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Research Article

DEVELOPMENT OF BIORELEVANT AND DISCRIMINATING METHOD FOR DISSOLUTION OF EFAVIRENZ AND ITS FORMULATIONS

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ABSTRACT

Dissolution process is considered as an important *in vitro* tool for evaluating the bioequivalence of products. Such a method, if properly mimic the *in vivo* conditions, it would surrogate the *in vivo* studies. Bioequivalence problems arise in class-II and class-IV categories of Biopharmaceutical Classification of Drugs (BCS). Efavirenz (BCS Class II drug) is used in active anti retroviral therapy. Saturation solubility of efavirenz bulk drug was evaluated in various surfactant media, pH solutions and bio-relevant media. We optimized the dissolution conditions for efavirenz with 900 ml of simulated gastric fluid with 0.25% w/v sodium lauryl sulphate (SGF-0.25% w/v SLS) as discriminating and bio-relevant dissolution medium at 50 rpm for 45 min (5 min time interval) with USP apparatus II. The optimized media contained a less amount of SLS (0.25% w/v) in SGF compared with 1% and 2% SLS concentration stated in the official monographs (IP, BP, USP) and FDA guidelines mimic the GI tract environment.
We studied the dissolution profiles of efavirenz bulk drug and its formulations in various concentrations of SLS alone (0.25% - 2.0% w/v) and with simulated gastric fluid with SLS. *In vitro* dissolution profiles of efavirenz and its formulations in optimized dissolution media (0.25% w/v SLS with SGF) followed zero order kinetics with diffusion mechanism drug release.

Keywords: Dissolution; Bio-relevant media; Efavirenz.

INTRODUCTION

The Biopharmaceutical classification system classified the drugs into four basic groups according to their solubility and permeability properties. The class-II category (low solubility and high permeability) drugs are identified as potential drug candidates for investigation. In fact, dissolution is a solubility related phenomena. *In vitro* media is formulated as bio-relevant media, which should be able to mimic the *in-vivo* environment. *In vitro* dissolution media is made bio-relevant by including varying levels of bile salts, lecithin and fatty acids[1-5]. Surfactants increase the solubility of low soluble drugs in the body and helps in the absorption process. Dissolution test conditions are more useful in QC/QA of drug product.

Efavirenz is an HIV-1 specific, non-nucleoside, reverse transcriptase inhibitor (NNRTI). It is chemically (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one [6] (Fig 1).

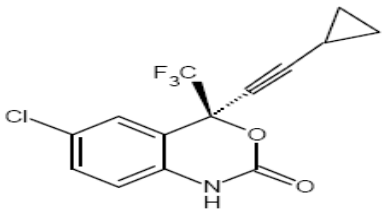

Fig 1:Chemical structure of efavirenz.

The pharmacopoeial (IP, BP, USP) and FDA guidelines stated dissolution media contained 1% and 2% w/v sodium lauryl sulphate for efavirenz formulations[7]. Low concentration of surfactant is more bio-relevant and easy to maintain the sink conditions than higher concentrations[8-11]. Literature survey disclosed the lack of bio-relevant and discriminating dissolution method for efavirenz formulations. The aim of this work was to develop a bio-relevant media with low concentration of surfactant as a discriminating method for dissolution study of efavirenz and its formulations. The strategy was based on the saturation solubility studies in presence of various surfactant media, pH solution and bio-relevant media. Secondly to compare the dissolution profiles of efavirenz bulk drug and its formulations in various concentrations of SLS alone (0.25-2.0% w/v) and simulated gastric fluids.

MATERIALS & METHODS

Chemicals

Efavirenz was procured from Hetero Drugs Limited, India. Hydrochloric acid, methanol, ortho-phosphoric acid, potassium dihydrogen orthophosphate, sodium hydroxide and sodium chloride were purchased from Sd Fine-Chem Ltd., Mumbai. Sodium lauryl sulfate (SLS) and Tween 80 were purchased from Hi-media Ltd., Mumbai. Double distilled water was used throughout the study. Three formulations of efavirenz were obtained from local market.

Instruments

Electro lab-TDT-08L dissolution test apparatus, double beam UV-Visible spectrophotometer-1800 (Shimadzu, Japan), analytical balance (Shimadzu AUX 220,Japan), orbital shaker bath (Bio-Technics, India) , pH meter (Elico,Hyderabad) and ultrasonic cleaner (Sonica) were used for the study.

