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Research Article

DEVELOPMENT AND VALIDATION OF A RP – LC METHOD FOR DISSOLUTION TEST OF EFAVIRENZ TABLETS AND ITS APPLICATION TO DRUG QUALITY CONTROL STUDIES

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ABSTRACT

A liquid chromatographic method is developed to evaluate a new dissolution profile of Efavirenz in tablet dosage form. The dissolution method is developed by considering different factors like medium composition, pH, surfactant concentration and rotation speed. The dissolution test is validated using a high-performance liquid chromatographic method (HPLC). HPLC method is developed using ammonium acetate buffer (pH 7.5): acetonitrile in the ratio 40:60 as mobile phase in a C18 column. The dissolution conditions are achieved using paddle, 900 ml of medium containing water with 2% (w/v) of sodium lauryl sulfate at a rotation speed of 50 rpm. The developed dissolution method follows zero order kinetics. The reverse phase chromatographic method is found to be accurate, precise and specific. Both the methods are validated according to ICH guidelines and applied successfully for the quality control of commercial Efavirenz tablets.

Keywords: Dissolution, Efavirenz, Method development, RP – HPLC, Validation

INTRODUCTION

Efavirenz (EFV), (4*S*)-6-chloro-4-(cyclopropylethynyl)-1,4- dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one [1] a non – nucleoside reverse transcriptase inhibitor (NNRTI) used in treatment of HIV type- I infection, but ineffective against HIV type- II infection and human cellular DNA polymerage α, β, γ and δ [2]. EFV is a lipophilic drug under the class II (less soluble, highly permeable) of biopharmaceutical classification system guidance of Food and Drug Administration [3]. Oral bioavailability of EFV is 45 – 50% and its intra and inter subject variability is about 55- 58% and 19 -24% respectively [4-5]. Several analytical methods have been reported for the estimation of EFV alone or in different combinations in pharmaceuticals, plasma and peripheral blood mononuclear cells using reverse phase HPLC [6 -29], HPTLC (30], LC/MS [31] and a capillary electrophoresis method [32]. Diego et al reported the pharmacokinetic and synergistic encapsulation of EFV within polymeric micelles [33, 34]. Tathagata et al reported the effect of EFV loaded tuftsin conjugated dendrimers against HIV infected macrophages *in vitro* [35]. Gregoire et al reported that EFV does not interact with ABCB1 transporter at blood brain barrier [36]. As EFV is poorly water soluble drug, many research articles are reported describing the techniques of enhancement of solubility and dissolution rate [37–48]. The poor oral bioavailability of EFV inspired the authors to develop a dissolution sink condition and evaluation by a new liquid chromatographic method. Though the developed sink condition is reported in many literatures, the chromatographic method is novel, rapid, accurate and precise.

MATERIALS AND METHODS

Chemicals and reagents

Standard drug of EFV was procured from Cipla Pvt. Ltd. as a gift sample. The solvents used for preparation of solutions and mobile phase were of HPLC grade. The ammonium acetate used for preparation of buffer system is of Analytical grade. Sodium lauryl sulfate used for dissolution medium was of analytical grade. Three brands of EFV tablets (Efavir, Cipla, 200mg; Efferven, Ranbaxy, 200mg and Evirenz, Alkem, 200mg) were collected from local market.

In vitro dissolution testing

Solubility determination and sink conditions

The *sink* conditions were determined in different media. HCl 0.1 N, HCl 0.01 N, 0.5%, 1% and 2% sodium lauryl sulfate, Tween – 20 and Tween - 80 were tested. The dissolution was carried out in Electrolab TDT 8L & 6L' (Electrolab, India), USP apparatus II at 50 rpm for 60 mins containing 900ml of 2% sodium lauryl sulfate. An aliquot (10 ml) was removed from each vessel after10, 15, 30, 45 and 60 mins and filtered in 0.45µm PVDF filter before injection. Volume of injection was 5µl and detected at 246nm in PDA detector equipped in HPLC system.

Chromatographic system and conditions for dissolution study

The chromatographic system consisted of a JASCO (Japan) chromatograph equipped with an LC – Net II/ADC, an MU – 2010 Plus PDA Detector, a PU – 2089 Plus quaternary pump, an online degasser and a Rheodyne model 7725 injector valve with 50µl sample loop. The chromatograph is coupled with "Chrompass" software. Separation of EFV was done on a ODS HYPERSIL RP 18 column (150mm x 4.6mm; 5µ) under reverse phase partition chromatographic conditions. The mobile phase consisted of a mixture of buffer (Ammonium acetate buffer, pH maintained at 7.5): acetonitrile in the ratio 40:60 (v/v).The buffer was prepared by dissolving Ammonium acetate in 1000ml of water, maintaining the pH at 7.5 ± 0.05. The flow rate was 1.5ml/min, injection volume was 5µl and the detection was done at 246nm.

