Research Article

Simultaneous Spectrophotometric Method for Determination of Emtricitabine and Tenofovir Disoproxil Fumarate in Three-Component Tablet Formulation Containing Rilpivirine Hydrochloride

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Received 20 May 2014; Accepted 24 October 2014; Published 16 November 2014

Academic Editor: Zsuzsanna Kuklenyik

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Developing a single analytical method for estimation of individual drug from a multidrug composition is a very challenging task. A complexation, derivatization, extraction, evaporation, and sensitive-free direct UV spectrophotometric method is developed and validated for the simultaneous estimation of some antiviral drugs such as emtricitabine (EMT), tenofovir disoproxil fumarate (TDF), and rilpivirine HCl (RPV) in tablet dosage form by Vierordt’s method. The solutions of standard and sample were prepared in methanol. The $\lambda_{\text{max}}$ for emtricitabine, tenofovir disoproxil fumarate, and rilpivirine hydrochloride were 240.8 nm, 257.6 nm, and 305.6 nm, respectively. Calibration curves are linear in the concentration ranges 4–12 $\mu$g/ml for EMT, 6–18 $\mu$g/ml for TDF, and 0.5–1.5 $\mu$g/ml for RPV, respectively. Results of analysis of simultaneous equation method were analyzed and validated for various parameters according to ICH guidelines.

1. Introduction

Around 33.4 million people were living with HIV in year 2008 and around 2 million people have died in the same year. Highly active antiretroviral therapy (HAART) has brought new hope for those people who live with HIV/AIDS by decreasing the morbidity and mortality among people infected with HIV. Highly active antiretroviral therapy also has improved the quality of life among the people who live with HIV/AIDS. Combination therapy is preferred to be the gold standard for the treatment of AIDS so as to maximize potency, minimize toxicity, and diminish the risk for resistance development and reduction of pill burden to once-daily dosing so as to optimize the patient’s compliance and reduce the treatment costs. The nucleoside reverse transcriptase inhibitors and nonnucleoside reverse transcriptase inhibitors as multidrug combinations are effective in the therapy of human immunodeficiency virus (HIV) infection and are used as a part of highly active antiretroviral Therapy, for the treatment of HIV 1, 2 [1]. The daily regimen containing emtricitabine, tenofovir disoproxil fumarate, and rilpivirine HCl is virologically and immunologically effective, well-tolerated, and safe with benefits in the lipid profile in the majority of patients (Figure 1) [2]. It is common practice in HIV treatment to give different drugs to the patient. In order to improve the comfort of the daily intake, manufacturers try to combine several active compounds in one dosage form. In this study a UV spectrophotometric method was developed for tablet containing EMT, TDF, and RPV.

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTIs). Chemically it is 5-fluoro-1-(2R, 5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine. EMT is the enantiomer of thio analog of cytidine which differs from other cytidine analogs, in that it has fluorine in 5th position.

Tenofovir disoproxil fumarate {9-[(R)-2-[[bis [[isopropoxy carbonyl] oxy] methoxy] phosphonyl] methoxy] propyl adenine fumarate} is a nucleotide analog reverse transcriptase
inhibitor (NRTI) and is used for treating HIV infection in adults, in combination with other antiretroviral agents [3, 4].

Rilpivirine HCl chemical name is benzonitrile 4-[[4-[[4-[(1E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]hydrochloride. It is a second-generation nonnucleoside reverse transcriptase inhibitor (NNRTI) with higher potency, longer half-life, and reduced side effect profile compared with older NNRTIs, such as efavirenz. It is treated with treatment of HIV-1 infection in conjunction with other antiretroviral [5, 6].

Literature indicates spectrophotometry [7–13], HPLC [14–17], HPTLC [18], and LC/MS/MS [19] methods for determination of TDF individually and in combination with other drugs in pharmaceutical formulations, drug substance, and biological matrices. Similarly for EMT individually and in combination with other drugs by UV [20, 21], HPLC in pharmaceutical formulations, drug substance and biological matrices [22–27], HPTLC, LC/MS/MS [28], and stability indicating liquid chromatographic methods [29] were reported. A detailed literature survey for RPV revealed that few analytical methods are available using spectrophotometric [30], HPLC [31], and HPTLC [32], individually. Literature are available to show the existence of HPLC method for the triple drug combination of TDF, EMT, and RPV as well [5, 6].

