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RESEARCH ARTICLE

Bioequivalence Study of the Two Products of Efavirenz by Validated Analytical Method

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ABSTRACT

The aim of this study was to find whether the bioavailability of a 600 mg efavirenz capsule (E.F.600 capsule, test) produced by Macneil and Argus Pharmaceutical Ltd. was equivalent to the tablet EFAVIR produced by the Cipla Ltd. (reference preparation). The pharmacokinetic parameters assessed in this study were area under the plasma –concentration time curve 0–96 h (AUCt), area under the plasma concentration time curve from time 0 to ∞ (AUCinf), the peak plasma concentration of drug (Cmax), time needed to achieve the peak plasma concentration (tmax), and the elimination half-life (t½). This was a randomized, single-blind, two-period, crossover study which included 20 healthy adult male and female subjects under fasting conditions. In each of the two study periods (separated by a washout of 1 week), single dose of test or reference drug was administered. Blood samples were taken up to 96 h past dose, the plasma was separated, and the concentrations of efavirenz were determined by high-performance liquid chromatography -UV method. Bioavailability and bioequivalence studies play a key role during the phase of drug development for both innovator drugs and generic drugs and thus have gained a great attention over the past few decades. Bioequivalence study is used to introduce generic drugs of innovator drugs at a lower cost. Hence, a thorough understanding of these bioavailability/ bioequivalence studies is required.

Keywords: Bioavailability, bioequivalence, pharmacokinetic parameters

INTRODUCTION

Ensuring uniformity in standards of quality, efficacy, and safety of pharmaceutical product is the fundamental responsibility of CDSCO. Reasonable assurance has to be provided that various products, containing same active ingredients, marked by different licenses, are clinically equivalent and interchangeable.

Accordingly, the bioavailability of an active substance from a pharmaceutical product should be known and reproducible. In most cases, it is cumbersome and unnecessary to assess this by clinical studies. Bioavailability and bioequivalence data are, therefore, required to be furnished with applications for new drugs, as required under schedule Y, depending on the type of application being submitted.

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Both bioavailability and bioequivalence focus on the release of drug substance from its dosage form and subsequent absorption into the systemic circulation. For this reason, similar approaches to measure bioavailability should generally be followed in demonstrating bioequivalence.

Bioavailability can be generally documented by a systemic exposure profile obtained by measuring drug and/or metabolite concentration in the systemic circulation over time.

Bioequivalence studies should be conducted for the comparison of two medical products containing the same active substance. The studies should provide an objective means of critically assessing the possibility of alternative use of them. Two products marketed by different licenses containing same active ingredients must be shown to therapeutically equivalent to one another to be considered interchangeable. Several test methods are available to assess equivalence, including comparative bioavailability (bioequivalence)

study, in which the active drug substance or one or more metabolites is measured in accessible biological fluid such as plasma, blood, or urine.

- Comparative pharmacodynamic studies in humans.
- Comparative clinical trials.
- *In vivo* dissolution tests (Guidelines for bioavailability and bioequivalence studies, CDSCO, 2005).[1]

Reversed-phase high-performance liquid chromatography (HPLC) is the separation method of choice for most pharmaceutical compounds, both hydrophilic and hydrophobic, due to the stable, reproducible nature of the HPLC columns, the largely aqueous composition of the mobile phase, and the relative ease of reproducing the methods in a variety of laboratories.[1,2]

Efavirenz, a non-nucleoside reverse transcriptase inhibitor, is being increasingly used in since 1998 in association with other antiretroviral agents in the treatment of HIV infection. Its long half-life allows once-daily dosing and therefore presents an advantage for treatment compliance and efficacy.[3-5] Efavirenz undergoes extensive metabolism, mainly by the cytochrome p-450 isoenzyme, CYP2B6, which is known to exhibit extensive interindividual variability. This could lead to heterogeneity in response to treatment. In addition, differences in efavirenz pharmacokinetics between various racial and ethnic groups have been reported.

