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Rapid Stability Indicating RP-HPLC Method for Simultaneous Quantification of Related Impurities of Antiretroviral Drugs

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A simple, rapid and stability indicating reverse phase liquid chromatographic method has been developed for the simultaneous quantitative determination of emtricitabine, rilpivirine and tenofovir disoproxil fumarate and their related impurities in a combined dosage form. The pharmaceutical formulation along with individual active ingredients were subjected to stress conditions of hydrolysis (acid and base), oxidation and thermal degradation as per ICH guidelines. The chromatographic separation was achieved using an Xterra RP C18 (250 × 4.6 mm, 5 µm stationary phase with a linear gradient elution and the detection wavelength was set at 254 nm. The mobile phase consists of buffer and acetonitrile delivered at a flow rate of 1 mL/min. The stress samples were assayed against a reference standard and the mass balance was found to be close to 99 %. The proposed RP-HPLC method was validated with respect to linearity, precision, accuracy and robustness.

Keywords: HPLC, Validation, Related impurities, Tenofovir disoproxil fumarate, Emtricitabine, Rilpivirine.

INTRODUCTION

The combinations of nucleoside reverse transcriptase inhibitors (NRTI's) and non nucleoside reverse transcriptase inhibitors (NNRTI's) as multi drugs are efficient in the treatment of human immunodeficiency virus (HIV) infection[1,2]. Fixed dose combination of three drugs comprising emtricitabine, rilpivirine and tenofovir disoproxil fumarate forms one of the first line regimen in HIV-treatment[3]. Emtricitabine is chemically known as emtricitabine (FTC; 5-fluoro-1- (2R,5S)-[2 9-hydroxymethyl]-1,3-oxathiolan-5-yl) is a nucleo-side reverse transcriptase inhibitor[4,5]. Emtricitabine has been estimated using different analytical methods[6,7] either in single or in combined dosage form. Tenofovir disoproxil fumarate is chemically 9-((R)-2-*bis*(((isopropoxycarbonyl) oxy) methoxy) phosphinyl) methoxy) propyl) adenine fumarate, is a nucleotide analogue reverse transcriptase inhibitor[4,5]. Tenofovir disoproxil fumarate has been determined individually or in combination with other drugs[8,9].

Rilpivirine is chemically 4-[[4-[[4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl] amino] benzonitrile monohydrochloride, is a diarylpyrimidine non-nucleoside reverse transcriptase inhibitor[4,5]. The assay of rilpivirine by few chromatographic methods were reported along with other antiretroviral drugs[9,10]. Few HPLC methods has been reported for the estimation of emtricitabine, tenofovir disoproxil fumarate and rilpivirine from pharmaceutical dosage form[11,12]. The purpose of this study was to develop a rapid stability indicating RP-HPLC method for the simultaneous quantification of emtricitabine, tenofovir disoproxil fumarate and rilpivirine with their related impurities.

EXPERIMENTAL

Tenofovir disoproxil fumarate, emtricitabine, rilpivirine, related impurity standards and tablet dosage forms were obtained from Strides Arcolab, Bangalore, India. The solvents and reagents used for the study were of HPLC or analytical grade. The HPLC grade Acetonitrile was purchased from Merck. High purity water obtained from Millipore MilliQ Plus water purification system was used.

The Agilent HPLC system equipped with a pump, auto sampler and a PDA detector. The data acquisition was monitored and processed using chemstation software. Xterra RP C_{18}(250 × 4.6 mm, 5 µm particle size) analytical column was used.

Optimization of chromatographic conditions: The method was developed on a reverse phase column Xterra RP C_{18}(250 × 4.6 mm, 5 µm) maintained at ambient temperature. The mobile phase selected was a gradient mixture of mobile phase A (acetate buffer) and mobile phase B (acetonitrile) delivered at a flow rate of 1 mL/min. The gradient programme [time (min)/% A] was set as 0/100, 15/100, 65/65, 75/52, 85/45,

90/40, 95/70, 100/100, 105/100. The injection volume was 5 µL and the detection was performed at 254 nm using photo diode array (PDA) detector. The typical retention times of emtricitabine, tenofovir disoproxil fumarate and rilpivirine are 19, 63 and 85 min, respectively.

Preparation of stock and sample solutions: 20 mg of emtricitabine and 30 mg of tenofovir disoproxil fumarate were weighed accurately and transferred in to 100 mL volumetric flask, 80 mL of methanol was added and sonicated for 10 min and made up to 100 mL with acetonitrile. 12.5 mg of rilpivirine HCl standard has been weighed and transfered into a 100 mL volumetric flask. 40 mL of 80 % acetonitrile was added and sonicated for 15 min and made up to 100 mL with 80 % acetonitrile. From these stock solutions working standard solutions containing 0.02 mg/mL of the emtricitabine, 0.03 mg/mL of tenofovir disoproxil fumarate and 0.005 mg/mL of the rilpivirine HCl in diluent were prepared.

