Research Article

Simultaneous Estimation and Validation of Emtricitabine, Tenofovir Disproxil Fumarate and Efavirenz in Pharmaceutical Dosage Form by UV-Spectrophotometry

K Vijaya Sri[1*], Dilipkumar Yadla[1], M. Shalem[1] and V. R. Sirisha. K[2]

[1]Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, (Affiliated to Osmania University), Maisammaguda, Secunderabad-500014, Telangana, India
[2]General Manager, Ocean Pharma coat Pvt Ltd, Co-operative Industrial Estate, Gandhi Nagar, Balanagar,Hyderabad

Abstract

The main objective of present research work was to develop and validate the UV- spectrophotometric methods for the simultaneous estimation of emtricitabine, tenofovir disproxil fumarate and efavirenz in bulk and pharmaceutical dosage form method is based on simultaneous equation analysis by using methanol as a solvent. The simultaneous equation method depends on mainly that amoung three components (emtricitabine, tenofovir disproxil fumarate and efavirenz) each of which absorbs at the λmax of each other, Results: The λmax of emtricitabine, tenofovir disproxil fumarate and efavirenz was found to be 260nm, 241nm and 240nm respectively the linearity range of emtricitabine, tenofovir disproxil fumarate and efavirenz was between 6.67-40, 10-60 and 20-120 µg/ml respectively. The method was validated for various parameters as per ICH guidelines and the results are found to be in acceptable limits. Conclusion: New, simple, accurate methods were developed for the simultaneous estimation of emtricitabine, tenofovir disproxil fumarate and efavirenz.

Keywords: UV, Emtricitabine, Tenofovir Disproxil Fumarate, Efavirenz, Simultaneous Equation Method

***Correspondence**
Author: K Vijaya Sri
Email: vijayasree_2002@yahoo.co.in

Introduction

Emtricitabine, Tenofovir disproxil fumarate and efavirenz is a new formulation consisting of two nucleoside analog HIV-1 reverse transcriptase inhibitors (emtricitabine, tenofovir disproxil fumarate) and one non nucleoside reverse transcriptase inhibitor (efavirenz)is indicated for use alone as a complete regimen for the treatment of HIV-1 infection in antiretroviral treatment-naive adults with HIV-1 RNA less than or equal to 100, 000 copies/ml [1]. Emtricitabine is chemically known as 5-fluoro-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5- yl]cytosine. Emtricitabine, a synthetic nucleoside analog of cytidine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 RT by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in chain termination. Tenofovir disproxil fumarate is chemically known as 9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy] phosphinyl]-methoxy] propyl] adenine fumarate (1:1). Tenofovir disproxil fumarate is an acyclic nucleoside phosphonate diester analog of adenosine mono phosphate. Tenofovir disproxil fumarate requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 RT by competing with the natural substrate deoxyadenosine 5′-triphosphate and, after incorporation into DNA, by DNA chain termination.Efavirenz is chemically known as (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2, 4-dihydro-1H-3, 1-benzoxazin-2-one.is a non-nucleoside reverse transcriptase inhibitor bind non competitively to an active site of the reverse transcriptase molecule. Reverse transcriptase directs the polymerization of DNA from viral RNA. The NNRTIs inhibit this polymerization by altering the position of critical amino acids within the catalytic site [2].The structures of emtricitabine, tenofovir disproxil fumarate and efavirenz are shown in **Figures 1-3**.

Literature survey reveals that there are several HPLC, UV, LC, LC-MS, HPTLC methods available for estimation of emtricitabine, tenofovir disproxil fumarate and efavirenz [3-7] and combination [8-12] of tenofovir disproxil fumarate and emtricitabine. Very few HPLC [13-15] methods available for simultaneous estimation of emtricitabine, tenofovir disproxil fumarate and efavirenz. Till date there is no reported on UV spectrophotometric method available for simultaneous estimation of emtricitabine, tenofovir disproxil fumarate and efavirenz. Hence

there is a need to develop UV spectrophotometric methods for simultaneous estimation of emtricitabine, tenofovir disproxil fumarate and efavirenz.