However, no spectrophotometric method for the triple drug combination of TDF, EMT, and RPV has yet been reported for simultaneous estimation of emtricitabine, tenofovir disoproxil fumarate, and rilpivirine HCl in tablet dosage forms. These methods mentioned in the literature, especially the chromatographic techniques, are time-consuming, costly, and require expertise. A simple and accurate UV spectrophotometric method developed can be highly useful for the routine analysis of tablet formulations. Hence, an attempt has been made to develop and validate in accordance with ICH guidelines [33].

2. Objective

The main objective of the present study is to develop simple, precise, accurate, and economical analytical method with a better detector range for simultaneous estimation of three-component tablet formulation by Vierordt’s method and to validate the above method as per the ICH guidelines.

3. Experimental

3.1. Apparatus. A double beam UV-visible spectrophotometer (Shimadzu, 1700), attached to a computer software UV probe 2.0, with a spectral width of 2 nm and pair of 1 cm matched quartz cell, was used.

3.2. Materials and Reagents. Authentic samples of emtricitabine (EMT) and tenofovir disoproxil fumarate (TDF) were kindly provided by Aurobindo Pharma Ltd. (Hyderabad,
India) while rilpivirine HCl (RPV) was kindly gifted from Strides arco Lab. (Bangalore, India). HPLC grade methanol (S.D fine chemical Ltd., Mumbai, India) was used throughout these experiments. Commercially available tablet dosage forms were assayed in the study Complera/Eviplera Gilead Sciences Inc., Canada, labeled to contain 200 mg EMT, 300 mg TDF, and 25 mg of RPV per tablet.

3.3. Study of Spectra and Selection of Wavelength. 10 μg/mL solution of all three drugs was scanned over the range of 200–400 nm in 1 cm cell against blank and the overlain spectra (Figure 2) were observed. While studying the overlay spectra it was observed that EMT shows maximum absorbance at 240.8 nm, TDF shows maximum absorbance at 257.6 nm, and RPV shows peaks at 305.6 nm, respectively. It was observed that there is no interference for each other at absorbance maxima and spectral characteristics are such that all three drugs can be simultaneously estimated by simultaneous equation method [34].

3.4. Standard Solution Preparations. The standard stock solution of EMT, TDF, and RPV was prepared by accurately weighed 20, 30 and 2.5 mg of each drug in 10 mL of volumetric flask separately with methanol. The standard stock solutions were further diluted to get the concentration of 8, 12, and 1 μg/mL of each.

3.5. Calibration Curve. A calibration curve was plotted over a concentration range of 4–12 μg/mL for EMT, 6–18 μg/mL for TDF, and 0.5–1.5 μg/mL for RPV individually. For each drug 6 replicates were made by individual weighing (Figure 3).

3.6. Simultaneous Equation Method. This method of analysis was based upon the absorption of drugs at wavelength maximum of each other. Three wavelengths of 240.8, 257.6, and 305.6 nm were selected which are the \( \lambda_{\text{max}} \) of three drugs for the development of the simultaneous equations [35, 36]. The absorbances of EMT, TDF, and RPV were measured and the absorptivity values were determined at all the three selected wavelengths. The concentrations of three drugs in mixture can be calculated using the following equations [37]:

\[
\begin{align*}
    C_{\text{EMT}} &= A_1 (a_y z_3 - a_z y_3) - a_y (A_2 a_z - a_z A_3) + a_z (A_2 a_y - a_y A_3) + a_x (a_y, a_z - a_z a_x) \\
    C_{\text{TDF}} &= a_x (A_2 a_z - a_z A_3) - A_1 (a_x a_z - a_z a_x) + a_z (a_x a_y - a_y a_z) + a_y (a_x a_z - a_z a_y) \\
    C_{\text{RPV}} &= a_x (a_y A_3 - A_3 a_y) - a_y (a_x A_3 - A_3 a_x) + A_1 (a_x a_y - a_y a_x) + a_y (a_x a_y - a_y a_z) + a_z (a_x a_y - a_y a_x)
\end{align*}
\]

where \( C_{\text{EMT}}, C_{\text{TDF}}, \) and \( C_{\text{RPV}} \) are the concentrations of EMT, TDF, and RPV, respectively, in mixture and in sample solutions.

