ISSN 2348-232X

Unique Research Journal of Chemistry

Available online: www.ujconline.net

Research Article

RP-HPLC AND UV QUANTIFICATION OF ANTIRETROVIRAL DRUGS IN FIXED DOSAGE FORMS

Kalpana J[1], Himaja M[1*], and Anbarasu C[2]

[1]Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore – 632014, Tamilnadu, India.
[2]Strides Arcolab, Bangalore – 560076, Karnataka, India

Received: 24-04-2014; Revised: 14-05-2014; Accepted: 05-06-2014

*Author for correspondence: **Dr (Mrs). M. Himaja**

Professor, Pharmaceutical Chemistry Division, VIT University, Vellore-632014.Tamil Nadu, India, Mobile: +919944796228.

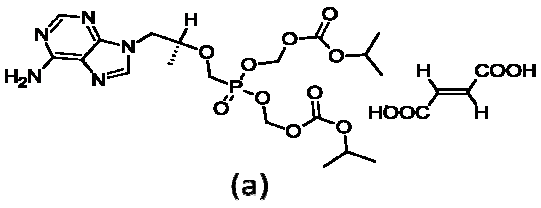
ABSTRACT

A simple and improved liquid chromatographic method with reduced run time and a cost effective UV spectrophotometric methods have been developed for the quantitation of antiretroviral drugs in a combined dosage form of three drugs. All the analytical parameters were determined as per ICH Q2B guidelines. Both the methods displayed good linearity, reproducibility and precision. No spectral or chromatographic interference were observed. The validated methods are reliable and can be suitably applied for the routine quality control analysis in the estimation of commercial formulations.

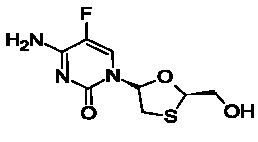
Keywords: Tenofovir disoproxil fumarate, Emtricitabine, Nevirapine, HPLC, Antiretroviral

INTRODUCTION

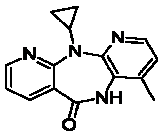
Recently, combination therapy has become the standard treatment to manage acquired immune deficiency syndrome (AIDS)[1,2]. The US Department of Health and Human Services has recommended highly effective combination regimens of antiretroviral drugs to combat mortality and morbidity in humans[3,4]. Tenofovir Disoproxil Fumarate (TDF), Emtricitabine (EMT) and Nevirapine (NVP) are the antiretroviral drugs that form the first line medicines for the treatment of HIV infected patients.



(a)



(b)



(c)

Chemical structures of (a) Tenofovir Disoproxil Fumarate (b) Emtricitabine and (c) Nevirapine

Chemically TDF is bis(isopropyloxy-carbonyloxymethylester of (R)-9-(2-phosphonomethoxy-propyl) adenine with fumaric acid[5] and EMT is 4-amino-5-fluoro-1-[(2S, 5R)-2-(hydroxymethyl)-1,3-oxathiolan-5- yl]-1,2-dihydropyrimidin-2-one. NVP chemically 11-cyclopropyl-4-methyl-5,11-dihydro-6Hdipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one, is a non-nucleoside reverse transcriptase inhibitor (NNRTI)[7].

TDF, EMT and NVP have been reported to be quantified individually or in combination with other drugs in biological fluids by various techniques [8-10]. The reported methods involve complicated procedures with sample pre-treatment and internal standard. So far in most of the existing reports the title drugs are determined either individually or in combination of two. However no single method has been reported for the simultaneous analysis of listed drugs as a combination of three.

In the present study, we developed a rapid RP-HPLC with short run times as well as a simple, cost effective UV spectrophotometric procedure to quantify TDF, EMT and NVP in pharmaceutical formulations without the requirement of sample pre-treatment. To our knowledge, this is the first report which addresses the quantification of all the three compounds with a single analytical method.

MATERIALS AND METHODS

Instrumentation:

The HPLC analysis was carried out on an Agilent HPLC system with an isocratic pump (G1310A), a DAD detector system (G1314A) and a thermostatted column (G1316A) compartment. The data acquisition was processed using Chemstation software. The UV method was carried out on Jasco V 670 UV/VIS/NIR diode array spectrophotometer (scan speed 400 nm/min and wavelength interval 1 nm), associated with Spectra manager software (Jasco, Japan) at 260 nm, 240 nm, 280 nm for TDF, EMT and NVP respectively. One centimetre quartz cells were used for measuring absorbance.