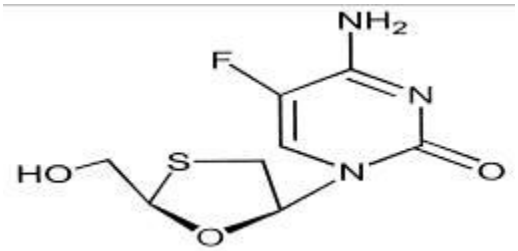

Figure 1 Structure of Emtricitabine

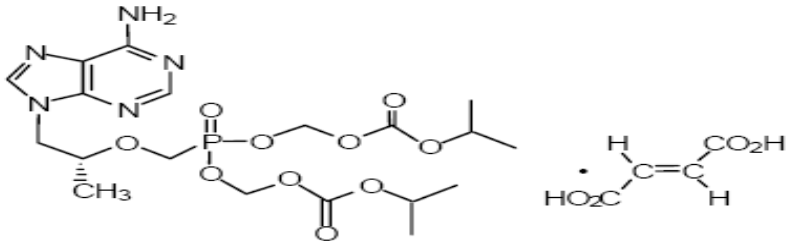

Figure 2 Structure of Tenofovirdisproxilfumarate

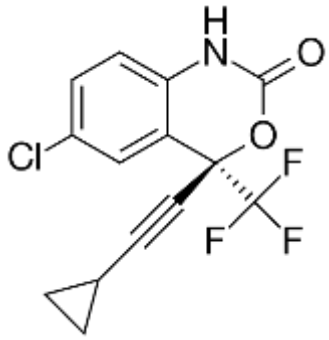

Figure 3 Structure of Efavirenz

Materials and Methods

Emtricitabine, tenofovir disproxil fumarate and efavirenz were gifted by Hetero Laboratories Ltd. (Hyderabad, A.P, INDIA). Methanol was analytical grade, purchased from Merck Chemical Company (India). The commercial available tablets of Atripla.

Instrument

Shimadzu Model 1800 UV-Visible double beam spectrophotometer with spectral bandwidth of 0.1nm and a pair of 1cm matched quartz cells was used in this study.

Preparation of standard stock solution

Stock I solution

Standard stock solution of emtricitabine, tenofovir and efavirenz were prepared separately by dissolving 20mg of emtricitabin, 30mg of tenofovir in methanol and made the volume up to 100 ml with same solvent and dissolving 60mg of efavirenz in methanol and made the volume up to 100 ml with same solvent.

Stock II solution

From the above stock solutions 1ml of the each aliquots were pipetted out ina 10ml volumetric flask separately and the volume was made up to the mark with same solvent to obtain the final concentration of 200µg/ml of emtricitabine, 300µg/ml of tenofovir and 600µg/ml of efavirenz.

Selection of wavelength

Working standard solutions of 20 µg/ml of emtricitabine, 30µg/ml of tenofovir and 60µg/ml of efavirenz were further prepared by appropriate dilution of standard stock solutions. Overlain spectrum of emtricitabine, tenofovir and efavirenz were scanned and from which the wavelengths of 241nm, 260nm and 246nm were selected for emtricitabine, tenofovir and efavirenz respectively for further studies.

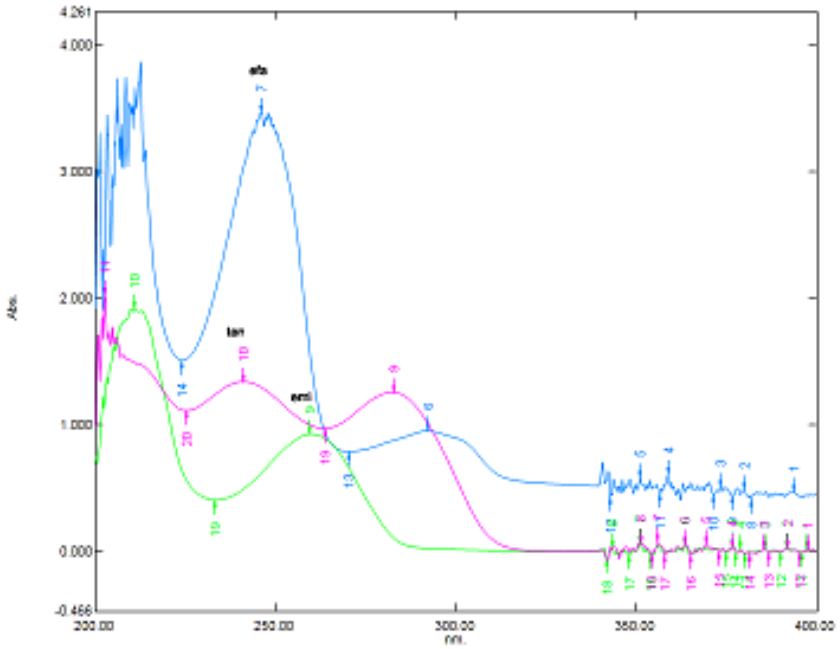

Figure 4 UV spectra of emtricitabine, tenofovirdisproxilfumarate, efavirenz in methanol

