STUDY OF DIFFERENTIATING ABILITY OF BIORELEVANT MEDIA OVER THE COMPENDIUM MEDIA EXEMPLIFYING WITH EFAVIRENZ AS A MODEL DRUG

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Summary

Simple compendia media are of value for quality control purposes and adequate only for predicting the performance of highly soluble compounds but inadequate for less soluble compounds. Compendial media normally failed to predict dissolution for *in vitro-in vivo* correlation (IVIVC) of class II drugs because their composition does not consider all the physiological parameters.

The objective is to study dissolution of poorly soluble drug Efavirenz in different biorelevant dissolution media and to evaluate the usefulness of the biorelevant dissolution testing in predicting the food and formulation effects on the absorption of poorly soluble lipophilic Efavirenz (EFV) drug.

Dissolution studies were studied using USP apparatus II in FaSSGF, milk and SLS (0.1, 0.2, 0.3, 0.5%). The *in vitro* data was compared with the *in vivo* data for the new formulation (NF) and the marketed formulation (MF) under fasted and fed conditions.

The NF developed was compared against the MF. The % release was studied using various biorelevant media in fasted and fed conditions. In the fed condition the Cmax for the NF (1454 ng/mL) is lesser than the MF (1698 ng/mL) indicating that the food effect generally observed in the MF is lowered in the NF. The T_{max} of the NF (2 hours) was found to be lesser than the MF (4 hours and 3 hours in fed and fasted conditions respectively) indicating that the NF reaches the system faster than the MF. An IVIVC for formulations derived based on the Pearson`s correlation coefficient was found to be in the range of 0.9 to 1.0.

The present study clearly demonstrates the utility of the biorelevant *in vitro* data in the prediction of the meaningful Invivo data of poorly soluble drug EFV.

Keywords: Lipophilic drug, *in vitro-in vivo* correlation (IVIVC), Fasted State Simulated Gastric Fluid(FaSSGF), Fed State Simulated Gastric Fluid(FeSSGF), Biorelevant dissolution testing, Efavirenz

Introduction

Efavirenz (EFV) is a lipophilic and non-nucleoside reverse transcriptase inhibitor for the treatment of the HIV infection and new drug application (NDA) warrants *in vitro* dissolution data together with bioavailability, chemistry, manufacturing and control (CMC) data. An appropriate medium has been chosen based on discriminatory capability, robustness, stability of the analyte in the test medium, and relevance to *in vivo* performance (1-3).

Simple compendia media are of value for quality control purposes and adequate only for predicting the performance of highly soluble compounds which is to a greater extent the basis of bio-waiver guidelines (4) but inadequate for less soluble compounds. Compendial media normally fail to predict dissolution for *in vitro-in vivo* correlation (IVIVC) of class II drugs because their composition does not consider all the physiological parameters mentioned (5).

Galia et al. (5) suggested that use of milk as a dissolution medium may be a useful tool for detecting specific food/formulation interaction on Class I drugs. They also proposed two new media representing the fed and fasted state in the upper jejunum while milk, 3.5% fat and the USP simulated gastric fluid with or without pepsin (1) were chosen to represent fed and fasted state conditions of the stomach respectively. Dressman et al. (6,7) showed that many factors such as surfactants, pH, buffer capacity, ionic strength, volume available for dissolution and the media used need to be controlled to dissolute class II drugs for a meaningful *in vitro-in vivo* correlation (IVIVC).

Jantratid et al. (8) showed that biorelevant Fasted state simulated gastric fluid (FaSSGF) and Fed state simulated gastric fluid FeSSGF media were stable for 72 hours under usual dissolution test conditions and can be used to predict formulation performance and food effects *in vivo* but difficulties in drug analysis limited the use of this approach. Alternative media such as milk (with 3.5% fat) and Ensure® Plus are similar to those of the standard meal recommended

by the American HHS-FDA for the effects of food in bioavailability (BA) and bioequivalence (BE) studies (9). It was found that drug binding to milk components was increased with the increased fat content of milk (10) whereas tablet disintegration was 5-fold more in milk than in FaSSGF (11). Other alternative media proposed are milk with an acidic solution of pepsin and snapshot media (8).

Nicolaides et al. (12) suggested that the prediction of the plasma profile of a lipophilic drug is possible by *in vitro* dissolution data which is better than those obtained with compendial media if its absolute bioavailability is known with dissolution limited absorption.