Methods

Saturation solubility studies

The solubility of efavirenz was determined in various media by preparing saturated solutions (double distilled water; 0.1, 0.5, 1.0, 1.5 and 2.0 % v/v Tween-80 in water; 0.25, 0.5,0.75, 1.0, 1.25, 1.5, 1.75, and 2.0% w/v SLS in water; 0.1 N HC1; acetate buffer, pH 4.5; phosphate buffer, pH 6.8; phosphate buffer, pH 7.4: tris buffer, pH 9.0 and different bio-relevant media such as SLS (0.25-2.0 % w/v) with simulated gastric fluid. An excess of efavirenz was taken in a 50 ml volumetric flask containing 10 ml of media. An orbital shaker bath was used for continuous shaking of the sample solutions in volumetric flasks, maintaining constant temperature of 37±0.5°C for 24 hours. The equilibrated solutions were filtered (0.45 µ whattmann filter paper) to separate the saturated solutions from excess undissolved drug. The saturated solutions were suitably diluted with 0.1 N sodium hydroxide solvent and the concentration of efavirenz was analyzed by spectrophotometrically at 305 nm (FIG 2).

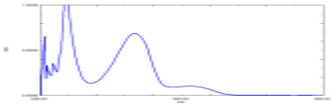

Fig 2: UV Spectrum of efavirenz (10 µg/ml) in 0.1 N sodium hydroxide solution.

Dissolution method-In vitro study

Dissolution testing of efavirenz bulk drug and formulations (Estiva-600 mg, Efcure-200 mg, and Efavir-200 mg) was carried out using paddle apparatus (USP apparatus II) at 50 rpm, 37±0.5° C; 900 ml of SLS alone in the concentration range of 0.25 to 2.0 % w/v and simulated gastric fluid (SGF) with SLS (0.25 to 2.0% w/v). The amount of drug dissolved was analyzed by spectrophotometric method at 305 nm.

We compared the dissolution behavior of efavirenz and its formulations in various concentrations of SLS (0.25-2.0% w/v) and various concentrations of SLS with simulated gastric fluid. Sampling aliquots of 5.0 ml were withdrawn at 5, 10, 15, 20, 25, 30, 35, 40 and 45 min and replaced with an equal volume of the fresh medium to maintain a constant total volume. The sample aliquots were filtered, diluted with 0.1 N sodium hydroxide solvent and quantified spectrophotometrically. Amount of the dissolved drug (efavirenz) was calculated using the regression equation (y=0.0085x-0.0039) of calibration curve (R^2 = 0.999). The cumulative percentage of drug dissolved was plotted against time. This release data in SGF- 0.25% w/v SLS media was subjected for kinetics by fitting the data into zero and first order to know the order of release and Higuchi, Hixon-Crowell kinetics to understand the release mechanism. The dissolution profile data of three formulations in 1% w/v SLS and 0.25% w/v SLS with simulated gastric fluid were compared. All the studies were done in triplicate.

RESULTS AND DISCUSSION

Efavirenz is a highly hydrophobic with functional groups such as -Cl, CF_3, cyclopropane and alkyl groups (FIG1). It has the group NH which can be protonated, but =NH-C=O makes it "enol" extended conjugation. Though the pKa is reported as 10.2., it is weakly basic. The log P of 4.6 also indicates low solubility. The solubility of efavirenz was found to be low in double distilled water 0.0176 mg/ml and was high in Tris buffer (pH 9.0), due to hydrogen bond formation between efavirenz and Tris. Although, the solubility was found to be high in Tris buffer, pH 9.0, the drug didn't show a pH dependent solubility in the GI tract pH range 1.2- 7.4. (FIG 3). The influence of surfactants on the solubility of efavirenz was studied and the data is shown in FIG 4. The non-ionic surfactant (Tween-80) improved solubility of efavirenz as the concentration of the surfactant increased. However, the solubility of efavirenz is higher in the SLS. The SLS (anionic surfactant) is the preferable dissolution media for efavirenz as per official monographs. The solubility of the drug increased proportionally with increase in concentration of SLS (FIG 4). This observation suggested micellar solubilization of drug, SLS must be ion-paired with the drug and enhanced the solubility of efavirenz. Saturation solubility studies were conducted in order to develop bio-relevant media, using different concentrations of the best surfactant (SLS) and simulated gastric fluid (SGF) (FIG 4). There is no much variation in the solubility enhancement of efavirenz in either medium. A slight decrease in solubility of the drug has been noticed in a mixture of SGF with SLS, in comparison to SLS alone, which is of not great concern, thus the combination of SGF-SLS was found to be suitable to use as a media for the dissolution studies of efavirenz commercial formulations.

