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Development and validation of UPLC method for simultaneous estimation of Efavirenz and Lamivudine in pharmaceutical formulations

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ABSTRACT

An accurate and precise UPLC method was developed for the simultaneous estimation of efavirenz and lamivudine in pharmaceutical dosage forms. The chromatographic analysis was performed on Acquity UPLC BEH Shield RP18 (50 × 3 mm, 1.7 µm) column with mobile phase consisting of 10% acetonitrile in methanol and 10 mM phosphate buffer (pH 4.0) in the gradient mode, at a flow rate of 0.4 mL/min, and eluents monitored at 254 nm. The calibration curves of peak area versus concentration, which was linear from 10-60 µg/mL for efavirenz and 5.0-30 µg/mL for lamivudine, had regression coefficient (r^2) greater than 0.999. The method had the requisite accuracy, precision, and robustness for simultaneous determination of efavirenz and lamivudine in tablets. The proposed method is simple, economical, accurate, and precise and could be successfully employed in routine quality control for the simultaneous analysis of efavirenz and lamivudine in pharmaceutical formulations.

INTRODUCTION

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of drugs that were introduced as antiretroviral agents for the treatment of infection with human immune deficiency virus (HIV). Additional drug classes were developed. They are protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), fusion inhibitors (Farmer *et al.*, 2001). Efavirenz, chemically (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2, 4-dihydro-1H-3, 1-benzoxazin-2-one, is a non-nucleoside reverse transcriptase inhibitor. It is used in the treatment of HIV infection (Figure 1). It binds directly and reversibly to the catalytic site of the reverse transcriptase enzyme and therefore, interferes with viral RNA to DNA directed polymerase activities (Adkins and Noble 1998). Lamivudine, chemically 4-amino-1-{(2R, 5S)-2-(hydroxyl methyl)-1, 3-oxathiolan-5-yl}-1, 2-dihydropyrimidin-2-one, is a nucleoside reverse transcriptase inhibitors with activity against human immune deficiency virus (HIV) and hepatitis B virus (Figure 2) (Zhou *et al.*, 2006; Van *et al.*, 2000).

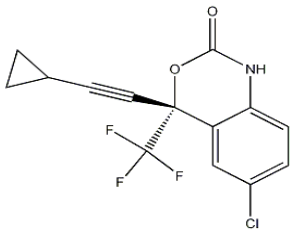

Fig1: Chemical Structure of Efavirenz.

The literature survey reveals that several analytical methods have been reported for the quantification of these drugs individually or in combination with other drugs in pharmaceutical dosage forms or in human plasma by high performance liquid chromatography (Manikanta *et al.*, 2012; Anandakumar *et al.*, 2013; Akula *et al.*, 2014; Naga Sandhya *et al.*, 2013), Liquid chromatography/tandem mass spectrometry (Manish *et al.*, 2012; Rower *et al.*, 2012; Li *et al.*, 2010; Kumar *et al.*, 2013), UPLC (Madeesh *et al.*, 2012; Marina *et al.*, 2011) and high performance thin layer chromatography (Hamrapurkar *et al.*, 2009).

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Present study involves development and validation of UPLC method for the simultaneous estimation of efavirenz and lamivudine in combined tablet dosage form, which is fast, sensitive with better resolution and peak symmetry. Finally, the developed method was validated to assess the validity of research data means determining whether the method used during the study can be trusted to provide a genuine, account of the intervention being evaluated.

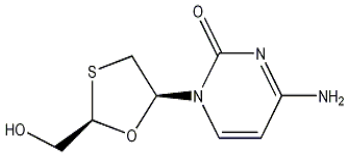

Fig. 2: Chemical Structure of Lamivudine.

MATERIALS AND METHODS

Materials

Pure efavirenz (EFV) and lamivudine (3TC) used as working standards, were gifts from Hetero Drugs Pvt. Ltd., Hyderabad, India. Methanol, acetonitrile and water (HPLC-grade) were purchased from Rankem, India. All other chemicals and reagents employed were of analytical grade, and purchased from Rankem, India. A commercial odivir tablets containing 300 mg of lamivudine and 600 mg of efavirenz were obtained from local pharmacies and used within their shelf life period.