\[
\begin{align*}
    A_1, A_2, \text{and } A_3 \text{ are the absorbances of sample at } &240.8, 257.6, \text{and } 305.6 \text{ nm, respectively,} \\
    a_x, a_y, \text{and } a_z \text{ are the absorptivity of EMT at } &240.8, 257.6, \text{and } 305.6 \text{ nm, respectively,} \\
    a_y, a_z, \text{and } a_x \text{ are the absorptivity of TDF at } &240.8, 257.6, \text{and } 305.6 \text{ nm respectively,} \\
    a_z, a_y, \text{and } a_x \text{ are the absorptivity of RPV at } &240.8, 257.6, \text{and } 305.6 \text{ nm respectively.}
\end{align*}
\]

The absorptivity of each solution was calculated by using the following formula [38]:

\[
\text{Absorptivity} = \frac{\text{Absorbance}}{\text{concentration (gm/100 mL)}} \quad (2)
\]

The developed method was validated as per ICH guidelines.

4. Results

4.1. Specificity. Specificity was studied by measuring the absorbance of EMT, TDF, and RPV individually at 240.8 nm, 257.6 nm, and 305.6 nm against the blank and comparing the absorbance of drugs solutions to the blank. No interference was observed.

4.2. Linearity. Linearity of the proposed method was determined by diluting the stock solution to give concentration range of 4–12 μg/mL for EMT, 6–18 μg/mL for TDF, and 0.5–1.5 μg/mL for RPV. The calibration curve was plotted between concentration verses absorbance (Tables 1, 2, and 3).
4.3. Accuracy. Accuracy was calculated as the percentage recoveries of blind samples of pure EMT, TDF, and RPV and it indicated the agreement between obtained results and those accepted as true, and detailed results are presented in Table 4. To ascertain the accuracy of the suggested methods, recovery studies were carried out by at three different levels (50%, 100%, and 150% level).

4.4. Precision. Intraday (within-day) and Interday (between-day) precision of the proposed methods were determined...
Table 3: Absorptivity value for RPV.

| Concentration | Absorbance $\lambda_1$—240.8 | Absorptivity $\lambda_1$—240.8 | Absorbance $\lambda_1$—257.6 | Absorptivity $\lambda_1$—257.6 | Absorbance $\lambda_1$—305.6 | Absorptivity $\lambda_1$—305.6 |
|---------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 0.5           | 0.021                         | 420.0                         | 0.024                         | 480.0                         | 0.062                         | 1240.0                       |
| 0.75          | 0.031                         | 413.3                         | 0.038                         | 506.6                         | 0.093                         | 1240.0                       |
| 1.0           | 0.041                         | 410.0                         | 0.049                         | 490.0                         | 0.121                         | 1210.0                       |
| 1.25          | 0.053                         | 424.0                         | 0.061                         | 488.0                         | 0.154                         | 1232.0                       |
| 1.50          | 0.062                         | 413.3                         | 0.072                         | 480.0                         | 0.185                         | 1233.3                       |

Absorptivity for $\lambda_1$ 416.1 Absorptivity for $\lambda_2$ 488.9 Absorptivity for $\lambda_3$ 1231.0

Table 4: Recovery studies for EMT, TDF, and RPV.

| Con (%) | Added amount (mg) | Amount recovered (mg) | Amount recovered (%) |
|---------|-------------------|-----------------------|----------------------|
|         | EMT               | TDF                   | RPV                   |
| 50      | 10                | 15                    | 2.5                  |
|         | EMT               | TDF                   | RPV                   |
| 75      | 15                | 22.5                  | 1.875                |
|         | EMT               | TDF                   | RPV                   |
| 100     | 20                | 30                    | 2.5                  |
|         | EMT               | TDF                   | RPV                   |
| 125     | 30                | 37.5                  | 3.125                |
|         | EMT               | TDF                   | RPV                   |
| 150     | 45                | 45                    | 3.75                 |

Table 5: Precision results for EMT, TDF, and RPV.

