	3.6334	3.9444
B	3.7947	3.7045
C	3.6830	4.3603
D	3.5262	4.3649
E	3.3499	3.9137
F	3.1935	3.0610
G	3.5206	3.4124
H	3.4345	3.2293
I	3.5161	3.2841
J	5.0706	3.7645
K	3.8842	3.1468
L	4.8940	3.9718
MEAN	3.7917	3.6798
SD	0.5875	0.4521

Table 16: Area under the plasma concentration time curve, auc $_{0-\infty}$ (ng/ml)

Vol. Code	Test Product	Reference Product
A	2929.8461	2627.9060
B	2878.0637	2756.1247
C	3036.5732	3071.6071
D	2983.4960	3749.8342
E	2727.6406	2738.3113
F	2577.3092	2815.4877
G	3103.7361	2662.0585
H	3259.6232	3147.8298
I	3582.8567	3247.1787
J	4121.3987	3826.8640
K	3554.2177	2538.8228
L	4080.9048	2753.3214
MEAN	3236.3055	2994.6122
SD	500.4633	428.7275

Table 17: Summary of Results (n=12)

S.NO	PARAMETERS	TEST PRODUCT	REFERENCE PRODUCT	% RATIO
1.	AUC_{0-24}(ng.h/ml)	3040.3670	2864.1394	106.15
2.	$AUC_{0-\infty}$ (ng.h/ml)	3236.3055	2994.6122	108.70
3.	C_{max} (ng/ml)	488.1953	501.8341	97.28
4.	t_{max} (h)	1.6667	1.8333	90.91
5.	k_{eli} (h^{-1})	0.1862	0.1909	97.53
6.	$t_{½}$ (h)	3.7917	3.6798	103.04

