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ORIGINAL ARTICLE

Development and validation of analytical method for quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC

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Abstract This paper describes the development and validation of a HPLC method for the quantitation of Emtricitabine, Tenofovir, and Efavirenz in pure form and pharmaceutical formulations. The Zorbax SB CN, (250 × 4.6 mm, 5 μm) column was used. UV detection was performed at 260 nm. The mobile phase consisted of methanol (A) and buffer at pH 4.5(B) using the gradient: 0–10 min (90% B), 10–22 min (35% B), and 22–25 min (90% B). The flow rate was 1.5 ml/min in ambient temperature. The injection volume of sample was 20 μl. The method showed to be linear ($r^2 > 0.999$), precise (RSD < 0.76%), accurate (recovery of 100.09% for Emtricitabine, 99.88% for Tenofovir and 100.04% for Efavirenz), specific and robust. Three batches of Emtricitabine, Tenofovir, and Efavirenz tablets were assayed by the validated method. The Emtricitabine contents in the tablet samples varied from 99.94 to 101.60%. The Tenofovir content in the tablet samples varied from 99.13 to 101.81% while Efavirenz content varied from 100.01 to 101.67%.

1. Introduction

Tenofovir Disoproxil Fumarate (TDF) is a fumaric acid salt of the bisisopropoxycarbonyloxymethyl ester derivative of tenofovir. Chemically it is described as 9-[(R)-2[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl] methoxy]propyl] adenine fumarate (Budawari, 2001). Fig. 1a shows the nucleotide reverse transcriptase inhibitor (NtRTI) used in combination with other antiretrovirals for the treatment of HIV infection (Martindale, 2002). Emtricitabine (FTC) is a nucleoside reverse transcriptase inhibitor (NRTI). Chemically it is described as 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine (Fig. 1b). FTC is the (−) enantiomer of thio analog of cytidine which differs from other cytidine analogs, in that it has fluorine in the 5th position. FTC is an antiviral agent used for the prevention of perinatal HIV-1 reverse transcriptase (Budawari, 2001). It is also active against Hepatitis B virus (Martindale, 2002; Gish et al., 2005).

Efavirenz is a human immunodeficiency virus type-I (HIV-I) specific non nucleoside reverse transcriptase inhibitor (NNRTI). Efavirenz is chemically described as (S)-6-chloro-4-(cyclopropylethynyl)-1, 4-dihydro-4-(trifluoromethyl)-2H-3,

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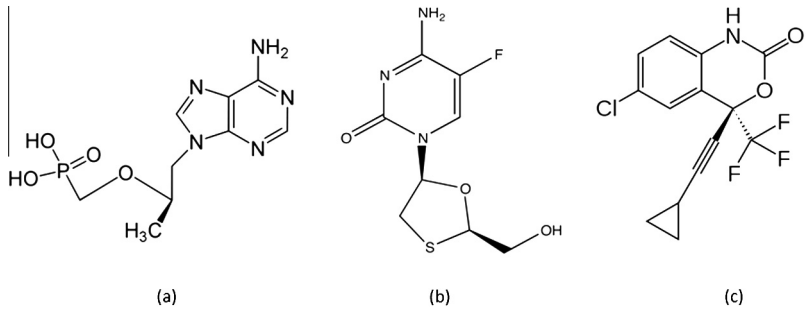

Figure 1 Chemical structure of (a) Tenofovir, (b) Emtricitabine and (c) Efavirenz.

1-benzoxazin-2-one (Fig. 1c) Physician's Desk Reference, 2008. A literature survey reveals that analytical methods based on HPLC (Malipatil and Nandedkar, 2009; Sharma and Pooja Gupta, 2009; Appala et al., 2008; Rolim-Neto, 2011; Montgomery et al., 2001), HPTLC (Joshi et al., 2009; Laxman et al., 2010), and UV spectrometry (Sockalingam et al., 2005; Shirkhedkar Atul et al., 2009) are available for the determination of these drugs individually and in combination with other drugs in different dosage forms. Some papers have described the analysis of combination with other drugs in plasma, based on HPLC (Kandagal et al., 2008; Deirdre Fox et al., 2011; Rezk et al., 2005), stability indicating (Sagar et al., 2009; Rao and Nikalje, 2009). However, there is no method reported regarding the quantitation of Emtricitabine, Tenofovir and Efavirenz.

Hence, the aim of this study was to develop a simple, precise, accurate, and validated HPLC method, using UV detection to quantify Emtricitabine, Tenofovir, and Efavirenz in pure form for pharmaceutical formulations. The molar absorptivity of Emtricitabine, Tenofovir and Efavirenz in the UV region was found to be at 260 nm. The validated method was applied to the analysis of tablets containing Emtricitabine, Tenofovir and Efavirenz (200 + 300 + 600 mg).

2. Experimental

2.1. Reagents and materials

Emtricitabine, Tenofovir, and Efavirenz reference standards were procured from Cipla Laboratories, Mumbai. Tablets were purchased from local pharmacy which were manufactured by Viraday, Cipla, Mumbai, India. Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA). Methanol (HPLC grade) was obtained from E-Merck (India) Ltd, Mumbai, India. All other chemicals used in the analysis were of AR grade.

2.2. Instrumental and analytical conditions

The HPLC analyses were carried out on Waters 2695 separation module (Waters Corporation, USA) equipped with auto sampler and Waters 2998 PDA detector, Zorbax SB CN, (250 × 4.6 mm, 5 μm) column. UV detection was performed at 260 nm. UV spectra scanning from 190 to 400 nm was recorded online for peak identification. The mobile phase consisted of methanol (A) and buffer at pH 4.5(B). The best separation was obtained between Emtricitabine, Tenofovir and Efavirenz using the gradient: 0–10 min (90% B), 10–22 min (35% B), and 22–25 min (90% B). The flow rate was 1.5 ml/min. The injection volume of sample was 20 μl. The separation of Emtricitabine, Tenofovir and Efavirenz was evaluated in different proportions of these solvents and for each condition, retention factor (k) and resolution (R) were calculated and data are presented in Table 1.

2.3. Preparation of buffer (pH 4.5)

Two grams of ammonium acetate was weighed and dissolved in 1000 ml of water. The pH of the solution was adjusted to 4.5 with orthophosphoric acid. The solution was filtered through 0.45 μm membrane filter.

2.4. Preparation of standard solution

About 100 mg of Emtricitabine, 150 mg of Tenofovir, and 300 mg of Efavirenz reference standards were accurately weighed and transferred into a 100 ml volumetric flask. 20 ml of methanol was added to ensure complete solubilization and the volume was adjusted with the mobile phase. Further dilutions were made to get a final concentration of 0.08 mg/ml of Emtricitabine, 0.12 mg/ml of Tenofovir and 0.24 mg/ml of Efavirenz.

Table 1 Retention factor (k) and Resolution (R) for Emtricitabine, Tenofovir and Efavirenz.

Emtricitabine Retention factor (K)	Tenofovir Retention factor (K)	Efavirenz Retention factor (K)	Resolution(R) for Tenofovir	Resolution(R) for Efavirenz
0.61	2.34	4.90	2.43	1.19
1.66	3.18	4.93	11.25	4.11
3.63	4.17	6.90	10.56	6.12
3.45	8.65	12.43	17.41	7.47
3.63	13.31	16.75	29.51	9.46

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