| Parameter | Sampling interval | EMT Amount present (mg) | EMT Amount present (%) | EMT %RSD | TDF Amount present (mg) | TDF Amount present (%) | TDF %RSD |
|-----------|-------------------|-------------------------|------------------------|----------|-------------------------|------------------------|----------|
|           | Within-day        |                         |                        |          |                         |                        |          |
|           | 0 hrs             | 0.1987                  | 99.35                  | 0.75     | 0.2991                  | 99.71                  | 0.90     |
|           | 8 hrs             | 0.2013                  | 100.68                 | 0.58     | 0.2970                  | 99.03                  | 0.54     |
|           | 16 hrs            | 0.1996                  | 99.84                  | 0.37     | 0.2967                  | 98.91                  | 0.32     |
|           | Between-day       |                         |                        |          |                         |                        |          |
|           | 1st day           | 0.1997                  | 99.85                  | 0.61     | 0.2966                  | 98.87                  | 0.74     |
|           | 2nd day           | 0.1996                  | 99.83                  | 0.78     | 0.2999                  | 99.98                  | 0.49     |
|           | 3rd day           | 0.1998                  | 99.90                  | 0.60     | 0.2955                  | 98.50                  | 0.36     |

Table 6: Ruggedness results for EMT, TDF, and RPV.

| Parameter | EMT Amount present (gm) | EMT Amount present (%) | EMT %RSD | TDF Amount present (gm) | TDF Amount present (%) | TDF %RSD | RPV Amount present (gm) | RPV Amount present (%) | RPV %RSD |
|-----------|-------------------------|------------------------|----------|-------------------------|------------------------|----------|-------------------------|------------------------|----------|
| Analyst 1 | 0.1995                  | 99.79                  | 0.65     | 0.2985                  | 99.50                  | 0.95     | 0.0251                  | 100.47                 | 0.74     |
| Analyst 2 | 0.2001                  | 100.05                 | 1.03     | 0.2996                  | 99.86                  | 1.28     | 0.0250                  | 100.25                 | 0.98     |
| Instrument 1 | 0.1999              | 99.67                  | 0.68     | 0.2981                  | 99.38                  | 0.79     | 0.0248                  | 99.33                  | 0.84     |
| Instrument 2 | 0.2000              | 100.04                 | 0.85     | 0.2989                  | 99.65                  | 0.81     | 0.0251                  | 100.58                 | 0.89     |
| Lab 1     | 0.2004                  | 100.20                 | 0.59     | 0.3000                  | 100.0                  | 0.58     | 0.0253                  | 101.24                 | 0.84     |
| Lab 2     | 0.1999                  | 99.97                  | 0.54     | 0.2977                  | 99.26                  | 0.84     | 0.0248                  | 99.38                  | 0.83     |

by estimating the EMT, TDF, and RPV three times on the same day to obtain repeatability and on three different days to obtain the reproducibility. The results are presented in Table 5.

4.5. Limits of Detection (LOD) and Quantitation (LOQ). They were calculated from the standard deviation (d) of the response and the slope of the calibration curve (S) in accordance with the following equations: LOD = 3.3 (d/S) and LOQ = 10 (d/S).

4.6. Ruggedness. A study was conducted to determine the effect of variation in analyst to analyst, lab to lab, and instrument to instrument in triplicate measurements as per the assay method. % RSD was calculated for each condition and results are presented in Table 6.
| Analyte | Wavelength (±nm) | Amount present (mg) | Amount present (%) | %RSD |
|---------|-----------------|--------------------|--------------------|------|
| EMT    | 239.8           | 0.2001             | 100.68             | 0.72 |
| TDF    | 256.6           | 0.2954             | 98.49              | 0.36 |
| RPV    | 304.6           | 0.0249             | 99.71              | 0.68 |

| DAY   | EMT Amount present (mg) | Amount present (%) | TDF Amount present (mg) | Amount present (%) | RPV Amount present (mg) | Amount present (%) |
|-------|-------------------------|--------------------|-------------------------|--------------------|-------------------------|--------------------|
| 1     | 0.2013                  | 100.66             | 0.3002                  | 100.07             | 0.0255                  | 102.11             |
| 2     | 0.2000                  | 100.00             | 0.2971                  | 99.06              | 0.0256                  | 102.41             |
| 3     | 0.1992                  | 99.64              | 0.2996                  | 99.88              | 0.0254                  | 101.68             |
| 4     | 0.1966                  | 98.34              | 0.3508                  | 101.95             | 0.0251                  | 100.49             |
| 5     | 0.2005                  | 100.03             | 0.2968                  | 99.06              | 0.0244                  | 102.41             |
| 6     | 0.1989                  | 99.64              | 0.2992                  | 99.88              | 0.0251                  | 101.68             |
| 7     | 0.1966                  | 98.34              | 0.3504                  | 101.95             | 0.0256                  | 100.49             |