Preparation of stock solution, working solution and calibration curve

Accurately 0.01gm of standard EFV was weighed and transferred to a 100ml volumetric flask. The standard dissolved with 5ml methanol, sonicated for 5mins and the volume was made up with 2% sodium lauryl sulphate. The stock solution was diluted further with 2% sodium lauryl sulphate to obtain six working solutions with concentrations of 10 - 40 µg/ml. The prepared samples were also filtered through 0.45µm PVDF filter before injection. The standard calibration curve was plotted by AUC Vs Concentration at 246nm.

Assay of formulations by in-vitro dissolution

Dissolution studies on three brands of EFV tablets were carried out in USP apparatus II (paddle method). The tablets were Efavir, Efferven and Evirenz having 200mg of label claim were placed in 900ml of dissolution medium. 2% sodium lauryl sulphate at 37 ± 0.5º C was used as the dissolution medium. Dissolution was carried out at 50rpm. Each time 10ml of aliquots were withdrawn at 10, 15, 30, 45 and 60 mins and replaced with equal volume of fresh medium to maintain constant total volume. The samples were filtered immediately by 0.45µm PVDF filter and 1ml of filtrate was diluted

with 2% sodium lauryl sulphate upto 10ml and assayed by HPLC method.

Evaluation of Release Kinetics

Four mathematical models were applied to evaluate the kinetics of drug release: zero order, first order, Higuchi and Hixson–Crowell, whose equations are shown in Table - 1. The curves were constructed applying the kinetic models. The mathematical model that best expressed the dissolution profile of EFV tablets was selected based on the coefficient of determination (r^2).

Method Validation

The developed methods were validated according to ICH guidelines [50]. The validation parameters were linearity and range, specificity, accuracy, precision, and Robustness. Intra-day and Inter-day precision values were estimated by assaying the pharmaceutical dosage form of EFV six times on the same day and on six different days. Accuracy was determined by recovery study by standard addition method. The standard was added to a predetermined concentration at 80%, 100% and 120% level and estimation of % of drug release was carried out by reverse phase chromatographic method.

System suitability of HPLC method

System suitability of the developed HPLC method was determined by replicate injection of dissolution samples at specified time interval. The parameters are retention time, asymmetrical factor and number of theoretical plates. The parameters are expressed in terms of mean ± standard deviation.

RESULT AND DISCUSSION

Optimization of Dissolution Sink Conditions

Physicochemical properties of a drug play an important role in dissolution of drug in dosage form. Noyes – Whitney equation reveals the importance of aqueous solubility in dissolution rate. Aqueous solubility of EFV in demineralised water proved the poor aqueous solubility, so the effect of surface tension was studied. Surfactants and wetting agents enhance the penetration dissolution medium in the matrix of EFV tablets. The effect of surfactants on solubility of EFV was studied using Tween-20, Tween-80 and Sodium Lauryl Sulphate in 0.5%, 1% and 2% concentration range. A very less drug release was found in demineralised water (30%). About 50% of drug release was found in Tween – 20 and Tween -80, whereas 92% of drug release was found in 2% Sodium lauryl sulphate medium at 60th mins. The drug release profile is summarised in Table – 2 and represented in figure-1. Other test conditions like selection of apparatus and speed of revolutions were selected from USP apparatus I and II at 50 and 75 rpm. The release profile is summarised in Table – 3 and represented in Figure – 2.

From the above summary the best result was found in dissolution medium containing 2% sodium lauryl sulphate. USP apparatus II was chosen as its acceptance as a standard procedure for tablet dosage form. The paddle speed was optimized at 50rpm and 75rpm by aliquots withdrawn at 10, 15, 30, 45 and 60 mins. But 50rpm was found to be most suitable for dissolution of tablet dosage form.