FIG.1 TYPICAL CHROMATOGRAM OF STANDARD SOLUTION

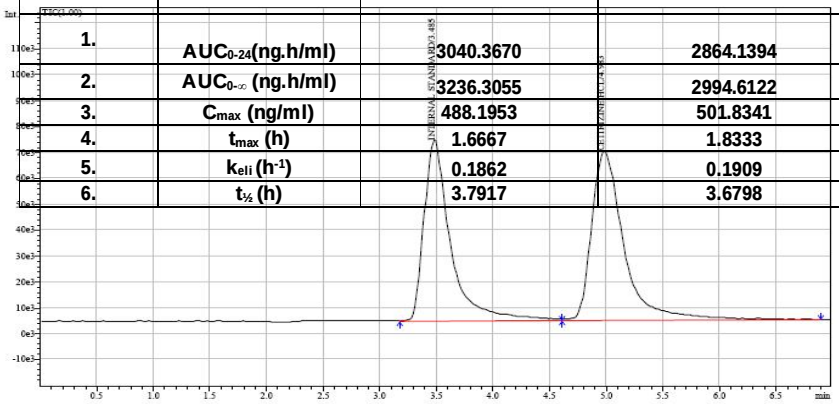


FIG .3 MASS SPECTRUM OF CETIRIZINE

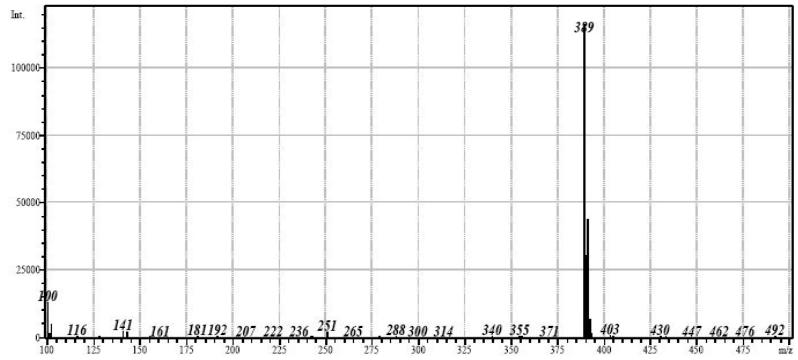


FIG .4 MASS SPECTRUM OF INTERNAL STANDARD

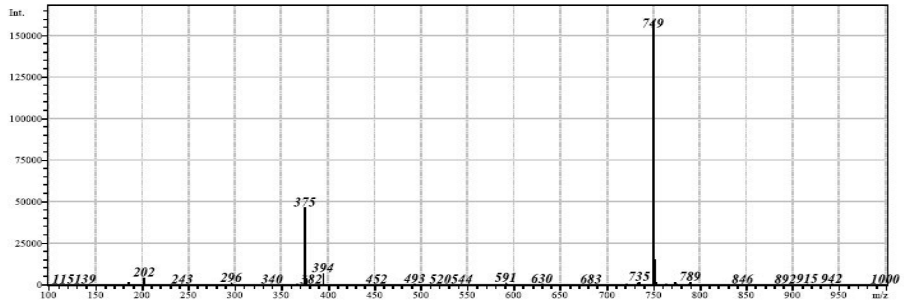


FIG 5. CALIBRATION CURVE OF CETIRIZINE

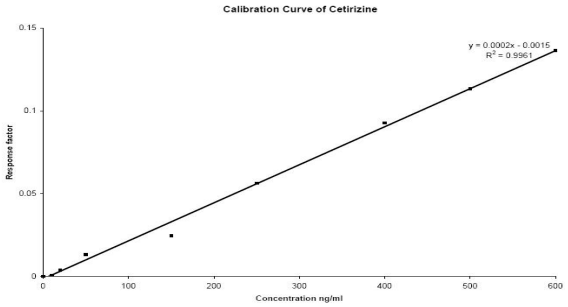


Fig 6. Concentration-time curve for Volunteer 1 (Reference & test product)

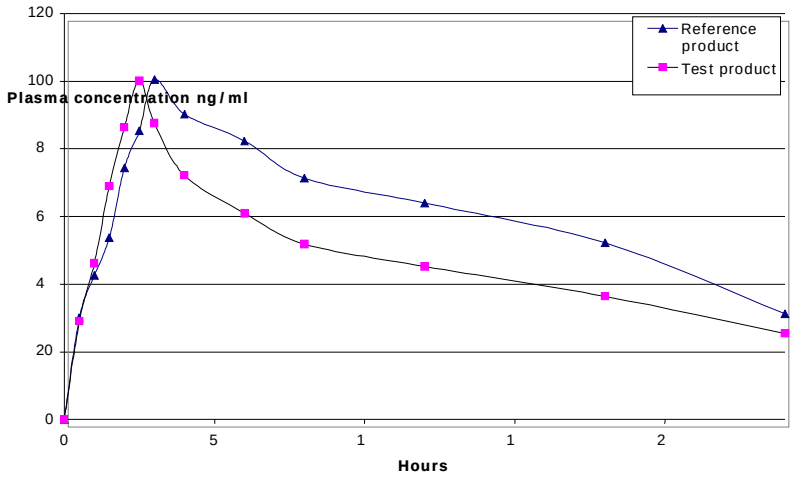


Fig 7. Concentration-time curve for Volunteer 2 (reference & test product)

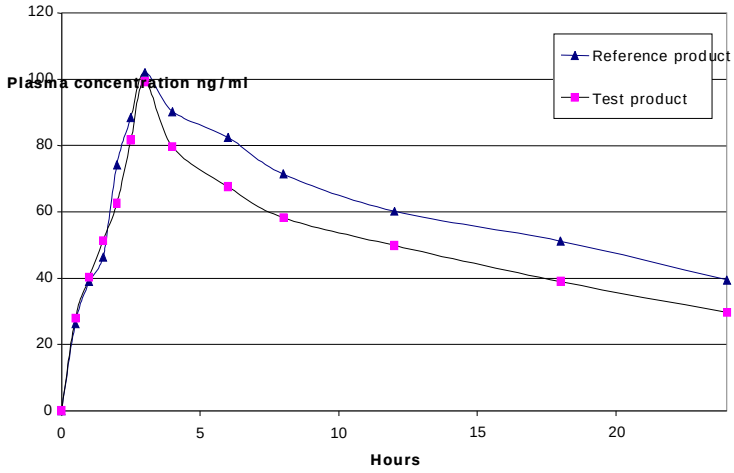


Fig 8. Concentration-time curve for Volunteer 3 (reference & test product)