Instrumentation and chromatographic conditions

The Waters Acquity™ UPLC M-class system consisting of a H05UPB062M binary gradient pump, an inbuilt auto sampler, Water 2996 PDA detector and column oven connected to a multi instrument data acquisition and processing system with Empower 2.1 version. The column used for chromatographic separations was Acquity UPLC BEH Shield RP18 (50 × 3 mm, 1.7 µm).

Table 1: Optimized Chromatographic Conditions.

Parameters	Conditions
Column	Acquity UPLC BEH Shield RP18 (50 x 3 mm, 1.7µm)
Mobile Phase A	10 mM Phosphate buffer (pH = 4.0)
Mobile Phase B	10 % Acetonitrile in Methanol
Gradient Elution	As per Table 2
Flow Rate	0.4 mL/min
Wavelength	254 nm
Injection Volume	10 µL
Column Oven Temp.	35°C
Run Time	3.0 Min

Table 2: Gradient Programming of proposed method.

Time (min)	Flow Rate (mL/min)	% Mobile Phase A	% Mobile Phase B
Initial	0.4	82	18
1.0	0.4	65	35
1.8	0.4	50	50
2.5	0.4	82	18

The UPLC system was operated gradient mode with mobile phase consisting of 10% acetonitrile in methanol and 10 mM phosphate buffer (pH adjusted to 4.0) at a flow rate of 0.4 mL/min within a run time at 3 min (Table 1-2). Prior to use, the mobile phase was degassed by an ultrasonic bath and filtered by a millipore vacuum filter system equipped with a 0.2 µm high vacuum filter. Both drugs were detected and quantified at 254 nm.

Preparation of standard solutions

A mixed standard stock solution of EFV (1000 µg/mL) and 3TC (1000 µg/mL) was prepared by accurately weighing 100 mg of each EFV and 3TC, and dissolved in 100 mL volumetric flask containing 30 mL methanol and the flask was sonicated to dissolve the contents and made up to the mark with methanol. Aliquots of these solution was transferred into 100 mL volumetric flask containing 30 mL methanol, sonicated for 2 min and the remaining volume was made up to mark with methanol to get final concentration of 100 µg/mL of each analyte.

Method Validation

The method was validated in accordance with ICH guidelines (ICH – Q2 (R1)).

System Suitability

To ensure the validity of the analytical procedure, a system suitability test was established. The following parameters like asymmetry factor, theoretical plate number (N), resolution (Rs) and retention time (t_R) were analyzed by using 10 µL of the working standard solution containing EFV (40 µg/mL) and 3TC (20 µg/mL) injecting six times into UPLC system.

Linearity

Calibration curves were constructed by plotting peak areas versus concentrations of EFV and 3TC, and the regression equations were calculated. The calibration curves were plotted over the concentration range 10-60 µg/mL for EFV and 5.0-30 µg/mL for 3TC. Aliquots (10 µl) of every solution were injected and analysed under the operating chromatographic conditions described as above.

Detection limit and quantitation limit

Limit of detection (LOD) and Limit of quantification (LOQ) were calculated based on the ICH guidelines.

Accuracy

The accuracy was carried out by adding known amounts of each standard drug corresponding to three concentration levels - 50, 100 and 150 % - of the labeled claim to the analytes. At each level, three determinations were performed and the results were recorded. The accuracy was expressed as percent analyte recovered by the proposed method.

Precision

The precision of the method was checked by repeatability of injection, repeatability (intra-assay), intermediate precision (inter-assay) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (% RSD) for ten determinations of peak areas of EFV (30 µg/mL)

and 3TC (15µg/mL), performed on the same day. For both intra- and inter-assay variation, sample solutions of EFV (20, 30 and 40 µg/mL) and 3TC (10, 15 and 20 µg/mL) were injected in triplicate.

Robustness

The robustness of the proposed method was determined by carrying out the analysis, during which flow rate (varied by ± 0.02 %), and buffer pH (varied by ± 0.1) were altered, and the peak areas, retention times and no. of theoretical plates were noted.