Chromatographic condition:

An Inertsil ODS-2 column (4.6 mm x 150 mm, 5 µm particle size) is used with gradient elution at a flow rate of 1.0 mL/min and UV detection at 254 nm. Separation was achieved using a mobile phase consisting of methanol and ammonium acetate buffer (pH 4.6) in gradient run. Peak homogeneity was expressed in terms of peak purity values, and was obtained directly from spectral analysis report obtained using the instrument software.

Standard solutions and calibration graphs for spectrophotometric measurements:

Primary stock solutions were prepared separately for each drug in the mixture of methanol and water (40:60, v/v) to obtain a concentration of 1 mg/mL, which were then diluted to 400µg/mL concentration for all three drugs. Calibration graphs were constructed in the concentration range of 4 – 24µg/ mL for TDF, EMT and NVP using methanol and water (40:60, v/v) as blank.

Standard solutions and calibration graphs for chromatographic measurements:

A primary stock solution of TDF (3 mg/ mL), EMT (2 mg/ mL), NVP (4 mg/ mL), was prepared using diluent (20:30 v/v of 50% acetonitrile and 50% acetic acid). The stock solution was protected from light and used within 24 hrs. Samples in triplicates for each concentration were prepared and peak areas were plotted against respective concentration to obtain the calibration graph.

Analysis of pharmaceutical formulations:

Sample preparation:

Tablets were weighed accurately and finely powdered. The active ingredients were extracted in mixture of methanol and water (40:60, v/v) for the spectrometric assay and in mixture of acetonitrile and acetic acid (40:60, v/v) for chromatographic assay. Appropriate dilutions were made and the respective samples were subjected to UV and HPLC analysis.

Assay procedure:

The developed UV and HPLC methods were successfully applied for the analysis of TDF, EMT and NVP in fixed dose combinations and the active contents in each sample were estimated by comparing with the appropriate standard solution of the drug. No interferences due to excipients was observed in the spectra or chromatograms obtained.

RESULTS AND DISCUSSION

UV Spectrophotometry:

TDF, EMT and NVP are compounds with specific chromophoric group in their chemical structure which absorbs at a particular wavelength and this concept has been successfully applied for their quantitative determinations by UV spectrophotometric method. The absorption spectra for the three drugs were clear without any overlap and hence were reliable for direct measurements.

Linearity, accuracy and precision:

Based on the experimental conditions mentioned earlier, linear regression equations (intercepts and slopes) for TDF, EMT and NVP were established. The correlation coefficients with high values and Y-intercepts close to zero states good linearity of the calibrations. Repeatability of the assay method was determined by analyzing 100% standard concentrations of the three drugs in six samples. The various validation parameters are summarized in Table 1.

Table 1: UV and HPLC method validation parameters

Method	Parameter	Tenofovir Disoproxil Fumarate	Emtricitabine	Nevirapine
UV	Range (µg / ml)	4-24	4-24	4-24
	Slope	0.0475	0.0253	0.0264
	Intercept	-0.0297	0.0021	-0.0198
	Reproducibility (% RSD)	0.25	0.27	0.68
	Correlation coefficient	0.9999	0.9998	0.9995
HPLC	Range (PPM)	73-219	59-179	120-360
	Slope	7.106	8.267	11.191
	Intercept	0.162	0.087	-0.121
	Reproducibility (% RSD)	0.5	0.2	0.1
	Correlation coefficient	0.9999	0.9999	0.9999

Concentrations in the linearity range were evenly distributed.
The absorbances were measured at 260 nm, 240 nm, and 280 nm for TDF, EMT and NVP respectively

Recovery studies were carried out by addition of standard drug to the sample at three different concentration levels taking into consideration percentage purity of added bulk drug samples and reproducibility was confirmed by repeating the methods (Table 2). The percentage recoveries ranged from 99.3 to 99.5% for TDF, 99.6 to 100.4% for EMT and 99.5–100.7% for NVP. Both the intra- and inter-day RSD values of the standards were less than 5% over the selected range thereby indicating the method is precise and accurate.