MATERIALS AND METHODS

Materials

- Chemical used
  1. Acetonitrile, HPLC Grade (Merck India, Mumbai)
  2. Potassium dihydrogen phosphate (Merck India, Mumbai).
3. Methanol HPLC Grade (Merck India, Mumbai).
4. Dichloromethane GR Grade (Merck India, Mumbai).

Methods

Analytical method

Preparation of standard curve of efavirenz using HPLC method

Accurately weighed 10 mg of efavirenz was dissolved in 10 mL of methanol separately to prepare a stock solution of 1 mg/mL. From this, 0.1 mL was taken and was further diluted to produce 10 µg/mL concentration. These stock solutions were further diluted with mobile phase to produce standard solutions of 0.05, 0.1, 0.25,.5, 1, 2, 4, and 6 mg/mL, and these solutions were analyzed by HPLC. The HPLC system used was Jasco. The column used was C8 250 i× 2.6 µm, 5 microparticle size. Isocratic mode of elution and reverse-phase liquid chromatography has been used for the study, and mobile phase used for the separation was 10 mm phosphate buffer (7.4):acetonitrile(36:65v/v). The flow rate was set at 1 mL/min. The injection volume was 50 µL and total run time was 15 min. The room temperature was maintained at ambient temperature, and both the analytes were eluted at a detection wavelength of 247 nm using UV detector. Wavelength was set at 247 nm during the HPLC analysis because it gives a good response at this wavelength [Figure 1].[6-8]

Validation[9,10]

The accuracy, sensitivity, precision, stability, recovery, and reproducibility of the analytical method were confirmed by validation in accordance with the USFDA guidelines.

System stability

It is defined by ICH as "the checking of a system before or during analysis of unknown to ensure system performance." System stability criteria may include such factors as plate count, tailing, retention, and/or resolution. System suitability criteria should also include a determination of reproducibility (%RSD) when a system suitability "sample" is run [Figure 2].

Linearity and LLOQ

To establish linearity, a series of calibration standards were prepared by a known concentration of efavirenz and IS to drug-free human plasma and analyzed. The lowest concentration on the standard curve with detector response 3 times greater than the drug-free (blank) human plasma was considered as the LLOQ [Figure 3].

Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample.

Accuracy and precision[11]

Both intraday and interday precision and accuracy were done in the same manner. A comparison was made between the obtained values and experimental values. Precision was expressed as %RSD. The value of accuracy should not be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%. The precision determined at such concentration level should not exceed 15% of RSD except for the LLOQ where it should not exceed 20% of RSD.

Extraction recovery

The extraction recovery of efavirenz (low, medium, and high) from plasma was evaluated by comparing the peak area responses from plasma samples spiked with particular standard working solution of analyte before extraction with those from drug-free samples extracted and spiked with the same concentration of analyte after extraction.

Freeze thaw stability[12]

The stability of the analytes after three freeze and thaw cycles was determined at low, medium, and high QC samples. The samples were stored at −20°C for 24 h and thawed unassisted at room temperature. After complete thawing, the samples were refrozen for 12–24 h. After three freeze thaw cycles, the concentrations were analyzed.

Bioequivalence study

The aim and objective of the present study were to evaluate the pharmacokinetic parameters and to compare the single-dose oral bioavailability of efavirenz containing efavirenz 600 mg (test preparation) prepared by Mencil and Argus pharmaceuticals Ltd., with the tablet efavir (reference preparation) prepared by Cipla Ltd.

Drug administration to human volunteers

The volunteers were randomized on the previous day of phase 1. In phase 1, each volunteer received either the test preparation or the reference preparation as a single dose at a fixed time. In phase 2, this order was reversed as per randomization. Study medications were administered at intervals of 2 min to groups of two subjects and were given with 240 mL water at room temperature.