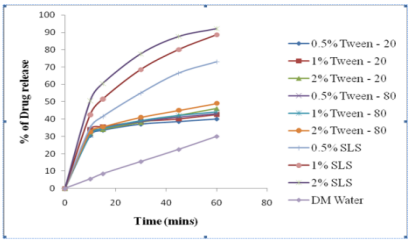

FIGURE 1: DISSOLUTION PROFILE OF EFAVIRENZ IN DIFFERENT DISSOLUTION MEDIUM

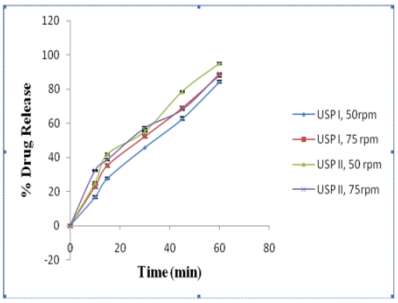

FIGURE 2: OPTIMIZATION OF APPARATUS AND REVOLUTIONS FOR DISSOLUTION PROFILE OF EFAVIRENZ TABLETS

Optimization of Chromatographic Conditions

The chromatographic method development was done by reviewing different solvent systems in literature. Various solvent systems were tried with acetonitrile, methanol and water including buffer systems at various pH. But ammonium acetate buffer and acetonitrile in the ratio 40:60 (v/v) were found to be most appropriate. pH of the buffer system was maintained at 7.5 ± 0.05 and the retention time was found at 3.38 ± 0.02 mins (Figure - 3). Isocratic mode was preferred to gradient elution as it requires long re-equilibrium time, perfect mixing. The analysis was done at normal room temperature.

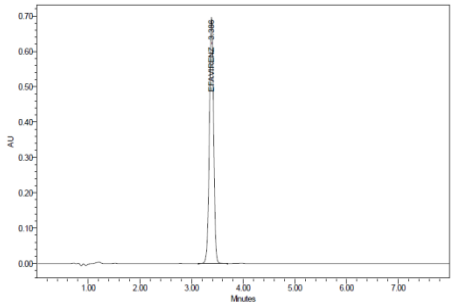

FIGURE 3: REPRESENTATIVE CHROMATOGRAM OF STANDARD EFAVIRENZ SHOWING R_T = 3.38 ± 0.02 MIN

Assay of formulations by in-vitro dissolution method

Three different formulations viz Efavir, Efferven and Evirenz were studied in developed dissolution medium containing 2% sodium lauryl sulphate at 37 ± 0.5º C. The dissolution was carried out in USP apparatus II at 50rpm. The samples withdrawn after time interval of 10, 15, 30 45 and 60 mins, diluted with 2% sodium lauryl sulphate solution, filtered through 0.45µm membrane filter. 5µl of sample is injected into the HPLC system and quantification was done from regression line equation of calibration curve. The % of drug release is listed in Table - 4 and the results were represented in terms of mean ± S.D.

Evaluation of Release Kinetics

The dissolution profile was used to evaluate the kinetics of drug release. The determination coefficient (r^2) for zero order, first order, Higuchi and Hixson-Crowell model are presented in Table - V. According to the determination coefficient (r^2), dissolution profile was better described by the zero order kinetics (Table - 5) and mechanism of drug release involved diffusion process.

Validation of method

Linearity

Linearity of the detector response of the dissolution test method was demonstrated in the range of 10 - 40µg/ml. The solutions were injected in triplicate and calibration curve was plotted of average area of chromatogram versus concentration. The coefficient of correlation was found to be 0.9998 ± 0.0002.

Specificity

The aim of the specificity study is to assess diluent interference as well as all the other excipients interference. Diluent and placebo mixture (in triplicate) were injected to check the interferences. No peak was found due to placebo at retention time of analyte peak. The study proves that test method is specific for quantification of dissolution of analyte without interference of diluents or any other excipients.

Accuracy

Accuracy of the proposed dissolution and HPLC method were verified by standard addition method. In the dissolution medium along with preanalyzed formulation, standard EFV were added at a level of 80%, 100% and 120%. Conditions of dissolution were maintained and the validation was carried out for a particular time of withdrawal of dissolved sample at 45th min and quantification was carried out in developed chromatographic conditions. The recovered quantity of EFV was expressed in terms of Mean ± S.D (Table - 6).

Precision

Precision of the developed dissolution and HPLC method were verified in terms of reproducibility and repeatability. The dissolution study carried out in six consecutive days and repeatability is checked by injecting the dissolution sample at 60th minute in triplicate and reproducibility is checked by comparison of dissolution result of six different days and expressed in terms of mean ± S.D and represented in Table – 7.

Robustness.

Robustness of developed methods were checked by little variation in experimental conditions like speed of paddle (40, 50 and 60 rpm) (Figure - 4), pH of solvent system (pH = 7.0, 7.5, 8.0) (Figure - 5), ratio of organic phase in solvent system (55, 60 and 65) (Figure - 6). The alteration in experimental conditions indicated the selectivity of the optimized conditions of dissolution and estimation of EFV in tablet dosage form.