RESULTS AND DISCUSSIONS

The UPLC method, as described, was validated and successfully employed for the simultaneous quantification of EFV and 3TC in tablets. There is need to consider the successive steps for the development of UPLC method. In particular, the problems relating to the standardization of sample preparations and selection of mobile phase needs to be emphasized. The optimized chromatographic conditions were selected based on sensitivity, retention time, peak shape and baseline drifts. The method was selective for the determination of EFV and 3TC since no interfering peaks appeared near the retention time of the compound of interest. A typical chromatogram recorded at 254 nm is shown in Figure 3. The retention times of EFV and 3TC at a flow rate of 0.4 mL/min were 0.921 and 1.981 min, respectively. The analyte peaks were well resolved and were free from tailing (< 2 for both the analytes). To ensure the validity of a system and analytical method, system suitability test was performed. The percent relative standard deviation (%RSD) of the retention times (RT) and peak areas of EFV and 3TC from the six consecutive injections of the standard solutions were 0.852 and 0.624, and 1.215 and 1.725, respectively. The tailing factor for EFV and 3TC peaks were 1.32 and 1.47, respectively, thus reflecting good peak symmetry. The resolution (Rs) between two analytes was 3.35, indicating good separation of both analytes from each other. The theoretical plate no. for EFV and 3TC were 12448 and 10275, respectively, thus indicating good column efficiency. The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 10-60 µg/mL for EFV and 5-30 µg/mL for 3TC. The regression coefficients of EFV (r^2 = 0.9991) and 3TC (r^2 = 0.9992) indicate a good linear relationship between peak area versus concentration over a wide range. LOD for EFV and 3TC was 0.12 and 0.04 µg/mL, respectively, while LOQ was 0.37µg/mL and 0.13µg/mL, respectively. The mean recoveries obtained for EFV and 3TC were 99.92-100.52% and 99.48-100.66 %, respectively, indicating that the developed method was accurate (Table 3).

Injection repeatability values (%RSD) of EFV and 3TC were found to be 0.813 and 1.266, respectively. Results for intra and inter-assay precision, expressed as %RSD, results were given in Table 4. The low values of %RSD indicate that the method is precise. Reproducibility was checked by analyzing the samples by another analyst using same instrument and same laboratory. There was no significant difference between %RSD values, which indicates that the proposed method was reproducible. There was no significant change in the peak areas, retention times and no. of theoretical plates of EFV and 3TC when the flow rate and pH of buffer were changed, indicate that the method was robust (Table 5). The results of the assay yielded 100.79% for EFV and 100.33 % for 3TC, of label claim of the tablets. The assay results show that the method was selective for the simultaneous determination of EFV and 3TC without interference from the excipients used in the tablet dosage form and the results were shown in the Table 6.