The main objective of the present study is to study dissolution of poorly soluble drug Efavirenz in different biorelevant dissolution media and to evaluate the usefulness of the biorelevant dissolution testing in predicting the food and formulation effects on the absorption of poorly soluble lipophilic drug, Efavirenz. The secondary objective was to check the usefulness of using the biorelevant media as a pre-cursor for the bio-studies in comparison to the compendia medium.

Experimental

Materials and reagents:

Sodium lauryl sulfate were obtained from Hi Media, Pepsin(95% purity) and Ammonium acetate (98% purity) from Loba Chemie Private Limited; Hydrochloric acid (36% purity), Sodium chloride (99.5% purity), acetic acid (99.8% purity), acetonitrile (HPLC grade) and ammonia (30% purity) were obtained from Qualigens; Efavirenz drug used in the analysis were obtained from Aurobindo Pharma Private Limited; marketed formulation (600 mg) of LOT# 6K17955A and the R&D new formulation RD039 formulated (600 mg) at International Specialty Products (ISP) were collected for the present study.

Instrumentation:

The HPLC system (Alliance, Milford USA) consisted of 2695 Separation module and 2998 Photodiode array detector with a thermostat for column and autoinjector. The Varian VK 7010 dissolution apparatus equipped with auto sampler VK 8000 unit were used for the dissolution studies. The cooling multispeed centrifuge from Thermo Electronic Corporation (IEC CL31R) was used for the centrifugation of the samples at higher rpm and lower temperature.

Preparation of Efavirenz tablets:

Efaverinz (50%), Microcrystalline cellulose (MCC) PH 101 (20%), cross carmellose sodium (CCS)(6%), Lactose (20%) and Plasdone K29/32 (3%) were pre-mixed with water for 1 minute inside the charge vessel of the Pro-C-Ept granulator at an impeller speed of 1000 rpm. The chopper was started at 2300-2500 rpm after 2 minutes of water addition. The granules were sieved through #18-mesh sieve and dried at 60°C. The final dried granules were sized through #30-mesh sieve, blended with superdisintegrant, magnesium Stearate (0.5%) and fumed silica (0.5%) in a V-cone blender (8 rpm) to form a homogenous mix and compressed using (for 600 mg) 19/9 mm capsule shaped standard concave punch set was used on a 16 station rotator tablet press (Cadmach model CMD4, India). Advanced Instrumentation monitor (AIM) software (Metropolitan Computing Corporation, USA) was used with the tablet press to determine the compression force required to give tablets of approximately equal hardness(breaking force) for the various doses used in the study (13).

Hardness and friability determination:

The hardness of the prepared tablets was determined 24 hours after compression using an Erweka hardness tester (Erweka TBH 310 MD, Germany), which also measures the tablet diameter. Ten tablets were tested for hardness from each batch, the mean and standard deviations were calculated.

Pre- weighed 20 tablets were placed in a plastic chambered friabilator (Erweka TAR 200, Germany) attached to a motor revolving at a speed of 25 rpm for 4 minutes. The tablets were then de- dusted, reweighed and percentage mass loss (friability) was calculated.

Content uniformity:
The prepared tablet formulation was assayed for drug content. Ten tablets were randomly selected from the batch and pulverized to a fine powder. Weighed aliquots containing an amount of powder equivalent to a single dose were taken in triplicate and assayed for the drug content using procedure mentioned in the analytical method section.

Composition and preparation of various dissolution media:

Preparation of Fasted State Simulated Gastric Fluid (FaSSGF): About 16 g of sodium chloride was weighed into 5000 mL of Milli-Q water (Elix, Millipore, USA) followed by the addition of 16 g of Pepsin and 35 mL of hydrochloric acid with constant stirring. The pH of the solution was found to be 1.2. (USP32)

Fed State Simulated Gastric Fluid (FeSSGF) Milk (with 3.5% fat) is used as dissolution medium to represent the fed state stomach.

Dissolution Studies using biorelevant media: For all dissolution tests the paddle method using USP apparatus 2 (Varian VK7010, with VK750D temperature controller and automated temperature sensor) was used. About 500 mL of dissolution medium for fasted state (14), and 1000 mL of dissolution medium for fed state at a temperature of 37 ± 0.5ºC were used at 50 rpm. Ten mL of samples were drawn at appropriate time intervals. Dissolution profiling was carried out for the time points of 5, 10, 15, 30, 45 and 60 minutes, respectively. All the dissolutions were carried out using 6 vessels. The samples except the fed stomach were drawn at the above mentioned time points and diluted by taking 1 mL from the aliquot to 10 mL and the solution was made to the mark with the mobile phase, filtered through 0.45 µm Millipore filters prior to injection to the HPLC.