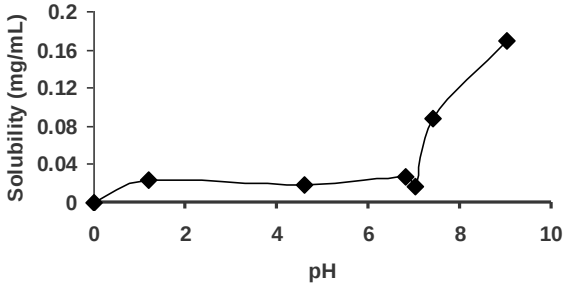

Fig 3:Solubility profile of efavirenz in different pH

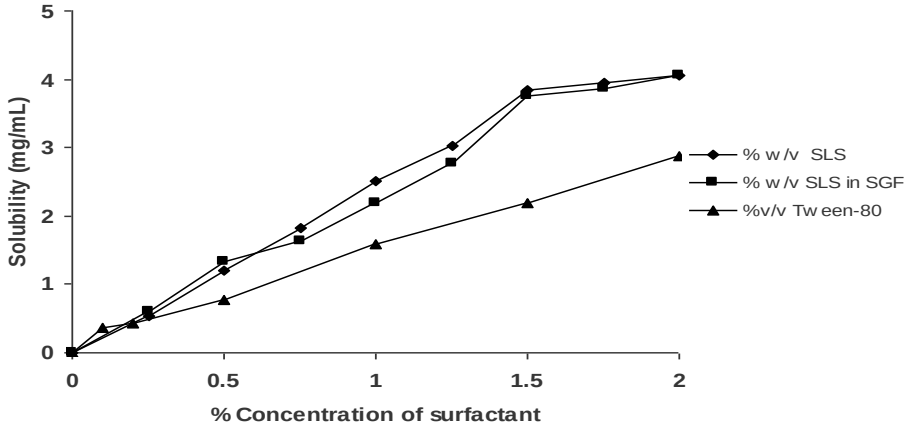

Fig 4: Solubility profile of efavirenz in surfactant and SLS in SGF media.

In vitro dissolution testing of efavirenz bulk drug and formulations (Estiva, Efcure, and Efavir) were performed. The dissolution study results in different concentrations of SLS with simulated gastric fluid and SLS alone for the above mentioned formulations are shown in the FIG 5 and 6 at 20 min time point. The % cumulative drug release for pure drug solid form proportionally increased with increase in concentration of SLS from 0.25 to 2.0% w/v, whereas, a different scenario was observed in formulations. There was a gradual increase in the cumulative % drug release up to 0.75% SLS concentration in SGF-SLS media followed by a slight decrease in drug release from 1-2% w/v SLS. It may be understood as the influence of various excipients in the formulation that led to the decrease in the cumulative % of drug release.

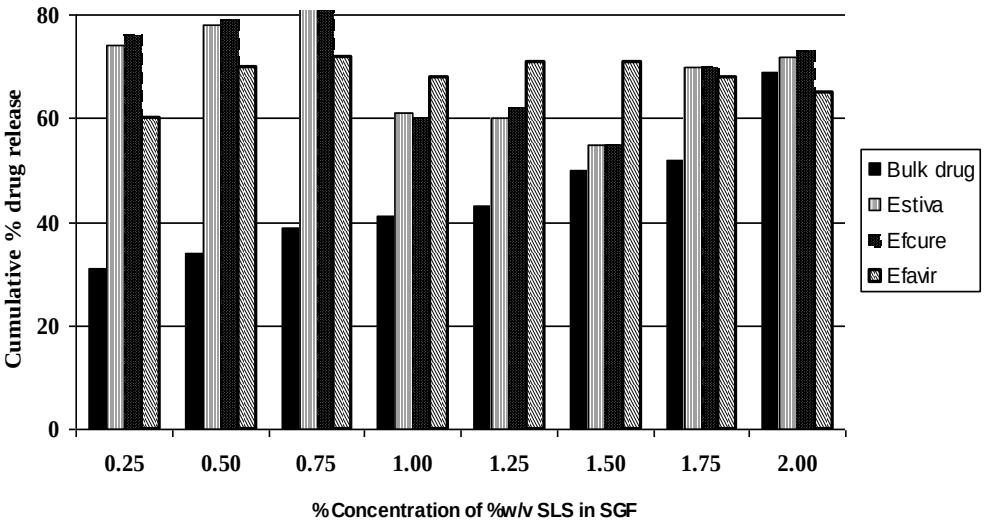

Fig 5:Comparison of % cumulative drug release of Efavirenz bulk drug and formulations in % w/v SLS in SGF at 20 min time point