Simultaneous Equation Method

Three wavelengths required in this method are selected at which one component shows maximum absorbance, while remaining shows considerable absorbance. Considering this fact, wavelengths 260nm, 241nm and 246nm (λmax of all three drugs) were selected for the estimation of emtricitabine, tenofovirdisproxilfumarate, and efavirenz by simultaneous equation method. At all selected wavelengths the absorbance and absorptivity values of three drugs were determined [16]. The absorbance of the mixture at 260 nm, 241 nm and 246 nm may be expressed as follows:

$$A1 = ax1bCx + ay1bCy + az1bCz\ldots\ldots\ldots \text{at 260 nm}$$
$$A2 = ax2bCx + ay2bCy + az2bCz\ldots\ldots\ldots \text{at 241 nm}$$
$$A3 = ax3bCx + ay3bCy + az3bCz\ldots\ldots\ldots \text{at 246 nm}$$

For measurements in 1cm cell b = 1. Hence

$$A1 = ax1Cx + ay1Cy + az1Cz$$
$$A2 = ax2Cx + ay2Cy + az2Cz$$
$$A3 = ax3Cx + ay3Cy + az3Cz$$

Apply the cramer's rule for above equations

$$D = \det\begin{bmatrix} ax_1 & ay_1 & az_1 \\ ax_2 & ay_2 & az_2 \\ ax_3 & ay_3 & az_3 \end{bmatrix} \quad D_{Cx} = \det\begin{bmatrix} A_1 & ay_1 & az_1 \\ A_2 & ay_2 & az_2 \\ A_3 & ay_3 & az_3 \end{bmatrix} \quad D_{Cy} = \det\begin{bmatrix} ax_1 & A_1 & az_1 \\ ax_2 & A_2 & az_2 \\ ax_3 & A_3 & az_3 \end{bmatrix}$$

$$D_{Cz} = \det\begin{bmatrix} ax_1 & ay_1 & A_1 \\ ax_2 & ay_2 & A_2 \\ ax_3 & ay_3 & A_3 \end{bmatrix}$$

$$D = ax_1(ay_2 az_3 - az_2 ay_3) - ay_1(ax_2 az_3 - az_2 ax_3) + az_1(ax_2 ay_3 - ay_2 ax_3)$$
$$D_{C_x} = A_1(ay_2 az_3 - az_2 ay_3) - ay_1(A_2 az_3 - az_2 A_3) + az_1(A_2 ay_3 - az_2 A_3)$$
$$D_{C_y} = ax_1(A_2 az_3 - az_2 A_3) - A_1(ax_2 az_3 - az_2 ax_3) + az_1(ax_2 A_3 - A_2 ax_3)$$
$$D_{C_z} = ax_1(ay_2 A_3 - A_2 ay_3) - ay_1(ax_2 A_3 - A_2 ax_3) + A_1(ax_2 ay_3 - ay_2 ax_3)$$

$$C_x = \frac{D_{C_x}}{D}$$

$$C_x = \frac{A_1(ay_2 az_3 - az_2 ay_3) - ay_1(A_2 az_3 - az_2 A_3) + az_1(A_2 ay_3 - ay_2 A_3)}{ax_1(ay_2 az_3 - az_2 ay_3) - ay_1(ax_2 az_3 - az_2 ax_3) + az_1(ax_2 ay_3 - ay_2 ax_3)} \ldots (1)$$

$$C_y = \frac{D_{C_y}}{D}$$

$$C_y = \frac{ax_1(A_2 az_3 - az_2 A_3) - A_1(ax_2 az_3 - az_2 ax_3) + az_1(ax_2 A_3 - A_2 ax_3)}{ax_1(ay_2 az_3 - az_2 ay_3) - ay_1(ax_2 az_3 - az_2 ax_3) + az_1(ax_2 ay_3 - ay_2 ax_3)} \ldots (2)$$

$$C_z = \frac{D_{C_z}}{D}$$

$$C_z = \frac{ax_1(ay_2 A_3 - A_2 ay_3) - ay_1(ax_2 A_3 - A_2 ax_3) + A_1(ax_2 ay_3 - ay_2 ax_3)}{ax_1(ay_2 az_3 - az_2 ay_3) - ay_1(ax_2 az_3 - az_2 ax_3) + az_1(ax_2 ay_3 - ay_2 ax_3)} \ldots (3)$$