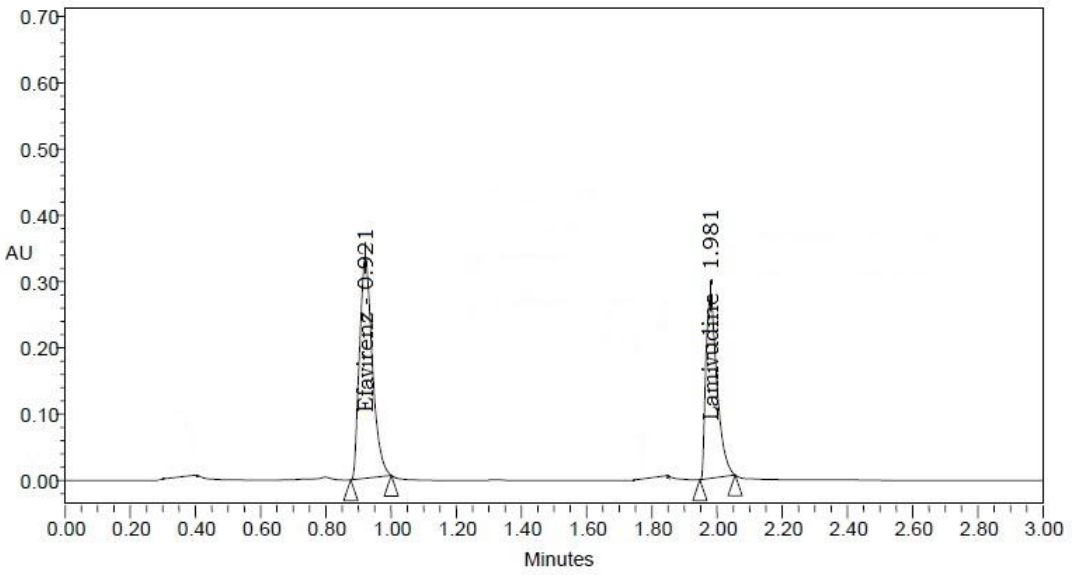

Fig. 3: A typical chromatogram of standard efavirenz (t_R: 0.921) and lamivudine (t_R: 1.981).

Table 3: Results of recovery studies by standard addition method.

Analyte	Amount of standard drug spiked		Amount of sample taken (mg)	% Recovery (Mean ± SD) {three replicates}	RSD (%)	Standard error of mean
	% Spiked	Quantity (mg)				
EFV	50	300	600	99.92±0.697	0.697	0.4023
	100	600	600	100.44±0.815	0.812	0.4707
	150	900	600	100.52±1.405	1.398	0.8111
3TC	50	150	300	99.48±1.360	1.367	0.7850
	100	300	300	100.66±1.665	1.654	0.9615
	150	450	300	99.84±1.101	1.103	0.6359

Table 4: Precision data of the proposed method.

Analyte	Analyte Conc. (µg/mL)	Intra-assay precision*	Inter-assay precision*	Reproducibility*	
				Analyst one	Analyst two
EFV	20	0.867	0.799	1.018	1.004
	30	1.302	1.243	0.985	1.438
	40	1.368	1.595	0.799	1.037
3TC	10	0.769	0.812	1.328	1.095
	15	1.024	1.215	0.611	0.478
	20	0.214	1.341	1.001	0.179

**% RSD Values.*

Table 5: Results for robustness of the proposed method.

Parameter	Used	Analyte	Retention time	Tailing Factor	No. of Theoretical Plates
			Mean± SD*	Mean± SD*	Mean± SD*
Flow rate (mL/min)	0.38	EFV	1.008± 0.003	1.23±0.020	11230±178
	0.4		0.924±0.004	1.33±0.015	12515±205
	0.42		0.911±0.006	1.45±0.021	10580±148
	0.38	3TC	1.856±0.009	1.34±0.021	9763±103
	0.4		1.783±0.005	1.46±0.006	10296±47
	0.42		1.762±0.006	1.39±0.015	9792±61
pH (Buffer)	3.9	EFV	0.931±0.014	1.21±0.015	10351±104
	4.0		0.921±0.008	1.34±0.010	12625±156
	4.1		0.915±0.007	1.41±0.023	10507±138
	3.9	3TC	1.826±0.022	1.31±0.015	9768±86
	4.0		1.775±0.009	1.45±0.015	10436±156
	4.1		1.756±0.015	1.39±0.015	9754±89

*Replicates of three injections.

Table 6: Assay results for efavirenz and lamivudine in tablets.

Product	Analyte	Label claim per tablet (mg)	% analyte estimated (Mean ±SD)*	RSD (%)	SEM
Odivir	EFV	600	100.79±0.513	0.509	0.296
	3TC	300	100.33±1.193	1.189	0.689

** n = 3; SEM = standard error of mean.*

CONCLUSION

A convenient, rapid, accurate and precise UPLC method was developed for the simultaneous determination of efavirenz and lamivudine in pharmaceutical formulations. The assay provides a linear response across a wide range of concentrations. This method can be said to be more economical as compared to other methods reported in literature. The method suitable for the determination of these drugs in tablets, and hence can be used for routine quality control of efavirenz and lamivudine in this dosage form.

REFERENCES

Adkins J.C., Noble S. Efavirenz. Drugs 1998, 56 (6), 1055.