For the fed stomach condition, the samples were drawn from the auto-sampler and it was observed that the drug was present in the non-aqueous layer of the milk. Hence the drug was extracted from the milk with ethyl acetate due to its high solubility (724 mg/mL) and low boiling point (78ºC) compared to other immiscible solvents. The organic layer collected was centrifuged using a cooling centrifugal device at 10°C and 6000 rpm for 30 minutes.

The solution was transferred to 50 mL bottles. The ethyl acetate was evaporated using incubator shaker for 60 minutes at 80°C. The solution was then reconstituted with 2 mL of acetonitrile and filtered through 0.45 µm filter. Similarly, all the other samples were prepared and were analyzed by HPLC. All the samples were stored at 4°C prior to analysis.

Dissolution Testing: The dissolution profiling of the prepared tablets was carried out in 1000 mL water with 2% SLS as recommended by U.S. FDA. The testing conditions were 50 rpm paddle speed of the apparatus USP Type II paddle at a temperature of 37.0ºC ± 0.5ºC with different time points of 0, 5, 10, 15, 30, 45 and 60 minutes respectively.

Methodology for content uniformity and in vitro dissolution studies (by HPLC):

A validated analytical method using the International Conference on Harmonization (ICH) guidelines (15) was used in the present study. The Waters HPLC system with a Thermo Hypersil BDS C18 reverse phase column (250 X 4.6 mm, 5 µ, 130 Å) maintained at ambient condition using a thermostat was used for the analysis. The column was equilibrated with 0.01 M ammonium acetate solution (pH 7.5) and acetonitrile in the ratio of 50: 50 (v/v) and a flow rate of 1.5 mL/min. A 20 µL aliquot of the sample solution was injected to the column.

Methodology for In vivo study:

The study was performed on 12 healthy male volunteers (subjects). The selection of subjects was performed based on Life Insurance Corporation of India's ideal height-weight chart for non-medical case and subjects weighed within ± 15% of the chart. The age group of the subjects was between 18 to 45 years and the body mass index was between 18 to 25 kg/m2. The subjects were selected on the basis of laboratory evaluations, medical history, clinical examination, X-ray, ECG recordings, screen for drugs of abuse and screen for alcohol breath test. The subjects meeting these criteria were selected for a two period cross over design study. Subject did not take any medication or alcohol at least 14 days prior to and during the entire study. Each subject gave his written consent to participate.

Subjects were randomly divided into two groups and one group received 600 mg oral dose of marketed formulation and another group received the new R& D formulation with 240 mL of water after an overnight fast (10 hours). Blood samples were collected just before the drug administration and at 0.50, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 8.0, 12.0, 24.0, 48.0, 72.0 and 120.0 hours following drug administration. After a washout period of 21 days (16), the volunteers received 600 mg of the marketed formulation and new R& D formulation after a meal. Plasma was separated and stored at -70° C.

Plasma assay:
Ten microliters of internal standard (Efavirenz d4) and 0.5 mL of ammonium acetate buffer (pH 4.5) were added to 2.5 mL of plasma extracted with methyl-tert-butyl ether (TBME) as extraction solvent. The mixture was vortexed for 10 minutes and centrifuged at 3000 rpm for 5 minutes. An aliquot of 200 µL of the supernatant was transferred to an auto sampler vial and injected in a Shimadzu (Kyoto, Japan) system using Thermo Hy- Purity advance (50 X 4.6 mm, 5 µm); and a mixture of 2 mM ammonium acetate pH 7.5 and acetonitrile (20: 80) as mobile phase. The temperature in the auto sampler and in the oven was 6 and 35ºC respectively. The analysis was performed using Analyst® software. The detection was made using mass/mass spectrometer (Applied Biosystems, Sciex, API-4000). The method was validated according to ICH guidelines.

Results

Physical parameters of the tablets:

Mass and thickness of the prepared tablets were within 1202.4 ± 21.2 mg and 3.54 ± 0.5 mm respectively. Further the hardness of the prepared tablets was maintained at 120 ± 5 N and the friability was much lower than 1%. New formulation demonstrated good content uniformity, varying from 99.6 ± 0.2%.