Where the absorbance of diluted sample solution at 260, 241 and 246nm are A1, A2and A3 respectively, The absorptivity of emtricitabine at 260, 241 and 246nm are ax1, ax2 and ax3 respectively, The absorptivity of tenofovir at 260, 241 and 246nm are ay1, ay2 and ay3 respectively, The absorptivity of efavirenz at 260, 241 and 246nm are az1, az2 and az3 respectively, Cx, Cy and Cz are the concentration of emtricitabine, tenofovir and efavirenz respectively in the diluted samples. Using above equations 1, 2 and 3 the concentrations of component X(emtricitabine), component Y (tenofovir) and component Z (efavirenz) in the sample mixture can be determined.

Application of the Proposed Method for Pharmaceutical Formulation

Twenty tablets were accurately weighed and ground into a fine powder using a glass mortar and pestle. The powder equivalent to about 60 mg of efavarinz (20 mg of emtricitabine and 30mg of tenofovir) was transferred to a 100ml volumetric flask. Approximately 75ml of diluent was added to the flask and mixed well by sonicating it for 15 min. Then flask was adjusted to volume with diluent. The solution was filtered using whatmann filter 5 paper into volumetric flask. The resulting solution was used as a working sample solution which contain200µg/ml of emtricitabne, 300µg/ml of tenofovir and 600µg/ml of efavirenz. 0.1ml of filtrate was taken in a 10ml volumetric flask and made the volume with methanol solvent. The above solution was analyzed at 260, 241 and 246nm wavelengths and values of the absorbance were substituted in respective equations 1, 2 and 3 to obtain the concentration of emtricitabine, tenofovir disproxil fumarate and efavirenz [17-18].

Validation of developed method

Linearity

Linearity was evaluated by preparing the solutions having the concentration range of 6.67-40µg/ml of emtricitabine, 10-60 µg/ml of tenofovir and 20-120 µg/ml of efavirenz. The calibration curves were obtained by plotting absorbance against concentration (µg/ml) for three different wavelengths (260nm, 241nm, 240 nm). Standard deviation (SD), slope, intercept, and correlation coefficient of determinations (r^2) of the calibration curves were calculated to ascertain the linearity of the method.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solution

(n=6) for emtricitabine (20µg/ml), tenofovir (30µg/ml) and efavirenz (60µg/ml) without changing the parameter of the proposed UV method. The %RSD was calculated.

Intermediate Precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses on the same day and next day for three different concentrations of standard solution of emtricitabine (6.67, 20, 33.3µg/ml), tenofovir (10, 30, 50µg/ml) and efavirenz (20, 60, 100µg/ml). The result was reported in terms of relative standard deviation (%RSD).

Accuracy

Accuracy of the proposed method was determined using recovery studies by spiking method. The recovery studies were carried out by adding different amounts (50, 100 and 150%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated.

Limit of Detection and Limit of Quantification

The limit of quantification (LOQ) and limit of detection (LOD) were based on the residual standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1) [18]. LOD = 3.3 × σ/S, LOQ = 10 × σ/S Where σ = the standard deviation of the response and S = slope of the calibration curve.

Ruggedness Studies

Ruggedness studies were performed by preparing three replicates of 20µg/ml of emtricitabine, 30µg/ml of tenofovir and 60µg/ml of efavirenz and analyzing by two different analysts and on two different instruments and the results are reported as %RSD.

Results and Discussion

The method was validated according to ICH guidelines [19] in order to determine the linearity, precision, accuracy and ruggedness of the method. The summary of optical parameters is shown in **Table 1**.