Twenty tablets were accurately weighed and powdered. Powder equivalent to 50 mg of emtricitabine, 75 mg of tenofovir disoproxil fumarate and 12.5 mg of rilpivirine HCl was weighed and prepared a sample solution containing 1 mg/mL of the emtricitabine, 1.5 mg/mL of tenofovir disoproxil fumarate and 0.25 mg/mL of the rilpivirine in diluent. The solutions were filtered through 0.45 µ nylon membrane filter and injected to HPLC system for the analysis.

RESULTS AND DISCUSSION

Optimum separation between the active compounds and all potential degradants was achieved with the optimized chromatographic conditions and the proposed method was validated as per ICH guidelines and current regulatory practices.

Method validation: The purpose of method validation is to confirm that the proposed method is suitable for its intended purpose[13]. The optimized method has been extensively validated in terms of specificity, precision, linearity, accuracy, limit of detection (LOD) and limit of quantification (LOQ) and robustness.

System suitability: System suitability test is used to verify that the resolution and reproducibility of the chromatographic systems are adequate for the analysis to be done. The limits for system suitability were set for theoretical plates, resolution and asymmetry (Table-1).

TABLE-1
SYSTEM SUITABILITY DATA

Parameter	Result
Theoretical plates (T)	Emtricitabine-8298 Tenofovir disoproxil fumarate-500043 Rilpivirine-270641
% RSD	Emtricitabine-0.6 Tenofovir disoproxil fumarate-0.4 Rilpivirine-0.4

Specificity: Specificity was tested by spiking with appropriate levels of impurities and demonstrating the separation of these impurities individually and from other components in the sample matrix. Identification of each impurity was confirmed with relative retention times by comparison with pure standards (Table-2).

TABLE-2
FORCED DEGRADATION DATA

Stress condition	Degradation observed (%)		
	Emtricitabine	Tenofovir disoproxil fumarate	Rilpivirine
Acid hydrolysis	2.10	26.93	0.9
Base hydrolysis	2.34	23.15	0.9
Oxidative stress	7.90	3.9	0.9

Forced degradation studies: Intentional degradation was performed under various stress conditions like acid hydrolysis with 1 N HCl, base hydrolysis with 1 N NaOH and oxidative degradation using 3 % hydrogen peroxide and evaluated the ability of the proposed method to separate and analyse the degradation products from each other and active ingredients as well. To ensure the peak purity of all peaks in stressed samples photo diode array detector was used.

In forced degradation studies it was found that tenofovir is susceptible for degradation in acid and base hydrolysis conditions, whereas emtricitabine and rilpivirine were stable in all three stress conditions (Table-2).

Linearity: The linearity of the detector response to different concentrations of impurities was studied by preparing a series of solutions using tenofovir disoproxil fumarate, emtricitabine, rilpivirine and their related compounds at five different concentration levels ranging from 0.05 to 0.5 % of the test concentration of respective active compound. The data were subjected to statistical analysis using linear regression model and the results indicates good linearity (Table-3).

TABLE- 3
LINEARITY DATA

Impurity name	Relative retention time	Correlation coefficient (r)	Limit of quantification
5-Flurocystosine	0.06	1.000	0.190
Emtricitabine acid	0.09	0.999	0.316
Sulfoxide isomer-II	0.12	1.000	0.16
Sulfoxide isomer-I	0.13	1.000	0.271
Lamivudine	0.21	1.000	0.174
Emtricitabine	0.29	1.000	0.232
Adenine impurity	0.15	1.000	0.199
Tenofovir impurity	0.17	1.000	0.137
9-Propyl adenine Impurity	0.82	1.000	0.504
Monoester impurity	0.88	1.000	1.264
Ethyl impurity	1.02	1.000	0.289
Isopropyl impurity	1.16	1.000	0.401
N-Propyl impurity	1.00	1.000	0.504
Carbonyl impurity	1.27	1.000	0.423
Tenofovir disoproxil dimer impurity	1.16	1.000	0.216
Rilpivirine related compound A	1.07	1.000	0.124
Rilpivirine related compound B	1.11	1.000	0.246
Rilpivirine related compound C	1.12	1.000	0.155
Rilpivirine related compound D	1.33	1.000	0.335

Limit of detection and limit of quantification: Limit of detection and limit of quantification for impurities were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively,

by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at LOQ level by injecting six individual preparations of impurities and calculating the percentage RSD (relative standard deviation) of the area (Table-3).

Precision: The precision of the developed method has been verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of the sample spiked with its known impurities. Percentage RSD for each impurity was calculated (Table-4).