Validation of In Vitro analytical method:

The developed HPLC method was validated by monitoring linearity and range, precision, accuracy, intermediate precision, limit of detection (LOD) and limit of quantification (LOQ) using a stock

solution of the drug at the strength of 1 mg/mL. Linearity and range were determined by injecting a 20 µL solution containing 1-200 µg/mL of the drug in triplicate into the HPLC column. It was found that the correlation coefficient (R^2) for linearity was 0.999. Precision was determined by injecting two different concentrations (1 and 100 µg/mL) six times on the same day as well as on different days. The percentage of relative standard deviation (R.S.D) was found to be less than 0.5% (intra-day) and less than1.5% (inter-day). The accuracy was evaluated by fortifying a mixture of degraded solutions with four known concentration of the drug. The recovery of the added drug was within the range of 98 to 100%. The intermediate precision (% RSD) was determined through a study on a different chromatographic systems using a different column (Qualisil®BDS C18, 250 X 4.6 mm, 5µ, 130 Å) to be less than 1.0%. LOD and LOQ were determined by using signal to noise ratio as 0.4 and 1 µg/mL. This was further confirmed by injecting the same concentration into the HPLC column.

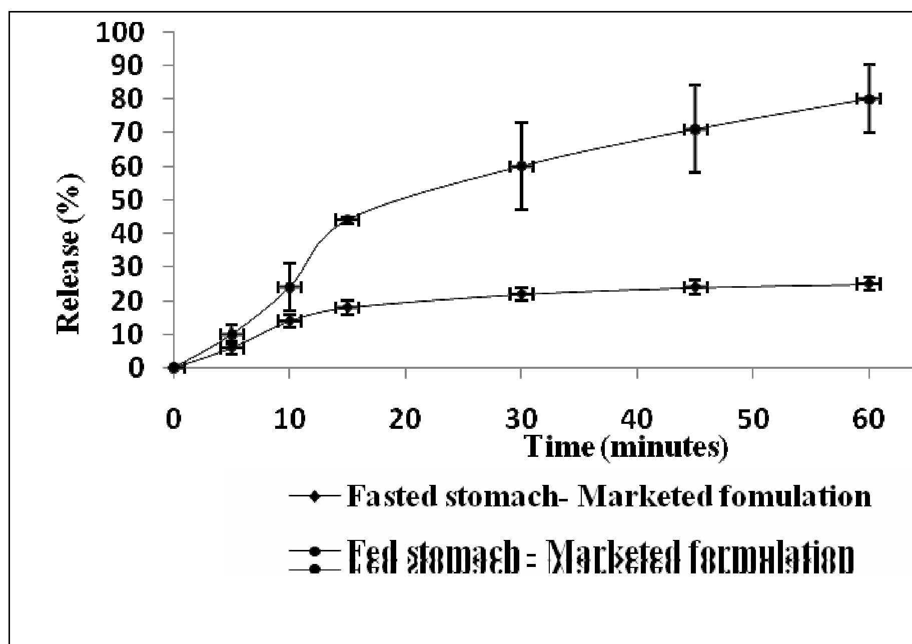

Figure 1: Dissolution Profiling of Efavirenz Marketed Formulation In biorelevant media with standard deviation bars.

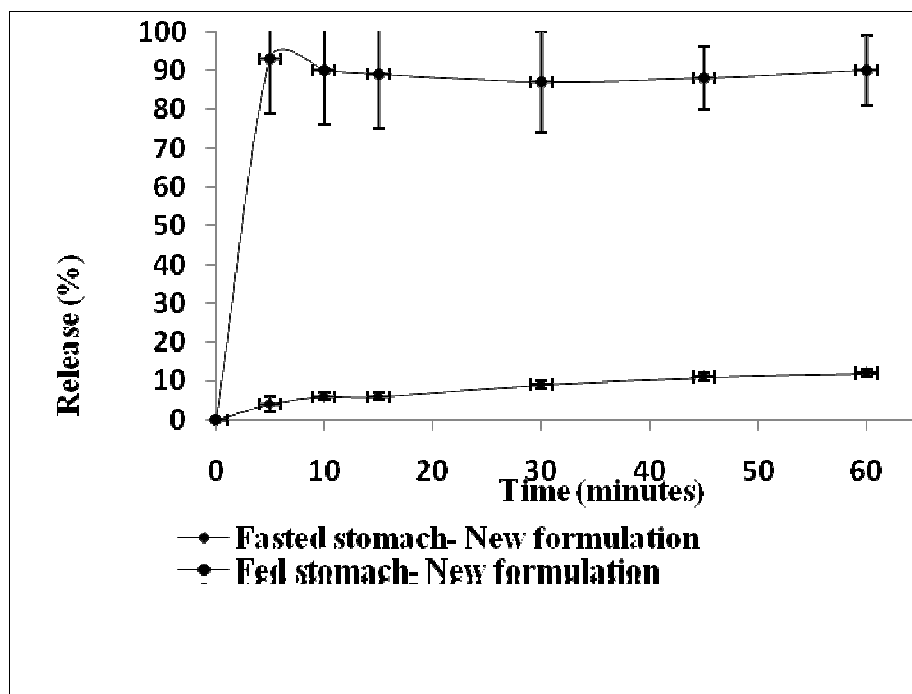

Figure 2: Dissolution Profiling of Efavirenz New Formulation in Biorelevant media with Standard deviation bars.