Table 1 Summary of optical parameters

Parameter	Emtricitabine at 260 nm	Tenofovir at 241 nm	Efavirenz at 246nm
Linearity range (µg/ml)	6.67-40	10-60	20-120
Regression equation (y=mx+c)	y = 0.044x + 0.017	y = 0.020x + 0.723	y = 0.018x+2.228
Slope	0.044	0.020	0.018
Intercept	0.017	0.723	2.228
Correlation coefficient (r)	0.999	0.999	0.999
Molar absorptivity (L mol^{-1} cm^{-1})	3.1140×10^4	2.9174×10^4	10.5115×10^4
Precision (%RSD)	0.294	0.479	0.256
Limit of detection	0.086	0.241	0.778
Limit of quantification	0.261	0.739	2.330
Sandell's sensitivity (µ cm^{-2}/0.001 absorbance units)	0.001701	0.005475	0.004471

Linearity

The absorbance of the solutions of emtricitabine, tenofovir and efavirenz was determined at three wavelengths i.e. 260nm, 241nm, 246nm and the calibration curves were plotted are shown in (**Figures 5-7)** and the overlay spectra's of emtricitabine, tenofovir and efavirenz are shown in (**Figure 8**).

Method precision (repeatability)

Repeatability was determined for all the drugs and % RSD was found to be less than 2 which has shown in **Table 2**.