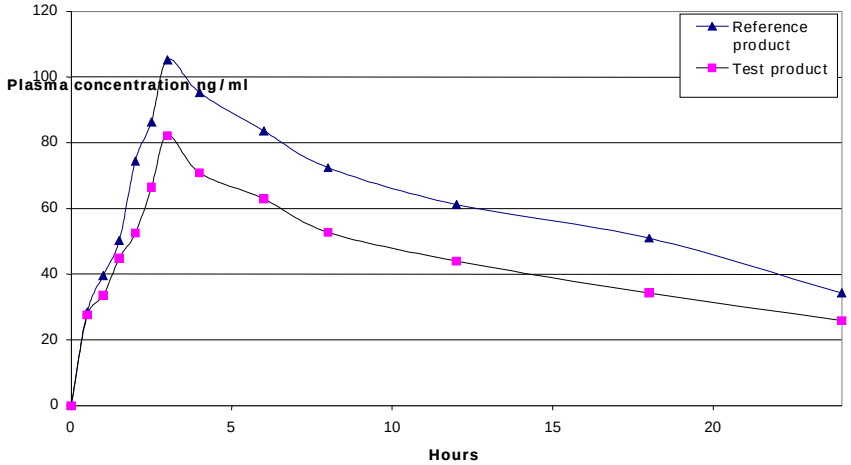


Fig 9. Concentration-time curve for Volunteer 4 (reference & test product)

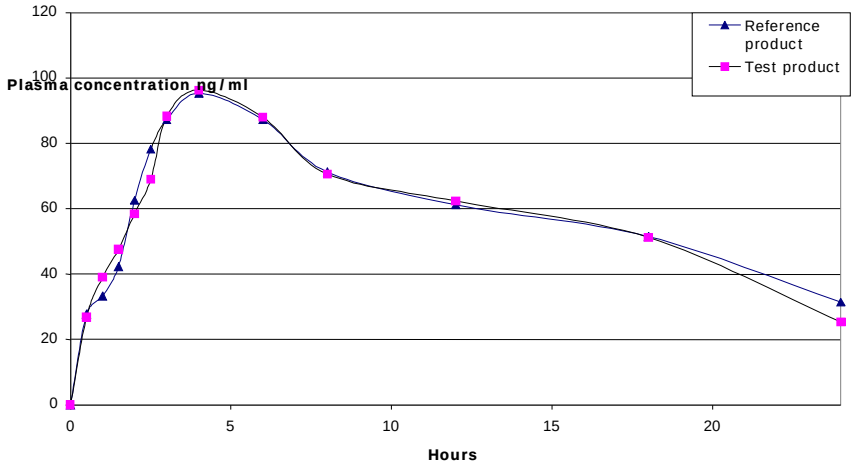


Fig 10. Concentration-time curve for Volunteer 5 (reference & test product)

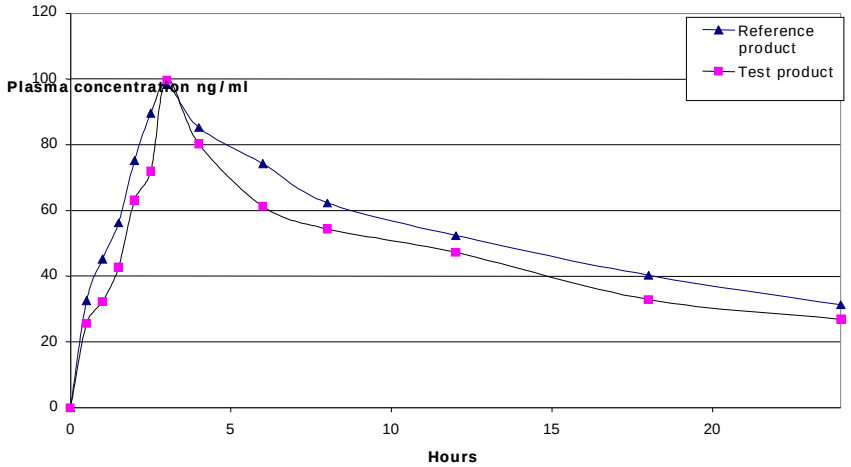


Fig 11. Concentration-time curve for Volunteer 6 (reference & test product)