The HPLC method was applied to dissolution studies of the marketed formulation and the new formulation of Efavirenz drug in different biorelevant dissolution media. The results, the mean dissolution profiles of Efavirenz marketed formulation in biorelevant dissolution media is presented in Figure 1. Similarly, Figure 2 shows the mean dissolution profiles of Efavirenz new formulation in biorelevant dissolution media.

In case of FaSSGF (pH~2), release from marketed formulation was significantly higher than the new formulation while the converse was true when the dissolution was carried out in FeSSGF (pH~5) (Figures 1 and 2). In other words, new formulation favors the release at higher pH. It is also evident from Figures 1 & 2 that both the marketed and new formulations showed increase in drug release in milk when compared to the corresponding release in FaSSGF. Additionally, drug absorption in fed state was observed to reach maximum faster for the new formulation in comparison to the marketed formulation.

Figures 1 & 2 depict the role of pH of the dissolution media on the release of Efavirenz from two different formulations. In the Efavirenz formulation the drug release clearly depends on the pH of the media. In the case of new formulation the drug release reaches the saturation point in 5 minutes and the release is maintained at other time points. However, in the case of the marketed formulation the drug release is delayed.

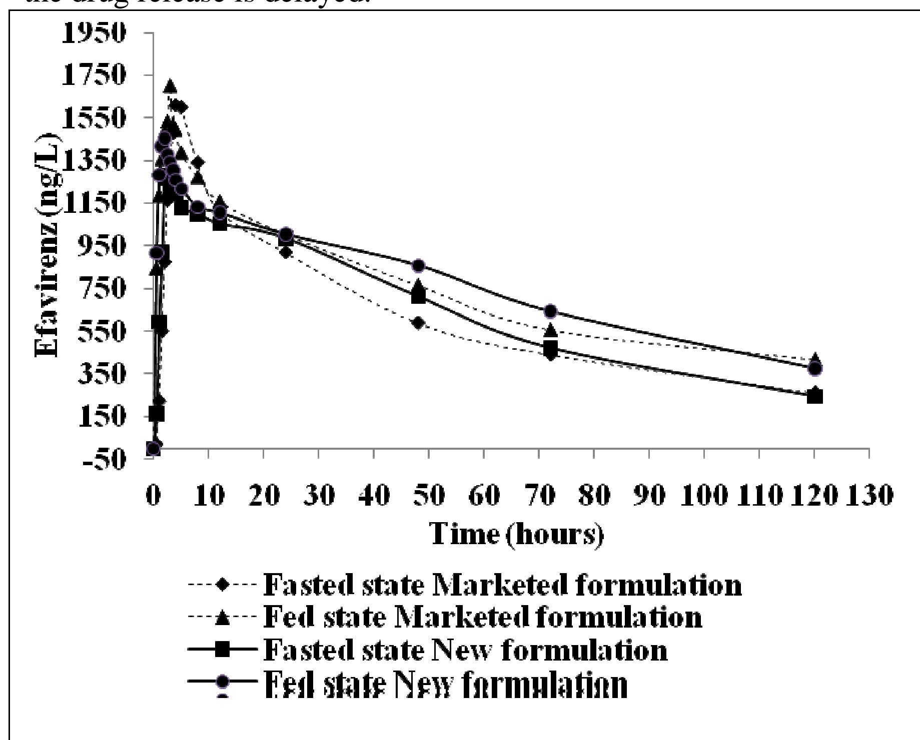

Figure 3: Pharmacokinetic Parameters of Efavirenz Marketed and New formulation in Human Plasma

Table 1: Pharmacokinetic Parameter of Efavirenz in Human

Condition	Sample Details	T_{max} (hour)	C_{max} (ng/mL)	AUC_{0-120h} (ng.h/mL)
Fasted	New formulation	2	1281	76397
	Marketed formulation	4	1608	73625
Fed	New formulation	2	1454	91538
	Marketed formulation	3	1698	88844