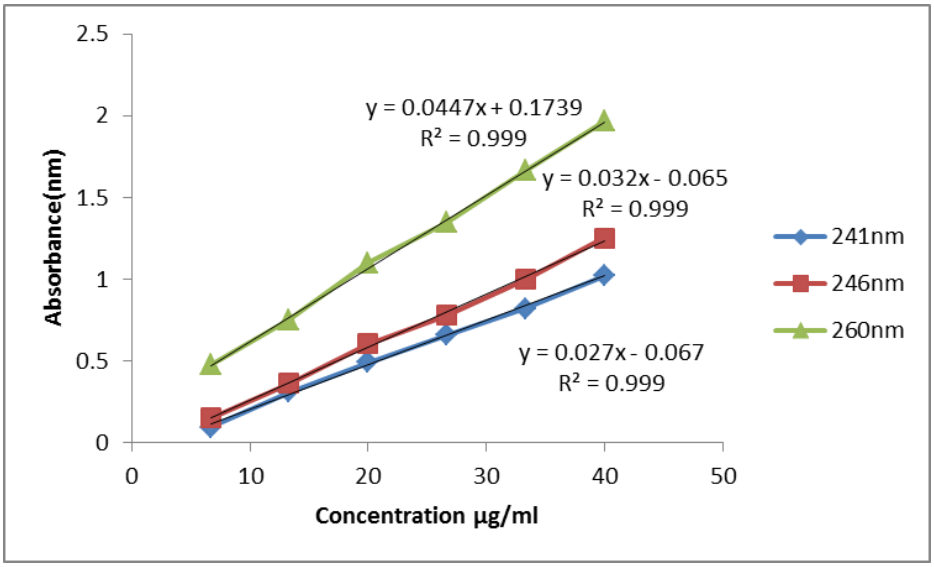

Figure 5 Calibration curve for Emtricitabine

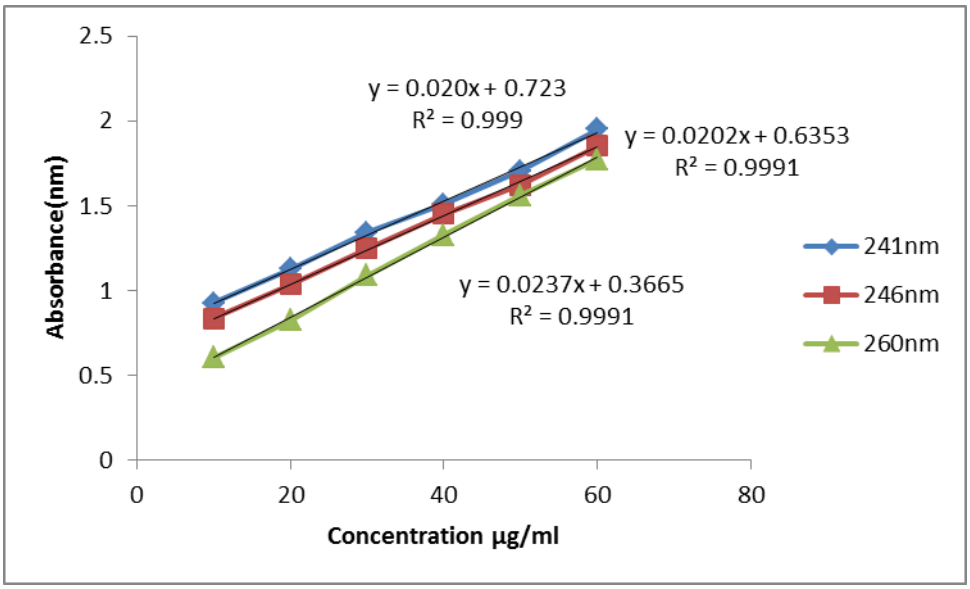

Figure 6 Calibration curve for Tenofovir disproxil fumarate

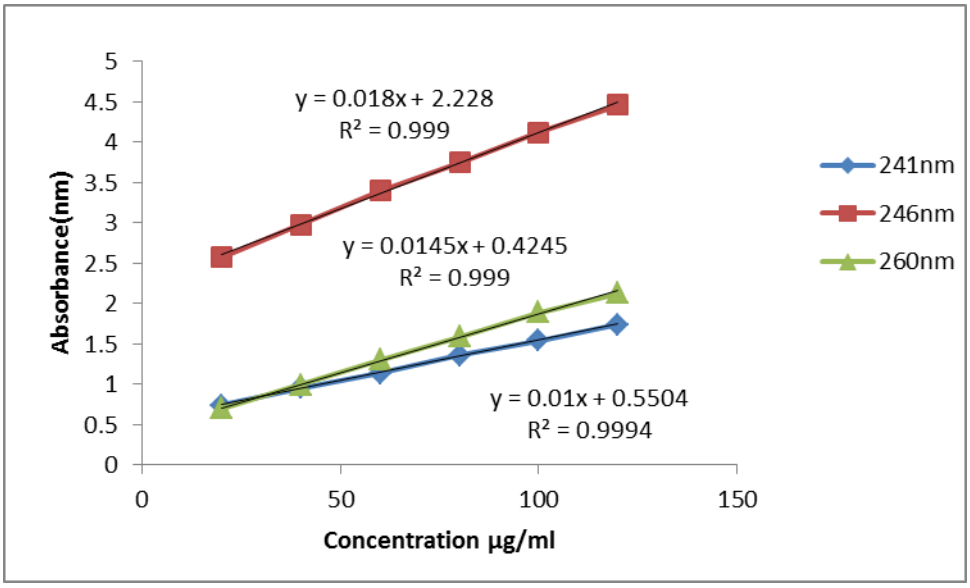

Figure 7 Calibration curve for Efavirenz

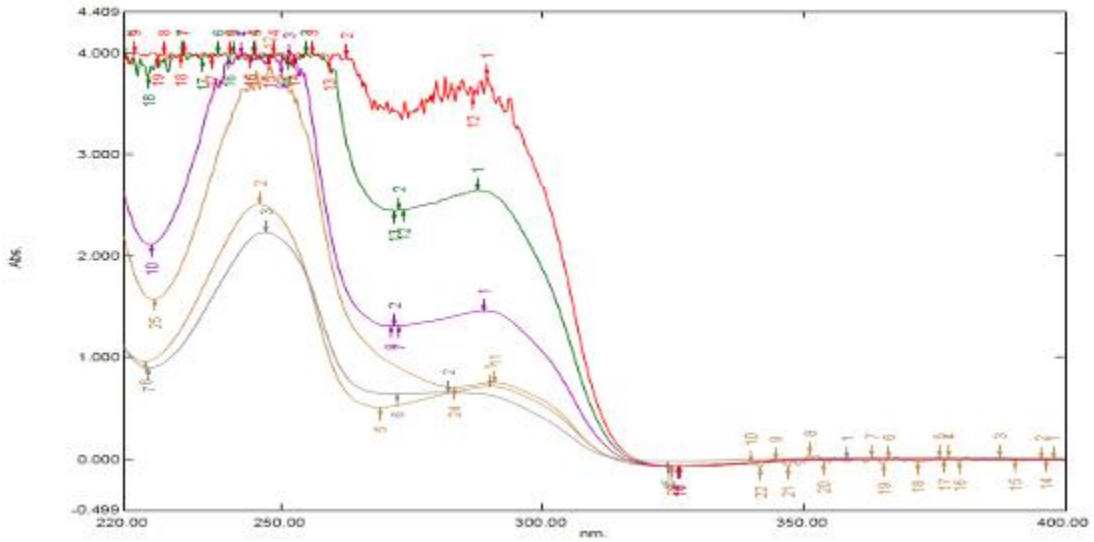

Figure 8 Overlay spectra of emtricitabine, tenofovirdisproxilfumarate and efavirenz

Table 2 Repeatability

Drug name	Conc µg/ml	Absorbance 1	2	3	4	5	6	Absorbance mean ± SD	% RSD
Emtricitabine	20	1.099	1.098	1.099	1.096	1.098	1.097	1.098±0.001169	0.170
Tenofovir	30	1.341	1.342	1.344	1.343	1.345	1.342	1.343±0.00147	0.109
Efavarinz	60	3.422	3.423	3.425	3.426	3.425	3.424	3.424±0.00147	0.402

Intermediate Precision (reproducibility)

The precision of the developed method was expressed in terms of percent relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values were found to be less than 2 that indicate this method precise for the determination of the pure form. The interday and intraday precision results were mentioned in **Table 3**.