Table 2: Intra- and inter-day precision and accuracy

Method	Conc.	Tenofovir Disoproxil Fumarate			Emtricitabine			Nevirapine		
		Intra-day		Inter-day	Intra-day		Inter-day	Intra-day		Inter-day
		Accuracy (%)	Precision (% RSD)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)	Precision (% RSD)
UV	8 µg/ml	99.5	0.82	1.24	99.6	3.01	3.36	100.5	2.71	2.39
	12 µg/ml	98.9	1.32	1.81	100.4	0.12	0.96	99.5	3.82	2.96
	16 µg/ml	99.3	0.92	1.64	100.1	0.83	0.69	100.7	1.53	1.34
HPLC	50%	100.2	0.6	0.9	100.6	0.8	1.7	101.5	0.5	0.9
	100%	101.3	0.4	0.6	100.5	1.7	2.3	101.0	1.0	0.7
	150%	102.0	1.2	0.9	101.5	1.6	2.1	101.6	1.0	1.0

Accuracy and precision were determined with QC samples. Triplicate samples were analyzed.
For intra-day determinations, two standard curves were prepared on the same day

Chromatographic methods:

In order to resolve the active drugs different combination of mobile phase at various flow rates were tried. The compounds were resolved using a mobile phase composition of methanol–ammonium acetate buffer (pH 4.6) with gradient elution at a flow rate of 1 mL/min and the optimum wavelength for detection was set at 254 nm. An Inertsil ODS-2 column (4.6 mm x 150 mm, 5 µm particle size), maintained at ambient temperature (25°C) was found to be suitable for the separation. Under optimized chromatographic conditions the compounds were well resolved with a resolution greater than 2.0. Tailing factor for all the active compounds was found to be less than 1.3. Linear regression parameters were satisfactory (Table 1)

Range and linearity:

The six point calibration curves, that were constructed, were linear over 50 to 150% of standard concentration. Peak areas were plotted versus concentration and linear regression analysis performed on the resultant curve. The correlation coefficients of TDF, EMT and NVP were found to be 0.9999 respectively with % R.S.D. values ranging from 0.1 to 0.5% were obtained following linear regression analysis.

Accuracy and precision:

The accuracy was determined at three different concentrations in the calibration range in triplicates. Added known quantity of TDF, EMT and NVP at 50%, 100% and 150% of sample concentration into the placebo and the recovery percentage was calculated. Repeatability was investigated by injecting six replicate of standards, where the % R.S.D. values was found to be less than 2%. The % R.S.D. values for intra- and inter-day assays in the cited formulations performed in the same laboratory by the two analysts did not exceed 3%, thus indicating the ruggedness of the method. The mean retention time of TDF, EMT and NVP was 7.899, 4.317, 6.768 minutes respectively with % R.S.D. of 0.1%.

Sensitivity and selectivity:

Limit of detection (LOD) and Limit of quantification (LOQ) decide about the sensitivity of the method. The LOQ was found to be 0.025%, 0.023%, 0.012% with a resultant % R.S.D. of 2.9%, 6.5%, 3.7 % (n = 5) for TDF, EMT and NVP respectively. The LOD for TDF, EMT and NVP was found to be 0.008%, 0.007%, 0.004% respectively. This data shows high sensitivity of the method. As shown in the chromatogram, all three drugs eluted forming symmetrical single peaks, well separated from the solvent front (Fig 2). Hence, the proposed method is selective and specific.

Application of the method for the analysis of tablet formulation:

Both the proposed methods can be satisfactorily applied for the quantification of TDF, EMT and NVP without the interference of the excipients. Satisfactory results (Table 3) were obtained for the recovery of the three drugs and were in good agreement with the label claims indicating that both the proposed techniques can be used for the quantification in routine quality control analysis of pharmaceutical dosage forms.

Table 3: Percentage recoveries of TDF, EMT and NVP in formulations

Product	Drug components	UV		HPLC	
		Mean ± S.D.	% RSD	Mean ± S.D.	% RSD
Tenofovir Disoproxil Fumarate (300 mg), Emtricitabine (200 mg)	Tenofovir Disoproxil Fumarate	99.7 ± 2.8	1.7	99.51 ± 1.5	1.2
	Emtricitabine	99.8 ± 3.5	2.1	101.32 ± 0.6	1.0
Nevirapine (400mg)	Nevirapine	102.5 ± 2.3	2.2	103.04 ± 2.4	0.7