Blood collection from human volunteers

All volunteers were assembled at 6 am on the study day 1 of each session after overnight fasting of at least 10 h. Their TPR and BP were recorded, and indwelling intravenous cannula was introduced with strict aseptic precaution in the antecubital vein for blood collection. The volunteers received either of the study preparation (test or reference) according to their code numbers with 240 mL of water. The

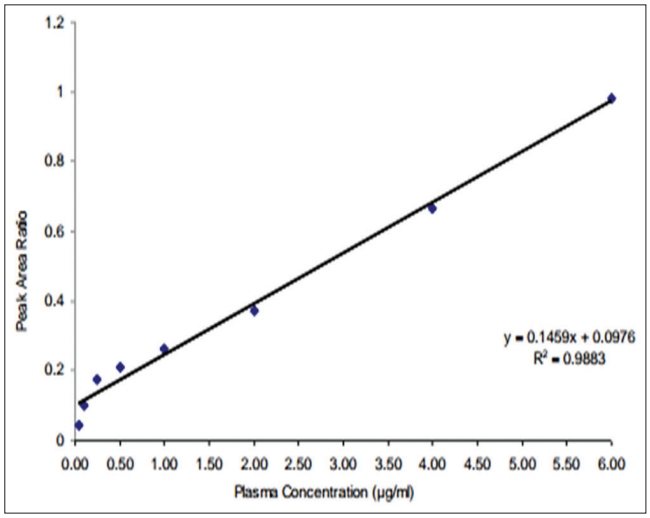

Figure 1: Plasma calibration curve of efavirenz

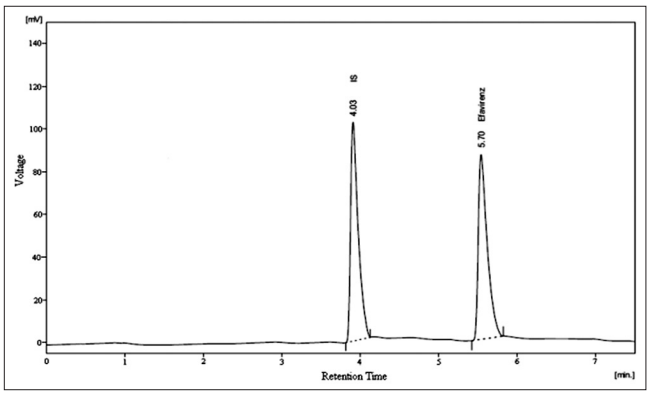

Figure 2: System suitability sample (efavirenz and IS)

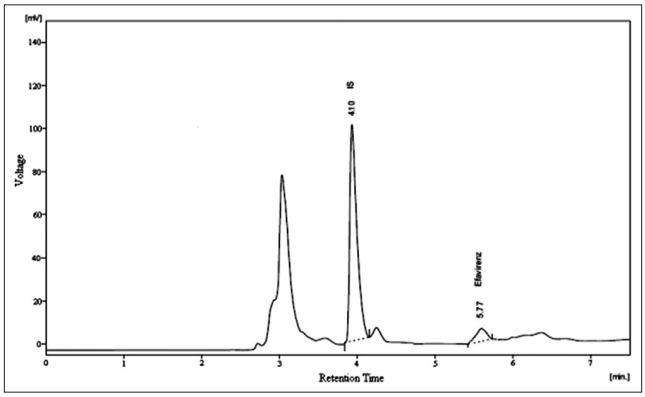

Figure 3: Blank human plasma spiked with IS and analyte (efavirenz) at LLOQ (50 ng/mL)

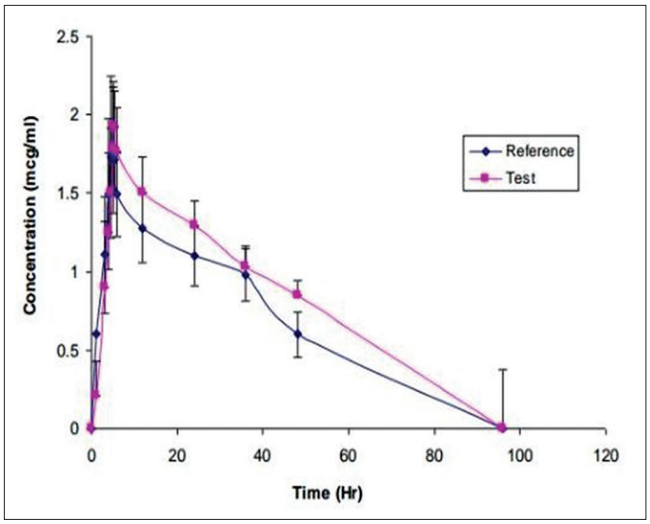

Figure 4: Plasma concentration (mean ± standard deviation) versus time profile of efavirenz in human plasma