Table 3 Intermediate Precision

Drug name	Conc µg/ml	Intraday precision Absorbance mean±SD	%RSD	Interday precision Absorbance mean±SD	%RSD
Emtricitabine	6.67	0.475±0.0024	0.524	0.472±0.0012	0.450
	20	1.095±0.0047	0.430	1.096±0.0029	0.397
	33.3	1.655±0.0077	0.470	1.654±0.0033	0.235
Tenofovir	10	0.93± 0.0037	0.402	0.93±0.003	0.302
	30	1.344±0.0024	0.185	1.344±0.002	0.135
	50	1.705±0.0021	0.126	1.703±0.002	0.198
Efavarinz	20	1.522±0.0478	0.829	1.512±0.004	0.627
	60	1.686±0.125	0.393	1.634±0.00115	0.757
	100	2.226±0.089	0.275	2.015±0.0079	0.819

Accuracy

Accuracy is determined by performing recovery studies at 3 levels in which known amount of analyte shall be added and recovery shall be carried out in three replicates of each concentration level and the % recovery was calculated. The accuracy results are shown in **Table 4**.

Limit of Detection and Limit of Quantification

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. LOD & LOQ values are 0.086, 0.261 for emtricitabine, 0.241, 0.731 for tenofovirdisproxilfumarate and 0.256, 0.778 for efavirenz.

Table 4 Results of Accuracy

Drug name	Level of % recovery	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found* (µg/ml) ± SD	%Recovery* ± SD
Emtricitabine	50	20	10	29.9±0.251	99.71±0.173
	100	20	20	40.07±0.231	101.1±0.388
	150	20	30	50.2±0.241	100.1±0.323
Tenofovir	50	30	15	44.96±0.361	98.43±0.448
	100	30	30	60.02±0.374	100.05±0.426
	150	30	45	74.99±0.394	99.92±0.400
Efavarinz	50	60	30	90.1±0.401	100.2±0.287
	100	60	60	199.31±0.432	99.72±0.577
	150	60	90	150.21±0.474	100.1±0.606

Ruggedness Studies

Ruggedness was performed by two different analysts and two different instruments and the results of the study were given in **Table 5** and % RSD obtained was less than 2 which is within the acceptance limits.

Analysis of Formulation

The % purity of emtricitabine, tenofovir and efavirenz was 99.54, 100.75 and 100.48 respectively and results are shown in **Table 6**.

Table 5 Ruggedness

Drug name	Conc (µg/ml)	Parameter (different analyst)	Absorbance mean*±SD	% RSD	Parameter (different instrument)	Absorbance mean*±SD	%RSD
Emtricitabine	20	Analyst 1	0.472±0.0010	0.450	Shimadzu(UV-1800)	0.294±0.0010	0.340
	20	Analyst 2	0.470±0.0020	0.449	Lab-India(UV®-3000)	0.294±0.0020	0.343
Tenofovir	30	Analyst 1	1.096±0.0020	0.847	Shimadzu (UV-1800)	0.479±0.0010	0.847
	30	Analyst 2	1.094±0.0015	0.845	Lab-India (UV®-3000)	0.479±0.0020	0.818
Efaverinz	60	Analyst 1	1.634±0.0015	0.358	Shimadzu (UV-1800)	0.161±0.0006	0.758
	60	Analyst 2	1.631±0.0010	0.321	Lab-India (UV®-3000)	0.160±0.0010	0.720

Table 6 Results of Assay

Drug name	Labeled claim (mg)	Amount found* (mg) ± SD	% Purity ± SD
Emtricitabine	200	199.76±0.004	99.54±1.665
Tenofovir	300	301.14±0.031	100.75±0.721
Efavirenz	600	600.06±0.061	100.48±1.812

Conclusion

This simultaneous equation method requires measurement of absorbance of all the three drugs at 260 nm, 241 nm and 246 nm and few simple manual calculations by using simultaneous equations. The above proposed UV methods are very simple, precise, accurate, rapid and cost effective for the simultaneous estimation of emtricitabine, tenofovir and efavirenz from its pharmaceutical dosage forms. Hence it can be utilized for routine analysis in bulk and pharmaceutical dosage forms.