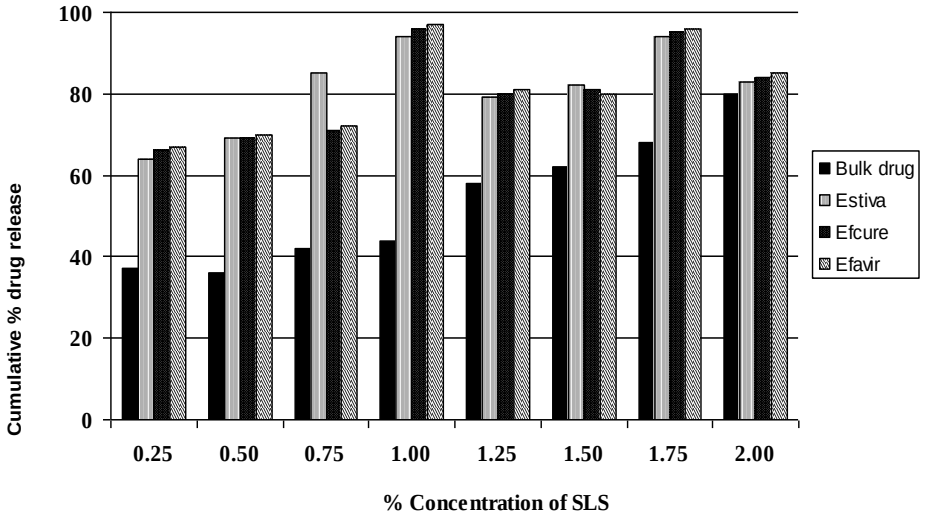

Fig 6:Comparison of % cumulative drug release of efavirenz bulk drug and formulations in % w/v SLS

Further, the % cumulative drug release data in 0.25% w/v SLS with SGF was processed for kinetics of drug release such as zero and first order as well as Higuchi and Hixon Crowell cube root equations. The regression equations and with its correlation coefficient are given in the Table 1. From Table-1, it is clearly understood that the dissolution of efavirenz alone and for formulations followed zero order kinetics and the mechanism related to drug release was diffusion process. The dissolution profile data of three formulations in 1% w/v SLS and 0.25% w/v SLS with SGF media shown in FIG 7 & 8 revealed that the 0.25% w/v SLS with simulated gastric fluid as dissolution media shows more discriminate between dissolution profiles of three formulations than 1% w/v SLS. The higher concentration of SLS (1% W/V) leads to faster dissolution (less than 25 min for all formulations); any potential correlation with *in vivo* performance is lost.

Table 1: Cumulative % drug release data in zero order, first order, Higuchi and Hixon-Crowell cube root equations

Fitting of release data into zero order, first order, Higuchi and Hixon-Crowell cube root equations				
Formulation	**Equations and r^2**			
	Zero	**First**	**Higuchi**	**Hixon-Crowell Cube root**
Bulk drug	y=1.0177x + 8.89 **r^2**= 0.935	y=0.025x + 0.8083 **r^2**= 0.5607	y=7.6103x + 1.064 **r^2**= 0.979	y=0.3392x + 2.963 **r^2**= 0.935
Estiva 600 mg tablet	y=1.782x + 26.63 **r^2**= 0.826	y=0.0267x + 1.068 **r^2**= 0.452	y=14.154x + 5.68 **r^2**= 0.974	y= 0.594x + 8.87 **r^2**= 0.826
Efcure 200 mg tablet	y=1.803x + 27.51 **r^2**= 0.817	y=0.0267x + 1.076 **r^2**= 0.4493	y=14.35x + 6.134 **r^2**= 0.970	y=0.601x + 9.1724 **r^2**= 0.8176
Efavir 200 mg capsule	y=1.974x + 16.212 **r^2**= 0.9301	y=0.0288x + 0.970 **r^2**= 0.5377	y=14.858x + 3.492 **r^2**= 0.986	y=0.6583x + 5.404 **r^2**= 0.9301