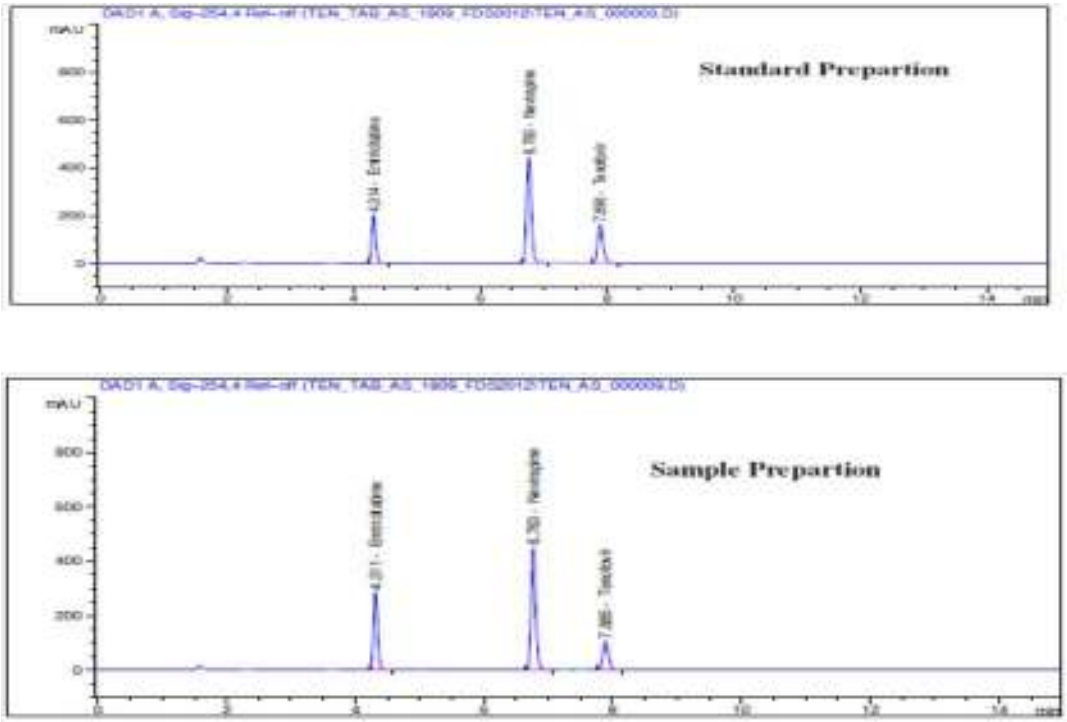


Figure 2: Resultant HPLC chromatogram following the analysis of a standard and sample solution of Tenofovir Disoproxil Fumarate, Emtricitabine and Nevirapine

CONCLUSION

The developed UV and HPLC methods were found to be rapid and reliable without any interference between the tablet excipients and active drugs. In HPLC method all the three analytes have been eluted within 10 minutes exhibiting a shorter analysis rate. The UV method exhibited a good precision and recovery. Both the established methods are convenient and can be applied for the routine quality control analysis of the examined drugs in both bulk and tablet dosage forms.

ACKNOWLEDGMENTS

The authors thank the management of VIT University, Vellore as well as the Strides Arcolab, Bangalore for constant support and encouragement during the course of the project.

REFERENCES

1. Clercq ED. New developments in anti-HIV chemotherapy. Biochem. Biophys. Acta., 2002; 1587: 258-275.
2. Beach JW. Chemotherapeutic agents for human immunodeficiency virus infection: mechanism of action, pharmacokinetics, metabolism, and adverse reactions, Clin. Ther., 1998; 20: 2-25.
3. Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents, US Department of Health and Human Services. 2006.
4. Gallant JE. Initial therapy of HIV infection, Journal of Clinical Vir J. Clin. Virol., 2002; 25: 317-333.
5. Indian pharmacopoeia, The Indian Pharmacopoeial Commission, Ghaziabad, New Delhi, 2007. p. 1071, 1782 and 1276.
6. The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, 14th edn. Merck Research Laboratories New Jersey, USA monograph, 2006. p.3565.
7. Staszewski S, Ramirez JM, Tashima KT. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults, N. Engl. J. Med., 1999; 341: 1865-73.
8. Montaner JSG, Reiss P, Cooper D. A randomized double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS trial, JAMA., 1998; 279: 930–937.
9. Podzamczer D, Ferrer E, Consiglio E. A randomized clinical trial comparing nelfinavir or nevirapine associated to zidovudine/lamivudine in HIV-infected naive patients (the Combine Study), Antivir. Ther., 2002; 7: 81.
10. Anbazhagan S, Indumathy N, Shanmugapandiyan P. Simultaneous quantification of stavudine, lamivudine and nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets, J. Pharm. Biomed. Anal., 2005; 39: 801-804.

Source of support: Nil, Conflict of interest: None Declared