Akula Srinath, B. Sneha, Akhila Alladi, Rayees Ahmed, R.G. Kulkarni. Method development and validation for simultaneous estimation of lamivudine, tenofovir and efavirenz in combined tablet dosage form by RP-HPLC and UV-spectroscopic method. Int J Pharm Sci Res, 2014; 5(12): 5491-7.

B. Naga Sandhya, A. Manikanta Kumar, Mahesh K Nasare, P. Vijay Kumar, Jetta Satish, Prakash. V. Diwan. RP-HPLC method for simultaneous estimation of lamivudine, didanosine and efavirenz in pharmaceutical dosage forms. Der Pharmacia Lettre, 2013; 5(3): 148-55.

Farmer Paul, Léandre Fernet, Mukherjee Joia, Gupta Rajesh, Tarter Laura, Kim Jim Yong. Community-based treatment of advanced HIV disease: introducing DOT-HAART (directly observed therapy with highly active antiretroviral therapy). Bull World Health Organ, 2001; 79(12): 1145-51.

Hamrapurkar P., Phale M., Shah N. Quantitative Estimation of Efavirenz by High Performance Thin Layer Chromatography. J Young Pharm, 2009; 1(4): 359-63.

International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedure: Text and Methodology {ICH – Q2(R1)} November 2005: 1-13.

K. Anandakumar, G. Abirami, S. Murugan, B. Ashok. RP-HPLC method for simultaneous estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz in tablet formulation. J Anal Chem, 2013; 68(9): 815-21.

Kumar V.R., Reddy B.P., Kumar B.R., Sreekanth K., Babu K.N. High throughput LC-MS/MS method for simultaneous determination of zidovudine, lamivudine and nevirapine in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci, 2013; 15(921-922): 9-14.

Li Z., Ding C., Ge Q., Zhou Z., Zhi X., Liu X. Simultaneous determination of lamivudine, stavudine and nevirapine in human plasma

by LC-MS/MS and its application to pharmacokinetic study in clinic. Biomed Chromatogr, 2010; 24(9): 926-34.

Manikanta Kumar A, B. Naga Sandhya, Mahesh Nasare, V. V. L. N Prasad, Prakash V. Diwan. Development and Validation of RP-HPLC method for simultaneous estimation of Lamivudine and Efavirenz in the Pharmaceutical Dosage Form. J Adv Pharm Edu Res, 2012; 2(4): 232-8.

Manish Yadav, Puran Singhal, Sailendra Goswami, Umesh C. Pande, Mallika Sanyal, Pranav S. Shrivastav. Selective Determination of Antiretroviral Agents Tenofovir, Emtricitabine, and Lamivudine in Human Plasma by a LC–MS–MS Method for a Bioequivalence Study in Healthy Indian Subjects. J Chromatogr Sci, 2010; 48: 704-13.

Marina V. Antunes, Julia Poeta, Jorge P. Ribeiro, Eduardo Sprinz, Rafael Linden. Ultra-performance liquid chromatographic method for simultaneous quantification of HIV non-nucleoside reverse transcriptase inhibitors and protease inhibitors in human plasma. J Braz Chem Soc, 2011; 22(1): 134-41.

Rower J.E., Klein B., Bushman L.R., Anderson P.L. Validation of a sensitive LC/MS/MS method for the determination of zidovudine and lamivudine in human plasma. Biomed Chromatogr, 2012; 26(1): 12-20.

Sk. Madeesh, Y. Ismail and V. Gunasekaran. Development and validation for simultaneous estimation of lamivudine, tenofovir and efavirenz by UPLC. Int J Pharm, 2012; 2(3): 656-60.

Van Rompay A.R., Johansson M., Karlsson A. Phosphorylation of nucleosides and nucleoside analogs by mammalian nucleoside monophosphate kinases. Pharmacol Ther, 2000; 87(2-3): 189-98.

Zhou Z., Rodman J.H., Flynn P.M., Robbins B.L., Wilcox C.K., D'Argenio D.Z. Model for intracellular Lamivudine metabolism in

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