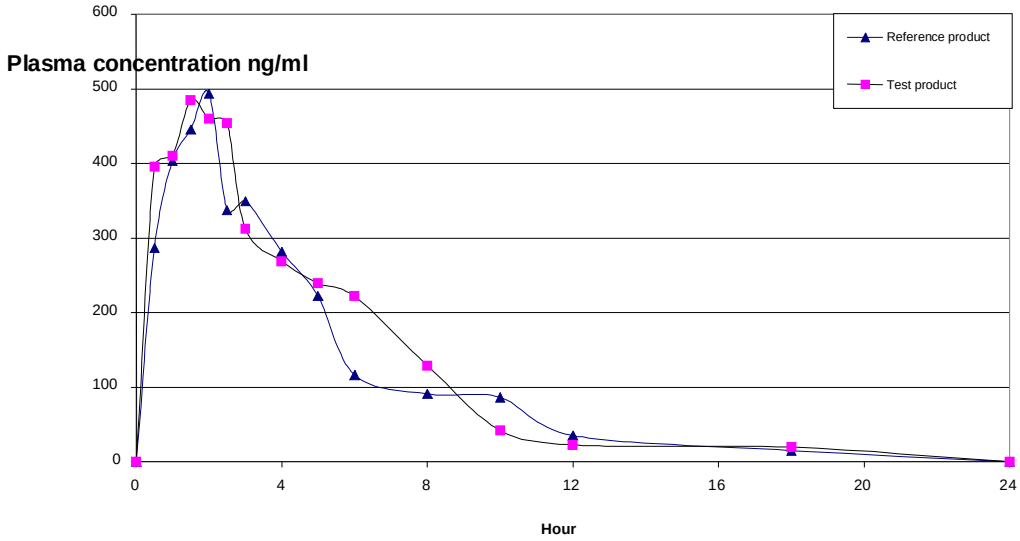


Fig 12. Concentration-time curve for Volunteer 7 (reference & test product)

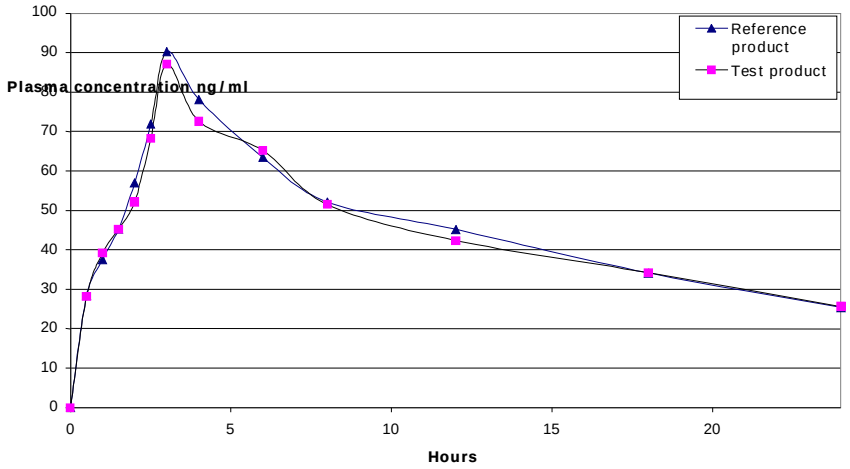


Fig 13. Concentration-time curve for Volunteer 8 (reference & test product)

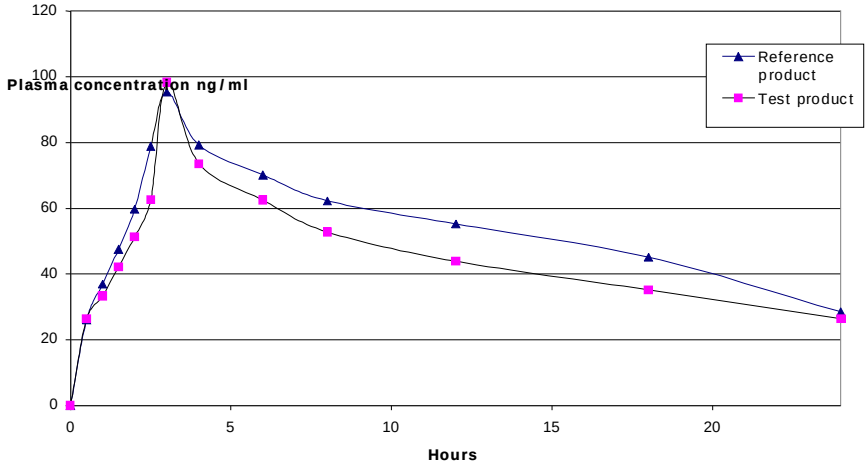


Fig 14. Concentration-time curve for Volunteer 9 (reference & test product)