Recommend future work

These UV-Spectrophotometric methods applied for ratio spectrophotometry, double divisor spectrophotometry, chemometrics.

Acknowledgement

The authors are grateful to Chairman, Principal Mallareddy College of Pharmacy for providing necessary research

facilities to carry out the research work and to Hetero drugs, India for providing the gift sample of the drug.

References

[1] Complera, Foster City, CA: Gilead Sciences, Inc. 2011
[2] Government of India. The Indian Pharmacopoeia. New Delhi, India: Controller of Publication; 2007.
[3] A SA., H BC., J SS., Application of UV-spectrophotometric methods for estimation of tenofovirdisproxil fumarate in tablets, Pak J Pharm Sci 2009;22(1):27-29.
[4] Rajesh S., Pooja G., A validated RP - HPLC method for simultaneous estimation of emtricitabine and tenofovir disoproxilfumarate in a tablet dosage form, Eurasian J Analyt Chem 2009;4(3):276-284.
[5] Arti S., Seema T., Simultaneous estimation of tenofovir and emtricitabine in human plasma using HPLC after protein precipitation extraction, J Analyt Bio analyt Techniques 2013:4(3).
[6] Skoog DA, Holler FJ and Nieman TA. Principles of instrumental analysis. 5th ed. Saunders college publications, 1998, pp no 1
[7] Kamboj PC. Pharmceutical analysis. Vallabh Prakashan Publishers. New Delhi, 2003, volume I, pp no 1.
[8] Arun Ramaswamy, Anton Smith Arul Gnana Dhas. Development and validation of analytical methodfor quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC Arabian Journal of chemistry 2014.
[9] Ashour H., Belal ST., New simple spectrophotometric method for determination of the antiviral mixture of emtricitabine and tenofovir disoproxil fumarate, Arabian J Chem 2013:1-7.
[10] A Lakshmana Rao. Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Efavirenz, Tenofovir and Emtricitabine in Pharmaceutical Formulations, A simple, precise and rapid HPLC method has been developed for the simultaneous determination of Efavirenz, Tenofovir and Emtricitabine in pharmaceutical dosage form. Indian Journal of Pharmacy and Pharmaclogy. vol 1. 2014:1-17.
[11] Prashant S. Devrukhakar, Roshan Borkar, NaliniShastri and K. V. Surendranath. A Validated Stability-Indicating RP-HPLC Method for the simultaneous determination of tenofovir, emtricitabine and efavirenz and stastical approach to determine the effect of variables. vol 1 2012:1-8.
[12] N Appala Raju and Shabana Begum. Simultaneous RP-HPLC Method for the estimation of the emtricitabine, tenofovir disproxil fumerate and efavirenz in tablet dosage form. Research Journal of Pharmacy and Technology. vol 1(4). 2008:522-525.
[13] ICH harmonized triplicate guide line text of validation of analytical procedures recommended for adaptation at September 4th of the ICH process on 27th October 1994.
[14] ICH Q2A, Validation of Analytical Procedures: Definitions and Terminology, Geneva, 1995, in 2005 incorporated in Q2(R1)
[15] Gopal Garg, Daharwal SJ, Swarnalatha Saraf and Saraf S. Various UV spectrophotometric simultaneous methods. Pharmainfo.net. 2006.
[16] Davinson AG, Backett AH, Stenlake JB. Practical Pharmaceutical chemistry. 4th ed. New Delhi: C.B.S. Publishers and Distributors; 1997.p. 275-278.
[17] Instruction Manual Operation Guide UV-1800, Shimadzu Spectrophotometer, Shimadzu Corporation, Kyoto, Japan.
[18] International Conference on Harmonization, Validation of analytical procedures: text and methodology. In ICH Harmonized Tripartite Guidelines Q2 (R1), November 2005.
[19] International Conference on Harmonization (ICH), Draft guidelines on validation of Analytical Procedure, Definition and Terminology. Geneva: Federal Registrar; 1995. p.11260-62.

Publication History
Received 12th Nov 2017
Revised 29th Nov 2017
Accepted 10th Dec 2017
Online 30th Dec 2017