System suitability of HPLC method

System suitability of the developed HPLC method was determined in terms of retention time, asymmetrical factor and number of theoretical plates. The parameters were evaluated from triplicate injection of the dissolution sample. The results are represented as mean of three determinations ± standard deviation. The retention time was found at 3.38 ± 0.02 mins, asymmetrical factor as 1.05 ± 0.004 and number of theoretical plates as 4256 ± 1.68.

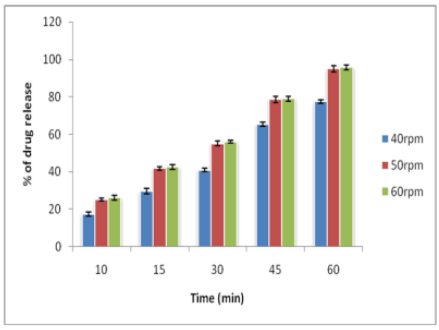

FIGURE 4: EFFECT OF REVOLUTIONS OF USP APPARATUS II ON % OF DRUG RELEASE

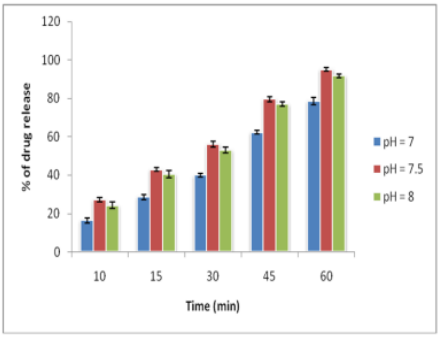

FIG 5: EFFECT OF *pH*OF DISSOLUTION MEDIUM ON % OF DRUG RELEASE.

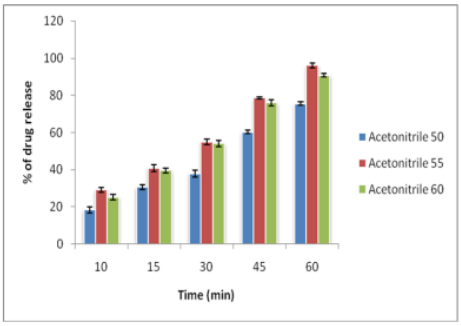

FIGURE 6: EFFECT OF ORGANIC PHASE COMPOSITION OF SOLVENT SYSTEM

TABLE - 1: MATHEMATICAL MODELS USED FOR DETERMINATION OF KINETICS [49]

Zero order kinetics	**$Q_t = Q_0 + K_0t$**
First order kinetics	$\log Q_t = \log Q_0 + (K_1t)/2.303$
Higuchi model	$f_t = K_Ht^{1/2}$
Hixson–Crowell model	$W0^{1/3} - Wt^{1/3} = K_st$

Q_t , amount of drug dissolved in time t; Q_0, initial amount of drug in the solution; K_0 and K_1, zero order and first order release constants, respectively; f_t , amount of drug released in time t by surface unity; K_H, Higuchi dissolution constant; W_0, initial amount of drug in the pharmaceutical dosage form; W_t , remaining amount of drug in the pharmaceutical dosage form at time t; K_s, a constant incorporating the surface–volume relation.

TABLE 2: SUMMARY OF DRUG RELEASE OF EFV IN DIFFERENT MEDIUMS OF TWEEN – 20, TWEEN – 80 AND SODIUM LAURYL SULPHATE

TIME POINTS (MIN)	**% DRUG RELEASED**									
	TWEEN-20			**TWEEN-80**			**SLS**			**DM WATER**
	0.5%	**1%**	**2%**	**0.5%**	**1%**	**2%**	**0.5%**	**1%**	**2%**	
10	30.5	34	32	31	30.5	32.5	35	42.5	50.5	5.5
15	33.5	35.5	34	34.5	34.5	35.5	41.5	51.5	60.5	8.5
30	37	38.5	38	38.5	39	41	55	68.5	77.5	15.5
45	38.5	40	42	41	42	45	66.5	80	87.5	22.5
60	40	42.5	46	43	44	49	73	88.5	92	30