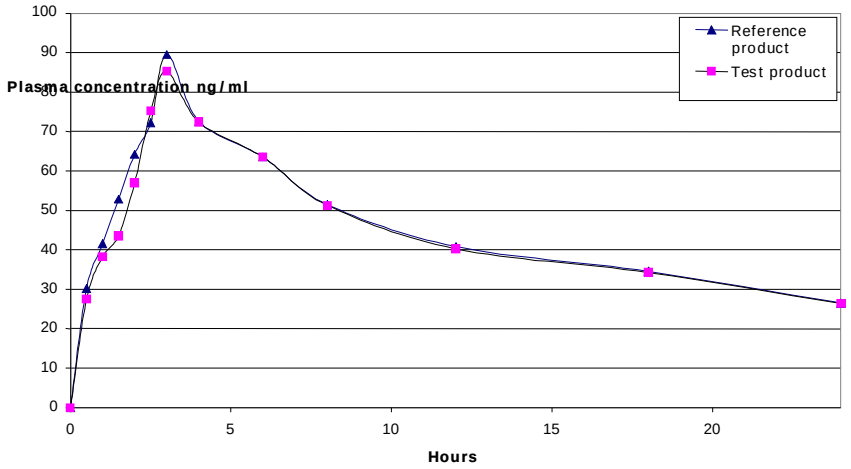


Fig 15. Concentration-time curve for Volunteer 10 (reference & test product)

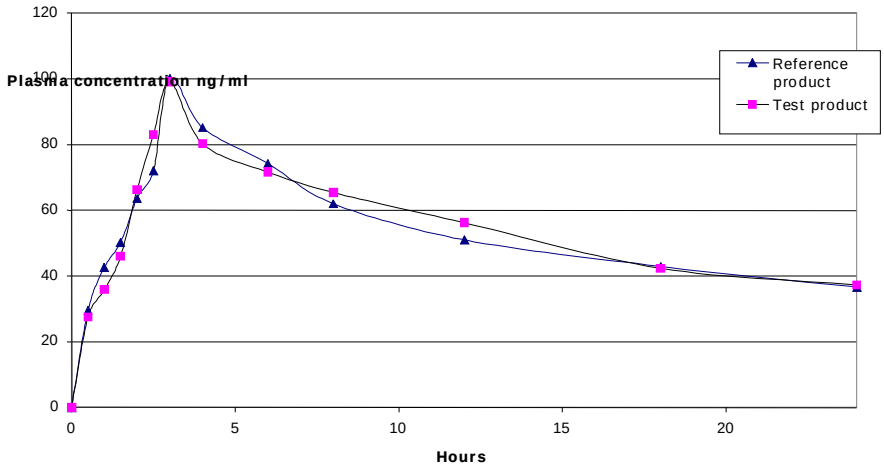


Fig 16. Concentration-time curve for Volunteer 11 (reference & test product)

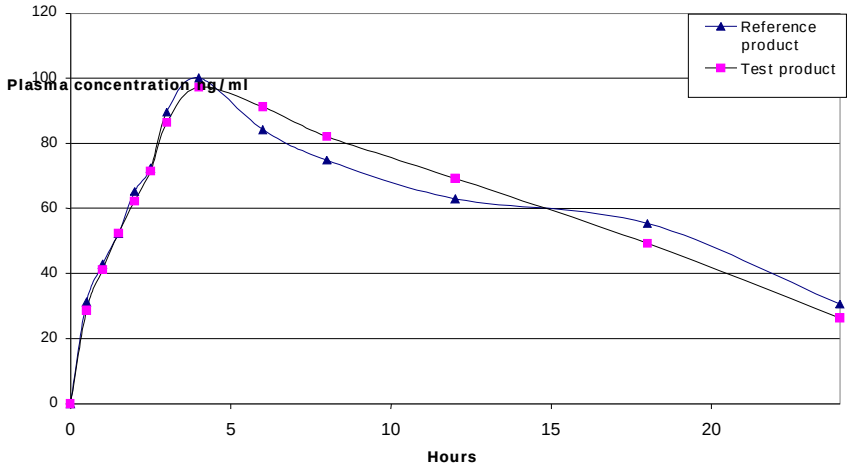


Fig 17.Concentration-time curve for Volunteer 12 (reference & test product)