The pharmacokinetic parameter of the marketed and the new formulation of Efavirenz by the human bio-studies in the fasted and fed state are represented in Table 1 and Figure 3. The analysis of all test samples was done using a calibration curve with quality control standards, distributed throughout each batch. A set of quality control samples was analyzed before and after all test samples of each period. The analyst did not have access to the randomization schedule until analysis was completed. In the case of marketed formulation the T_{max} for the fasted and the fed state was observed to be 4 and 3 hours respectively. Similarly for the new formulation the T_{max} for the fasted and the fed state was observed to be 2 hours. This indicates that the new formulation reaches the blood stream earlier compared to the marketed formulation regardless of it is taken with or without food. However the Cmax for the new formulation is lesser than the marketed formulation indicating that the food effect generally observed in the marketed formulation is lowered in the new formulation. The AUC for the new formulation was found to be higher than that of the marketed formulation indicating that the new formulation is available more to the body compared to the marketed formulation.

An *in vitro-in vivo* correlation (IVIVC) for the marketed and the new formulation was derived based on the Pearson’s coefficient (17). The *in vitro* dissolution profile in the various biorelevant media in fasted and fed state was correlated to the *in vivo* data of fasted and fed state respectively and a meaningful correlation was obtained. The correlation coefficient was found to be in the range of 0.9 to 1.0 (Table 2).

Table 2: In vitro-in vivo Correlation

Biorelevant Media	**Pearson’s Coefficient**	
	MF	**NF**
FaSSGF	0.93	0.93
FeSSGF (Milk)	0.89	0.92

Discussion

The selection of appropriate drug separation and analytical methods are necessary to obtain meaningful intended results considering the complexity of the biorelevant media compositions (8). In the present study retention time of Efavirenz was found to be 10.5 minutes at λ_{max} of 252 nm. Further, free fatty acids produced from digestion of lipid dosage forms can induce a pH shift in the media (18) as well as lipolysis products enhance wetting characteristics and hence solubilization (19), but the effects are especially apparent for poorly soluble lipophilic drugs (20). Meals can enhance solubility and dissolution of poorly soluble drugs and hence bioavailability (20, 21, 22).

Selection of the most appropriate media conditions is based on discriminatory capability, robustness, stability of the analytes in the test medium, and relevance to *in vivo* performance, were possible. When selecting the dissolution medium, the chemical and physical properties e.g. the solubility and solution state stability of the drug substance and drug products as a function of the pH value need to be considered.

Using the compendial dissolution medium with 2% SLS which was designed for routine analysis one neither can differentiate the release between different formulation nor one can forecast the food effects on drug absorption, since no distinction is made between fasted and fed state in the design of the compendial media. In the present study the quasi-sink media was used to study release pattern of drugs for differentiating their dissolutions and it was found satisfactory with 0.2% SLS (Figure 4).

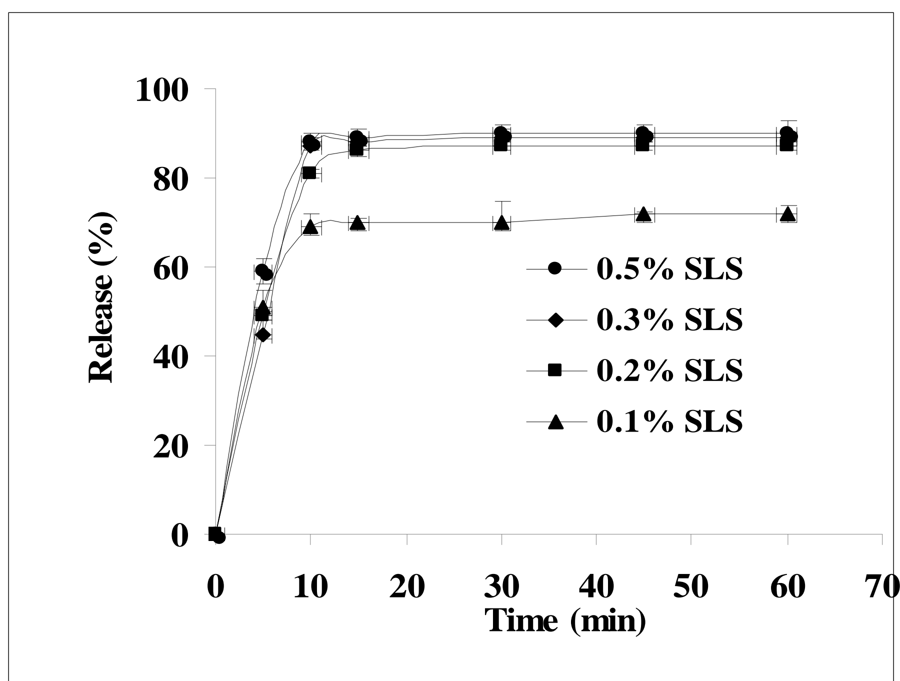

Figure 4: Dissolution Profiling (Time vs Release) of Efavirenz Test Formulation in quasi sink media. Experimental parameters were the same as those described in the text.