exact time was calculated according to drug administration schedule. The first blood sample (t = 0) was collected immediately before drug administration. The exact time of the collection of all blood samples was recorded and reported. A total of 12 blood samples were collected at 0 h (before drug administration), 0.5, 1, 1.5, 2, 2.5, 3, 6, 9, 12, 15, 18, and 24 h in coded centrifuge tubes containing EDTA. Blood samples were centrifuged immediately, the plasma separated into duplicate polypropylene tubes containing EDTA and stored deep freezer at −20°C. The tubes were labeled with volunteer code number, sampling time, and study date.

Dietary control of volunteers during *in vivo* study[13-15]

A standardized breakfast lunch and dinner were served to subjects at 3, 6–8, and 14 h, respectively, after drug ingestion. Water was provided *ad libitum* until 1 h pre-dose. Drug was given with 240 mL of water at room temperature, and no fluids except on cup of noncaffeine-containing soft drink were allowed until 3 h postdose. On the study day, volunteers were permitted normal activities excluding strenuous exercise.

Record of adverse events during *in vivo* study

Abnormal signs of symptoms or adverse reactions if any were monitored during the study, and it should be recorded.

Analyses of the blood samples

The concentration of efavirenz in blood samples were analyzed by validated HPLC method.

Evaluation of pharmacokinetic parameters[16]

The plasma levels produced by the administration of the efavirenz in each volunteer were used to establish the pharmacokinetic profile of test and reference preparation. The following pharmacokinetic parameters were calculated for each subject.

1. Peak plasma concentration (Cmax).
2. Time to maximum plasma concentration (tmax).
3. Area under curve from time 0 to 96 h (AUCt).
4. Area under curve from time 0-∞ (AUC 0-∞).
5. Elimination half-life.
6. Elimination rate constant.

RESULT

Administration of the reference preparation, tablet efavir as a single dose in the fasting state, produced the maximum plasma concentration of 2.076 ± 0.229 mcg/mL (Cmax) at a time 4.675 ± 0.520 h (tmax), whereas the test preparation of E.F.600 CAPSULE as a single dose in the fasting state produced the maximum plasma concentration 2.122 ± 0.280 mcg/mL (Cmax) at the time 5.250 ± 0.574 h (tmax) [Table 1].

Administration of the reference preparation, tablet efavir, produced the area under plasma concentration time curve (AUC0-t) 54.255 ± 9.285 mcg/mL, whereas administration of the test preparation of CAPSULE E.F.600 produced the area under plasma concentration curve (AUC0-t) 57.484 ± 6.331 mcg/mL [Table 1].

When administered as a single dose in the fasting state, the reference preparation, tablet efavir, produced the area under plasma concentration time curve up to infinity (AUC0-∞) 96.653 ± 16.426 mcg/mL, whereas administration of the test preparation of E.F.600 CAPSULE produced area under plasma concentration time curve up to infinity (AUC0-∞) 103.51 ± 14.50 mcg/mL [Table 1].