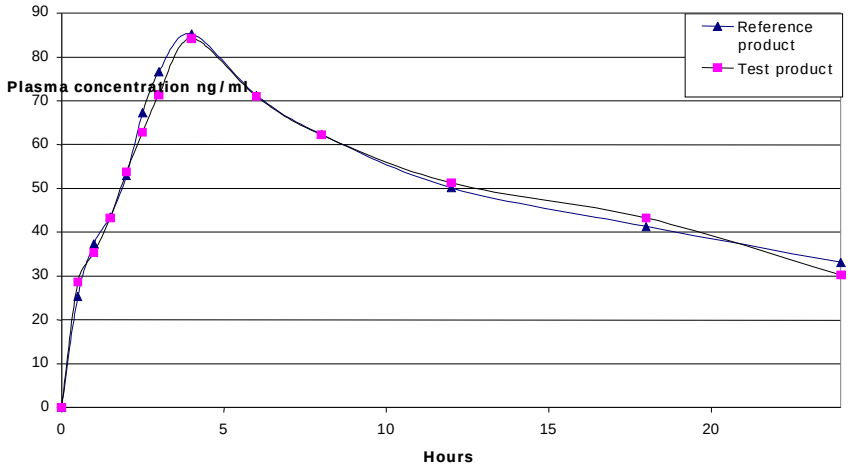


FIG 18. MEAN CONCENTRATION - TIME CURVE FOR 12 VOLUNTEERS (REFERENCE AND TEST PRODUCT)

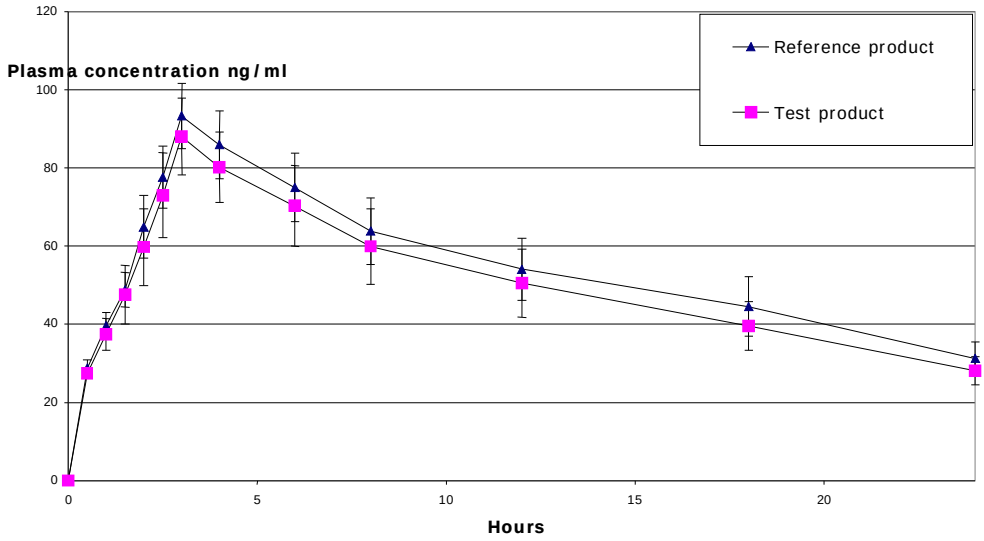
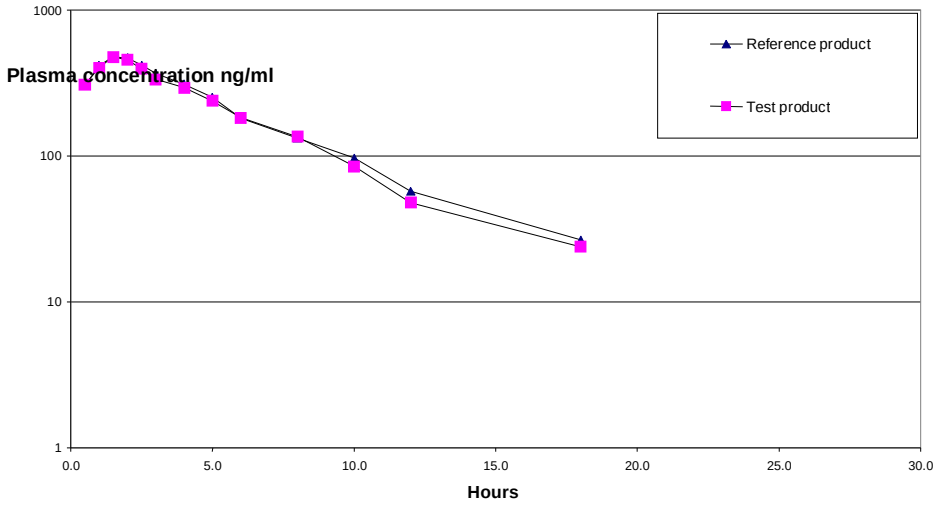


Fig.19 MEAN CONCENTRATION - TIME CURVE FOR 12 VOLUNTEERS (REFERENCE AND TEST PRODUCT) - LOG SCALE



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