The biorelevant media were used to simulate the conditions of fasted stomach and fed stomach. Hence, biorelevant media were able to predict trends in the *in vivo* data for different formulations and also the food effects. Dressman and Reppas (7) showed that the biorelevant media combinations had a clear advantage over the compendial media, which calculated plasma concentrations much closer to those observed values and predicted the possible food effects on dissolution and absorption.

Composition of gastric components in the fed state is highly dependent on the ingested meal and therefore it is difficult to design a medium which would be universally applicable. Milk is a logical starting point, since its ratios of fat : protein : carbohydrates are similar to those in a typical diet (23, 24). Solubilization of drugs in milk is due to non-specific binding to casein micelles and partitioning into the lipid components for highly lipophilic compounds.

The results of the present study (Figures 1 & 2) supported the findings of Galia et al. (10). The drug release and drug absorption of the new formulation both were increased and reached maximum within 5 minutes when milk was used as dissolution medium compared to that of FaSSGF which was supported by others (10, 11, 23 and 24).
The pH of FaSSGF and FeSSGF is 1.2 and 5.0 respectively. In FaSSGF the marketed formulation released a dose (25% at 60 min) which was higher than the test formulation (12% at 60 min). Similarly in the FeSSGF the new formulation (90% at 60 minutes) was found to higher than the marketed formulation(80% at 60 minutes). These results clearly show that the release of the new formulation was higher at higher pH (pH 5 vs pH 1.2) Figures 1 & 2.

In addition, T_{max} (time where a drug is present at the maximum concentration in blood plasma/serum) in the fed state of the marketed formulation was at 4 hrs and that of the new formulation was at 2 hrs. This clearly indicated that the new formulation could maintain drug level in blood for longer time than the marketed formulation and the food controlled the optimum dissolution of drug with a longer time presence in the blood. The AUC for the new formulation is higher than the MF indicates that the NF is available more to the body compared to the marketed formulation (Figure 3).

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Moreover, the new formulation would relief the patients immediately after intake (Table 1). Besides, the utilization of milk as an inert vehicle of drug delivery systems (25) would be an additional reason for performing dissolution experiments in the presence of milk.

Case examples of lipophilic drugs, where substantial differences in dissolution were observed between compendial and biorelevant media include albendazole (26), danazol and ketoconazole (27), and atovaqone and troglitazone (28). As expected from atovaquone's high lipophilicity, its dissolution in milk was higher than in all other media and reached a mean maximum (12%) of its oral bioavailability in the fed state (29). The present study also supported the facts that biorelevant media was better than compendial media for dissolution and absorption of less soluble lipophilic drugs like EFV.

The biorelevant media was used to differentiate between formulations and the food effect on absorption of Efavirenz.

Further, a bio-study was also conducted of the marketed formulation and a test R&D formulation. The results of the present study (Table 1 & 2 and Figure 3) clearly supported the findings of others (26, 27 and 29). An IVIVC correlation was drawn between the biorelevant dissolutions and it was within the range. It was found that the biorelevant dissolution studies could successfully assess the food effect.

Conclusions

The present study clearly demonstrates the utility of *in vitro* dissolution testing using biorelevant media as a tool for predicting the *in vivo* absorption of a poorly soluble drug EFV. Additionally, it can also be employed to forecast the food and the formulation effect of the poorly soluble lipophilic drug. Hence the biorelevant *in vitro* data assist in the prediction of the meaningful *in vivo* data.

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References

1. The United States Pharmacopeia USP 32. United States Pharmacopeial Convention Inc., Rockville, Marryland: US
2. Surendra KB, Thomas L, Ernest DB, Marta H et al. Qualification of Analytical Instruments for use in the pharmaceutical industry: A Scientific Approach. AAPS PharmSciTech 2004; 5(1): 22.
3. Swartz ME. Analytical Instrument Qualification, Pharmaceutical Regulatory Guidance. Newjersy: American
4. CPMP note for guidance on the investigation of bioavailability and bioequivalence (CPMP/EWP/QWP/1401/98 Rev 1), January 2010: London.
5. Galia E, Nicolaides E, Horter D, Lobenberg R, Reppas C, Dressman JB. Evaluation of various dissolution media for predicting In vivo performance of class I and II Drugs. Pharm Research 1998; 15: 698-705.