TABLE- 4
PRECISION AND INTERMEDIATE PRECISION DATA

Impurity name	RSD (%)	
	Precision data	Ruggedness data
5-Flurocystosine	0.5	5.9
Emtricitabine acid	0.8	4.6
Sulfoxide isomer-II	0.3	8.3
Sulfoxide isomer-I	0.3	4.9
Lamivudine	1.2	3.5
Emtricitabine	0.8	6.5
Adenine impurity	0.2	7.4
Tenofovir impurity	0.8	6.8
9-Propyl adenine impurity	0.1	5.5
Monoester impurity	0.7	3.7
Ethyl impurity	1.2	4.6
Isopropyl impurity	0.4	2.5
N-Propyl impurity	1.4	4.9
Carbonyl impurity	0.9	4.5
Tenofovir disoproxil dimer impurity	0.4	3.8
Rilpivirine related compound A	0.4	2.9
Rilpivirine related compound B	0.4	4.9
Rilpivirine related compound C	0.4	7.2
Rilpivirine related compound D	1.1	5.7

N = 6 sample preparations

Accuracy: Standard addition and recovery experiments were studied on sample to confirm the accuracy of the related substance method. The accuracy was studied at different levels, at each level it was carried out in triplicate for impurities and active compounds. Known quantities of impurities were spiked into the sample and the percentages of recoveries for the sample and their impurities were calculated (Table-5).

Robustness: To determine the robustness of the developed method, experimental conditions were altered and the resolution between active compounds and their impurities were recorded with their tailing factor. There were no dramatic changes in the chromatographic behaviour of impurities and all parameters have been observed within the limits required for system suitability tests.

Stability in analytical solutions: Solution stability was studied by injecting the standard preparation and sample preparation at regular interval. Percentage difference in area response between initial period and after the specified period was studied. The solution stability experiment data confirms that the sample solutions used for the related substance determination were stable for 29 h.

Conclusion

The proposed method provides good resolution between all the impurities and potential degradants and was found to be stability indicating. The validated stability indicating HPLC method has proved to be simple, precise and sensitive and can be used for the routine analysis of combined release tablet dosage form of emtricitabine, rilpivirine and tenofovir disoproxil fumarate also to check the purity and shelf life stability.

TABLE-5
ACCURACY DATA

Impurity name	Recovery (%)			
	50 (%)	100 (%)	200 (%)	300 (%)
5-Flurocystosine	98.9	99.1	97.2	98.3
Emtricitabine acid	100.7	99.4	98.9	100.1
Sulfoxide isomer-II	99.4	100.1	99.7	100.0
Sulfoxide isomer-I	100.9	99.4	99.9	100.3
Lamivudine	100.6	99.5	99.1	99.4
Emtricitabine	98.5	99.8	100.7	100.3
Adenine impurity	99.0	99.0	100.1	99.5
Tenofovir impurity	99.5	98.9	99.0	99.2
9-Propyl adenine impurity	99.2	98.9	100.1	99.3
Monoester impurity	99.1	98.6	98.9	98.3
Ethyl impurity	99.1	99.9	99.3	100.1
Isopropyl impurity	99.4	98.5	99.8	100.2
N-Propyl impurity	98.4	98.4	99.4	98.5
Carbonyl impurity	99.3	99.6	98.9	99.7
Tenofovir disoproxil dimer impurity	99.9	99.3	99.6	99.8
Rilpivirine related compound A	98.9	98.9	99.1	98.5
Rilpivirine related compound B	98.3	98.7	99.2	99.4
Rilpivirine related compound C	98.4	98.4	99.1	99.0
Rilpivirine related compound D	98.2	98.4	99.1	99.1

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REFERENCES

1. A. Rouzes, K. Berthoin, F. Xuereb, S. Djabarouti, I. Pellegrin, J. Pellegrin, A. Coupet, S. Augagneur, H. Budzinski and M. Saux, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **813**, 209 (2004).
2. M.R. Induri, M.B. Raju, Y.R. Prasad, K.P.K. Reddy and C.H.S. Raidu, *Indian J. Pharm. Educ. Res.*, **45**, 305 (2011).
3. S. Raffanti and D. Haas, Antimicrobial Agents: Anti-Retroviral Agents, McGraw-Hill, New York (1990).
4. N.L. Rezk, R.D. Crutchley and A.D.M. Kashuba, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **822**, 201 (2005).
5. S. Sentenac, C. Fernandez, A. Thuillier, P. Lechat and G. Aymard, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **793**, 317 (2003).
6. A.A. Shirkhedkar and B.C.H. Surana, *Pak. J. Pharm. Sci*., **22**, 27 (2009).
7. R.W. Sparidans, K.M. Crommentuyn, J.H. Schellens and J.H. Beijnen, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **791**, 227 (2003).
8. K. Mangaonkar and A. Desai, *Indian Drugs*, **45**, 119 (2008).
9. R. Sharma and K. Mehta, *Indian J. Pharm. Sci.*, **72**, 527 (2010).
10. J.M. Molina, P. Cahn, B. Grinsztejn, A. Lazzarin, A. Mills, M. Saag, K. Supparatpinyo, S. Walmsley, H. Crauwels, L.T. Rimsky, S. Vanveggel and K. Boven, *J. Lancet,* **378**, 238 (2011).
11. K.Y. Kavitha, G. Geetha, R. Hariprasad, R. Venkatnarayana and G. Subramanian, *Int. J. Comp. Pharm*.,**1**, 7 (2013).
12. M. Pendela, G.W. Kahsay, G.V. Mooter, L. aert, J. Hoogmartens and E. Adams, *Chromatographia*, **73**, 439 (2011).
13. ICH Guidelines: Q1AR Stability Testing of New Drug Substances and Products (2000).