TABLE 3: SUMMARY OF DRUG RELEASE OF EFV IN DIFFERENT APPARATUS AT DIFFERENT REVOLUTIONS

TIME POINTS (MINS)	% OF DRUG RELEASE			
	USP APPARATUS I		USP APPARATUS II	
	50 RPM (MEAN* ± S.D)	75 RPM (MEAN* ± S.D)	50 RPM (MEAN* ± S.D)	75 RPM (MEAN* ± S.D)
10	16.7 ± 0.97	22.96 ± 0.76	25.08 ± 0.55	32.38 ± 0.50
15	27.6 ± 0.92	42.26 ± 0.45	41.86 ± 0.68	40.7 ± 0.67
30	45.7 ± 0. 90	52.22 ± 0.30	55.08 ± 0.37	57.26 ± 0.65
45	62.4 ± 1.33	68.9 ± 0.40	78.56 ± 0.48	68.06 ± 0.36
60	84.2 ± 1.06	88.18 ± 0.58	95.06 ± 0.49	89.08 ± 0.40

***Mean of five determinations**

TABLE 4: % OF DRUG RELEASE DETERMINED BY HPLC METHOD

NAME OF THE FORMULATION	% OF DRUG RELEASE AT 10^{TH} MIN (MEAN* ± S.D)	% OF DRUG RELEASE AT 15^{TH} MIN (MEAN* ± S.D)	% OF DRUG RELEASE AT 30^{TH} MIN (MEAN* ± S.D)	% OF DRUG RELEASE AT 45^{TH} MIN (MEAN* ± S.D)	% OF DRUG RELEASE AT 60^{TH} MIN (MEAN* ± S.D)
Efavir	18.7 ± 1.15	36.7± 1.52	67.3 ± 0.57	78.7 ± 1.52	93.7 ± 1.52
Efferven	17.3 ± 1.52	38.3 ± 0.57	66.7 ± 0.57	78.7 ± 0.57	94.3 ± 1.52
Evirenz	19.3 ± 0.57	36.3 ± 1.15	67.3 ± 1.52	80.3 ± 0.57	94.3 ± 0.57

***Mean of three determinations**

TABLE 5: APPLICATION OF DRUG RELEASE DATA FOR DETERMINATION OF RELEASE KINETICS

NAME OF FORMULATION	COEFFICIENT OF DETERMINATION (r^2)			
	ZERO ORDER	FIRST ORDER	HIGUCHI	HIXON – CROWELL CUBE ROOT
Bulk drug	0.987 ± 0.002	0.875 ± 0.003	0.842± 0.002	0.704 ± 0.003
Efavir	0.973 ± 0.001	0.854± 0.002	0.753 ± 0.001	0.715 ± 0.002
Efferven	0.959 ± 0.002	0.732 ± 0.001	0.594 ± 0.003	0.628 ± 0.002
Evirenz	0.986 ± 0.001	0.793 ± 0.002	0.684 ± 0.002	0.786 ± 0.001

TABLE 6: ACCURACY OF THE DISSOLUTION METHOD WITH HPLC

NAME OF THE FORMULATION	CONCENTRATION OF FORMULATION (MG)	AMOUNT OF STANDARD ADDED (MG)	MEAN* (MG) ± S.D.
Efavir	200	160	342.75 ± 0.79
	200	200	380.38 ± 0.88
	200	240	418.43 ± 0.83
Efferven	200	160	341.53 ± 0.44
	200	200	380.98 ± 0.49
	200	240	418.87 ± 0.87
Evirenz	200	160	341.67 ± 0.93
	200	200	380.98 ± 0.8
	200	240	419.51 ± 0.82

TABLE 7: PRECISION OF DISSOLUTION AND HPLC METHOD

NAME OF THE FORMULATION	INTRADAY PRECISION [MEAN* (MG) ± S.D]	INTERDAY PRECISION [MEAN* (MG) ± S.D]
Efavir	190.65 ± 0.74	191.1 ± 0.77
Efferven	190.2 ± 0.93	190.06 ± 0.62
Evirenz	190.25 ± 0.44	190.41 ± 0.82

***Mean of six determinations**

CONCLUSION

The developed dissolution method and its evaluation by RP – LC method were validated by ICH guidelines. The in vitro dissolution profile for EFV was obtained using 900 ml of dissolution medium containing 2% sodium lauryl sulphate, USP apparatus II at 50rpm and 37±0.5 ◦C. Kinetics of drug release was best described by the Zero order kinetic model. Poor bioavailability of EFV inspired to develop the simplest methods of dissolution and evaluation by a simple chromatographic method. The validation results demonstrate that the in vitro dissolution method was accurate, precise, linear and specific. Both the HPLC analytical method and in vitro dissolution test were validated and can be used to evaluate the release profile of EFV tablets. The methods are so easy and cost effective that can be used for day to day quality control of raw material and finished formulations in small scale laboratories.

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