6. Dressman JB, Amidon GL, Reppas C, Shah VP. Dissolution testing as prognostic tool for oral drug absorption: immediate release dosage forms. Pharma Res 1998; 15: 11-22.
7. Dressman JB, Reppas C. In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs. E Pharm Sci 2000; Suppl. 2: S73–S80.
8. Jantratid E, Janssen N, Reppas C, Dressman JB. Dissolution Media Simulating Conditons in the Proximal Human Gastrointestinal Tract: An Update. Pharm Res 2008; 25: 1663-1676.
9. Klein S, Butler J, Hempenstall JM, Reppas C, Dressman JB. Media to simulate the postprandial stomach Matching the physicochemical characteristics of standard breakfasts. J Pharm Pharmacol 2004; 56: 605–610.
10. Macheras PE, Koupparis MA, Antimisiaris SG. Effect of temperature and fat content on the binding of hydrochlorothiazide and chlorothiazide to milk. J Pharm Sci. 1988; 77: 334–336.
11. Anwar S, Fell JT, Dickinson PA. An investigation of the disintegration of tablets in biorelevant media. Int J Pharm 2005; 290:121–127.
12. Nicolaides E, Symillides M, Dressman JB, Reppas C. Biorelevant dissolution testing to predict the plasma profile of Lipophilic Drugs after Oral Administration. Pharm Res 2001; 18: 380-388.
13. Yelchuri VR, Jagadish B, Koduri B et al. Impact of super disintegrants on Efavirenz release from tablet formulations. Acta Pharm. 2010; 60: 185-195.
14. Thomas Z and Sandra K. Simplified Biorelevant Media for Screening Dissolution Performance of Poorly Soluble Drugs. Dissolution technologies 2007: November
15. ICH Guidelines, Validation of Analytical Procedures: Text and Methodology.
16. Ramachandran G, Hemanth Kumar AK, Swaminathan S, Venkatesan P, Kumaraswami V, Greenblatt DJ. Simple and rapid liquid chromatography method for determination of Efavirenz in plasma. J Chrom B 2006; 835: 131-135.
17. Cardot JM., Besyssac, and Alric M. Invitro- Invivo Correlation: Importance of Dissolution in IVIVC. Dissolution technologies 2007: February

18. Porter CJ, Charman WN. In vitro assessment of oral lipid based formulations. Adv Drug Deliv Rev 2001; 50(1): S127–S147.
19. Luner PE, Vander Kamp D. Wetting characteristics of media emulating gastric fluids. Int J Pharm 2001; 212: 81–91.
20. Jones HM, Parrott N, Ohlenbusch G, Lave T. Predicting pharmacokinetic food effects using biorelevant solubility media and physiologically based modelling. Clin Pharmacokinet 2006; 45: 1213–1226.
21. Fleisher D, Li C, Zhou Y, Pao LH, Karim A. Drug, meal and formulation interactions influencing drug absorption after oral administration. Clinical implications Clin Pharmacokinet 1999; 36: 233–254.
22. Charman WN, Porter CJ, Mithani S, Dressman JB. Physicochemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and pH. J Pharm Sci 1997; 86: 269–282.
23. Macheras PE, Koupparis MA, Antimisiaris SG. Drug binding and solubility in milk. Pharm Res 1990; 7: 537–541.
24. Macheras PE, Koupparis MA, Antimisiaris SG. Effect of temperature and fat content on the solubility of hydrochlorothiazide and chlorothiazide in milk. J Pharm Sci 1989; 78: 933– 936.
25. Macheras P, Reppas C. Studies on drug-milk freeze-dried formulations I: Bioavailability of dicumarol and sulfamethizole formulations. J Pharm Sci 1986; 75: 692-696.
26. Galia E., Horton J, Dressman JB. Albendazole generics—a comparative in vitro study. Pharm Res 1999; 16:1871–1875.
27. Galia E, Nicolaides E, Horter D, Lobenberg R, Reppas C, Dressman JB. Evaluation of various dissolution media for predicting In vivo performance of class I and II Drugs. Pharm Research 1998; 15: 698-705.
28. Nicolaides E, Galia E, Efthymiopoulos C, Dressman JB, Reppas C. Forecasting the in vivo performance of four low solubility drugs from their in vitro dissolution data. Pharm Res 1999; 16: 1877-1883.
29. Dressman JB, Fleisher D. Mixing-tank model for predicting dissolution rate control of oral absorption. J Pharm Sci 1986; 75: 109 –116.