| Amount present (mg) | Amount present (% label claim) | Amount present (mg) | Amount present (% label claim) | Amount present (mg) | Amount present (% label claim) |
|---------------------|-------------------------------|--------------------|-------------------------------|--------------------|-------------------------------|
| 0.2004              | 100.20                        | 0.2943             | 98.10                         | 0.0251             | 100.61                        |
| 0.2032              | 101.60                        | 0.2940             | 98.01                         | 0.0251             | 100.77                        |
| 0.2016              | 100.81                        | 0.2951             | 98.38                         | 0.0250             | 100.32                        |
| 0.1996              | 99.81                         | 0.2976             | 99.22                         | 0.0256             | 100.41                        |
| 0.1989              | 99.46                         | 0.2975             | 99.18                         | 0.0250             | 100.13                        |
| 0.2013              | 100.68                        | 0.2968             | 98.94                         | 0.0255             | 102.01                        |
| S.D                | 0.767508                      | S.D                | 0.543                         | S.D                | 0.9390                        |
| % RSD              | 0.764205                      | % RSD              | 0.550                         | % RSD              | 0.9293                        |

4.7. Robustness. As per ICH norms, small, but deliberate, variations by changing the wavelength in ±1 nm from 240.8 nm, 257.6 nm, and 305.6 nm and the results are presented in Table 7.

4.8. Stability. The stability of EMT, TDF, and RPV standard and sample working solutions in methanol during handling was verified by keeping them at room temperature for 0, 8, and 16 hrs. No significant degradation was observed. The stock solutions were also stable when kept refrigerated at 4°C for at least one week and the absorbance of sample solution in each day was measured. Results are presented in Table 8.

4.9. Preparation for Analysis of Tablet Formulation. Twenty tablets were weighed accurately, the average weight of each tablet was determined, and then they were ground to a fine powder. A powder quantity equivalent to 20 mg of EMT, 30 mg of TDF, and 2.5 mg of RPV was transferred to a 10 mL volumetric flask and sufficient methanol was added to dissolve it. Then the solutions were sonicated for 15 min. Then final volume was adjusted with methanol and filtered by Whatman filter paper (no. 41). The filtrate was centrifuged at 10,000 RPM for 30 min. Then clear supernatant solutions were transferred to a separate flask without disturbing the sediment. From the clear solution, transfer 0.4 mL of solution to 100 mL volumetric flask. Now the tablet sample solution was scanned in multiphotometric mode and the concentration of all three drugs was obtained from the equation. Results of tablet analysis are reported in Table 9.

5. Discussion

The proposed method was validated for precision, accuracy, specificity, linearity and range, limit of detection (LOD) and limit of quantitation (LOQ), robustness, and ruggedness. Validation of the proposed method was carried out in
accordance with the International Conference on Harmonization [33] guidelines. The linearity of the calibration plots was confirmed by the high value of the correlation coefficients ($r^2 = 0.9996$ for EMT, $0.9997$ for TDF, and $0.9994$ for RPV). Recovery was in the range of 98–102%; the values of standard deviation and % RSD were found to be <2% showing the high accuracy of the method. The limit of detection and limit of quantification were theoretically calculated which were found to be 0.1392 and 0.4220 for EMT, 0.226 and 0.685 for TDF, and 0.041 and 0.124 for RPV, respectively. Robustness and ruggedness were also carried out and percentage RSD was found to be less than 2.0%. The assay of EMT, TDF, and RPV was found to be 100.42%, 98.63%, and 100.70%. Stability of EMT, TDF, and RPV in methanol was found to be stable up to 7 days at room temperature.

6. Conclusion

The Vierordt’s method has been successfully applied for simultaneous determination of EMT, TDF, and RPV in combined sample solution, and they were found to be accurate, simple, rapid, and precise. Once the equations were constructed, analysis required only measuring the absorbance values of the sample solution at the selected wavelengths followed by few simple calculations. The proposed method was completely validated showing satisfactory data for all the method validation parameters tested. SE method comparably noted to be very efficient in every aspect. Unlike HPLC, by using Simultaneous equation method (UV) the data can be generated applying simple calculations. So these methods can be easily and conveniently adopted for routine quality control analysis of these cited drugs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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