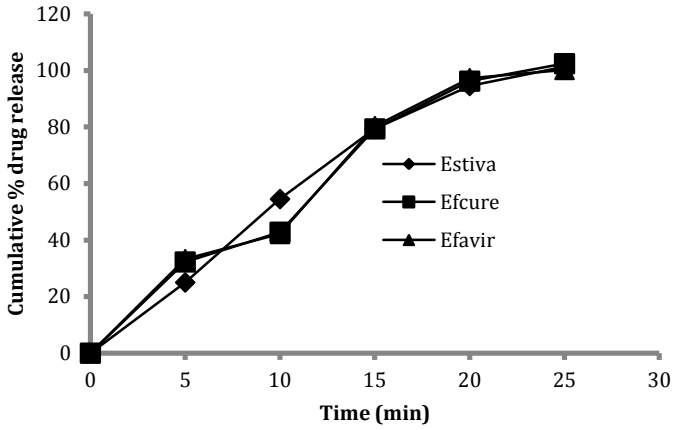

Fig 7: Dissolution profiles of three efavirenz formulations in 1% w/v SLS

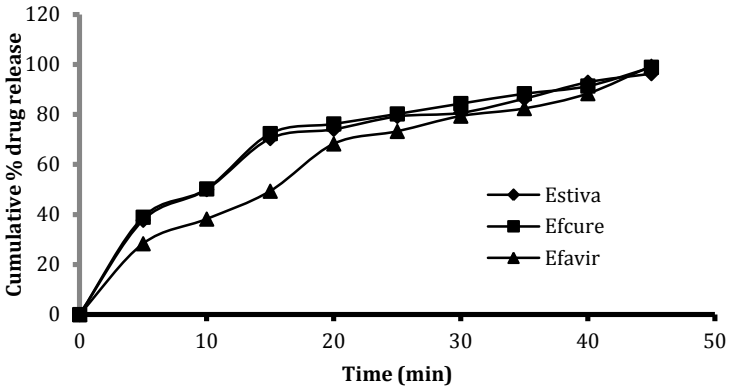

Fig 8: Dissolution profiles of three efavirenz formulations in 0.25% w/v SLS with SGF

CONCLUSION

In this work, we optimized the dissolution parameters for efavirenz bulk drug and its formulations. The dissolution medium of 900 ml of 0.25% w/v SLS in stimulated gastric fluid (SGF + 0.25% w/v SLS) is considered to be the discriminating and bio-relevant dissolution medium. The optimized parameters for the dissolution studies are found to be 50 rpm for 45 min. Thus the optimized media contains a lower concentration of SLS (0.25% w/v) in SGF media as compared with the 1% and 2% w/v SLS concentration stated in the IP monograph and FDA guidelines respectively and mimics the GI tract environment.

The dissolution data of efavirenz showed to follow zero order kinetics. It indicated the solubility related dissolution. The release mechanism was diffusion process. *In-vitro* dissolution studies of efavirenz and its formulations indicated that SGF with 0.25% w/v SLS may be discriminating media and possibly bio-relevant for predicting the *in-vivo* performance.

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REFERENCE

1. Ekarat Jantratid, Jennifer Dressman, Biorelevant dissolution media simulating the proximal human gastrointestinal tract, Dissolution technologies 2009 Aug; 21-25.
2. Galia E, Nicolaides, Horter D, Lobenberg R, Reppas C, Dressman J. Evaluation of various dissolution media for predicting *in-vivo* performance of class I & II drugs, Journal of pharmaceutical research 1998; 15(5): 698-705.
3. Hitesh jogia, Tushar Mehta, Madhabhai Patel. Evaluation of dissolution media containing a novel synthetic surfactant by in-vitro testing of BCS class II drugs, Dissolution technologies 2009 Aug; 14-19.
4. Sung Hyun Park, Hoo Kyun Choi. The effects of surfactants on the dissolution profiles of poorly water-soluble acidic drugs. International Journal of Pharmaceutics 2006; 321: 35-41.
5. Horter D, and Dressman J.B. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract, Adv. Drug Deliv. Rev 2001; 46:75-87.
6. Anthony Mottat C, David Osserton M and Brian widdop, Clarke's analysis of drugs and poisons 3rd edn, Pharmaceutical press, p. 968.
7. Indian pharmacopoeia, The Indian pharmacopoeia commission, Ghazianad (2007), volume I & II, 477-480, 1071-1073.
8. Jermy G, Paul A, Michal J, Hageman, and James N.H. Rapid throughput solubility screening method for BCS Class II drugs in animal GI fluids and simulated human GI fluids using a 96-Il well format, J.Pharm. Sci 2009; 97 (4): 1427-1442.
9. Karen J.M, Jonathan K.E, Jon E.L, Anne P, Linda J.S, Harry S, and Colin W.P. Influence of lipolysis on drug absorption from the gastro intestinal tract, *Adv. Drug Del. Rev* 1999; **25**: 33-46.
10. Singla N, Gupta GD, Kohli K, Singla AK. A discriminatory and biorelevant dissolution test method for simvastatin drug products, Dissolution technologies 2009 Nov; 11-13.
11. Thomas Zoeller and Sandra Klein. Simplified bio-relevant media for screening dissolution performance of poorly soluble drugs, Dissolution technologies 2007 Nov: 8-13.