Administration of the reference preparation, tablet efavir, produced the plasma elimination half-life (t½) 37.218 ± 5.053 h, whereas administration

Table 1: Pharmacokinetic parameters in 20 volunteers with the test and reference preparation

Pharmacokinetic parameters	Reference preparation (A) Mean ± SD	Test preparation (B) Mean ± SD
Cmax (mcg/mL)	2.076 ± 0.229	2.122 ± 0.280
tmax	4.675 ± 0.520	5.2500 ± 0.574
AUC_{0-t} (mcg.h/mL)	54.255 ± 9.285	57.484 ± 6.331
$AUC_{0-\infty}$ (mcg.h/mL)	96.653 ± 16.426	103.51 ± 14.50
Kel (h^{-1})	0.0189 ± 0.0025	0.0190 ± 0.00295
$t^{1/2}$ (h)	37.218 ± 5.053	37.436 ± 6.096
Relative bioavailability (%)	100	105.95

Table 1a: 90% Confidence interval with the test and reference preparation

Cmax	
Untransformed data	0.9456216-1.11426
Ln transformed data	0.922557-1.161968
AUC_{0-t}	
Untransformed data	0.9798676-0.15259
Ln transformed data	0.997789-1.03512
$AUC_{0-\infty}$	
Untransformed data	0.9997887-0.147583
Ln transformed data	1.001656-1.0302842

of the test preparation of E.F.600 CAPSULE produced the plasma elimination half-life (t½) 37.436 ± 6.09 h [Table 1].

Administration of the reference preparation tablet efavir produced the plasma elimination constant (Kel) 0.0189 ± 0.0025/h, whereas administration of the test preparation of E.F.600 CAPSULE produced the plasma elimination constant (Kel) 0.0190 ± 0.00295/h [Table 1] [Figure 4].

On the basis of comparison of the AUC0-t for efavirenz 6OOMG, after single dose administration, the relative bioavailability of the test preparation of E.F.600 capsule was 105.95% of that of the reference preparation, tablet efevir [Table 1].

90% confidence interval for Cmax, values of test preparation of E.F.600 capsule, was 0.94562162–1.11426 of that of reference preparation. 90% confidence interval for AUC0-t values of the test preparation of E.F.600 CAPSULE was 0.9798676–1.15259 of that of the reference preparation. 90% confidence interval for AUC0-inf values of the test preparation of E.F.600 CAPSULE, was 0.9997887–1.147583 of that of reference preparation [Table 1a] [Figure 2].

Statistical inference[17]

ANOVA (subject, period, and treatment) was applied to the Cmax, Ln Cmax, AUC0-t, and Ln AUC0-t values. There was no statistically significant difference for the treatment values of Cmax, Ln Cmax, AUC0-t, and Ln AUC0-t.

90% confidence interval for Cmax, Ln Cmax, AUC0-t, and Ln AUC0-t values of test preparation of E.F.600 CAPSULE was within the accepted limit of that of the reference preparation (i.e., 0.8–1.2).

Difference and ratios of Cmax, Ln Cmax, AUC0-t, and Ln AUC0-t were within the normal limits for both the test and the reference preparation of efavirenz 600MG.

DISCUSSION

The single dose bioequivalence study of E.F.600 CAPSULE containing efavirenz 600MG conducted in 20 adult healthy, human, male volunteers with two preparations of efavirenz. Values of Cmax, tmax, and AUC0-t were comparable for the reference and test preparation in the fasting state.

Efavirenz was detected in plasma from 1 h to about 48 h in the reference preparation as well as in the test preparation. Peak plasma levels of efavirenz with both the preparations were achieved between 1 and 2 h. The mean peak plasma levels of efavirenz 600 mg with reference preparation, tablet efavir[18,19] on the study day, ranged between 1.723 and 2.280 mcg/mL, while the test preparation of E.F.600 CAPSULE ranged between 1.722 and 2.576 mcg/mL. On the basis of comparison of the AUC0-t for efavirenz after single dose administration, the relative bioavailability of the test preparation of E.F. 600 CAPSULE was 105.95% of that of the reference preparation, tablet efavir [Figures 1 and 3].

CONCLUSION

On the basis of the pharmacokinetic parameters studied, it can be concluded that the test preparation of E.F.600 capsule containing efavirenz 600MFG manufactured by Mcneil and Argus Pharmaceuticals Ltd., AMBALA CANTT. 133001 is bioequivalent with reference preparation, tablet EFAVIR of M/S Cipla Ltd., district Solan 173205, India.

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