Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Bictegravir, Emtricitabine, and Tenofovir Alafenamide Fumarate

Bictegravir, Emtricitabine and Tenofovir Alafenamide Fumarate’s Simultaneous Estimation for the Development of Stability-Indicating RP-HPLC Method

ABSTRACT

Objectives: The focal intent of the current research work is to develop and validate a novel and reliable stability-indicating reverse-phase high performance liquid chromatographic method for the simultaneous estimation of a few anti-retrovirals, i.e., bictegravir, emtricitabine, and tenofovir alafenamide fumarate (AF).

Materials and Methods: The novel method employs inertsil octyldecysilyl C18 (4.6×250 mm, 5 mm) using 0.2% triethylamine buffer and methanol in a ratio of 40:60% (v/v) as the mobile phase to attain optimal elution. The detection wavelength was 260 nm with a 1.2 mL/min flow rate and a 20 μL injection volume.

Results: The linearity ranges for bictegravir, emtricitabine and tenofovir AF were 25-125 μg/mL, 100-500 μg/mL, and 12.5-62.5 μg/mL, respectively. The retention times for bictegravir, emtricitabine, and tenofovir AF were found to be 5.998 min, 2.805 min, and 4.537, min respectively. The percent recoveries of bictegravir, emtricitabine, and tenofovir AF were within the range of 98-102% w/w.

Conclusion: The novel method was successfully validated as per International Conference on Harmonization guidelines. In forced degradation studies, emtricitabine was found to be sensitive to thermal conditions; bictegravir and tenofovir AF, to oxidative conditions. The developed method is economical and reliable for routine analysis concerning all validated parameters.

Key words: Bictegravir, emtricitabine, tenofovir AF, RP-HPLC, validation, forced degradation studies

ÖZ

Amaç: Mevcut araştırmaların odak amacı, birkaç anti-retroviralın [bictegravir, emtrisitabin ve tenofovir alafenamid fumarat (AF)] eş zamanlı tahmini için yeni ve güvendi bir stabilite gösteren ters fazlı yüksek performanslı sıvı kromatografik yöntemi geliştirmek ve doğrulamaktır.

Gereç ve Yöntemler: Yeni yöntemi, optimal elüsiyon ulaşmak için mobil faz olarak %0,2 trietiylamin tamponu ve %40:60 (h/h) oranında metanol kullanılarak inertsil octyldecisil C18 (4,6×250 mm, 5 mm) kullanmaktadır. Deteksiyon dalga boyu 260 nm, akış hızı 1,2 mL/dk ve enjeksiyon hacmi 20 μL idi.

Bulgular: Bictegravir, emtrisitabin ve tenofovir AF’nin doğrusallık rançı sırasıyla 25-125 μg/mL, 100-500 μg/mL ve 12,5-62,5 μg/mL idi. Bictegravir, emtrisitabin ve tenofovir AF’nin retansiyon zamanları sırasıyla 5,998 dk, 2,805 dk ve 4,537 idi. Bictegravir, emtrisitabin ve tenofovir AF’nin yüzde gerilsekanımız %98-102 a/a aralığındadır.


Anahtar kelimeler: Bictegravir, emtrisitabin, tenofovir AF, RP-HPLC, validasyon, zorla bozunma çalışmaları

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Received: 26.04.2020, Accepted: 08.09.2020
INTRODUCTION

Human immunodeficiency virus (HIV) is a fatal viral infection that targets and alters the immune system, increasing the risk and impact of other infections and diseases. If left untreated, the infection might progress to an advanced disease stage called acquired immunodeficiency syndrome (AIDS). With the use of multiple specialized anti-retroviral medications that are commercially available, the state of HIV/AIDS infection can be controlled, de-escalated, and treated. Among numerous anti-retroviral formulations and combinations available, bictegravir is an oral tablet that contains three anti-retroviral drugs [bictegravir + emtricitabine + tenofovir alafenamide fumarate (AF)] under the brand name “BIKTARVY”. Bictegravir belongs to the class of HIV-1 integrase strand transfer inhibitors (INSTIs); emtricitabine and tenofovir alafenamide, to the class of HIV-1 nucleoside analog reverse transcriptase inhibitors. Hence “BIKTARVY” can be considered as the sole regimen for HIV-1 (type 1) infected patients.\(^1\-^3\) The INSTIs comprise two nucleoside reverse transcriptase inhibitors and recommended components during the initial stages of anti-retroviral therapy. Bictegravir is an effective INSTI with a high in vitro barrier that shows strong resistance toward the clinically relevant drug-drug interactions and possesses specific activity against HIV-1 and HIV-2. Bictegravir is metabolized by cytochrome P450 3A4 and a uridine diphosphate glucuronosyl transferase 1A1. It binds to the active site of HIV integrase and prevents HIV replication. Compared with other INSTIs, bictegravir possesses a high barrier to in vitro resistance and a lower potential to drug interactions among other readily available anti-retrovirals.\(^4\-^5\) Emtricitabine and tenofovir AF act on DNA synthesis via HIV reverse transcriptase, resulting in viral DNA chain termination and preventing the replication of HIV.\(^6\-^7\) The US Food and Drug Administration has approved “bictegravir” as a fixed-dose regimen (once daily) to treat HIV-1 infection.\(^8\-^9\) The chemical structures of the three active pharmaceutical ingredients are shown in Figure 1-3. The dosage regimen is as follows:

Bictegravir (50 mg) + emtricitabine (20 mg) + tenofovir AF (25 mg).

According to the “Department of Health and Human Services”.\(^10\-^11\) the current combination regimen is intended to treat HIV-1 patients. Biktarvy can be administered with or without food and is not recommended with other anti-retrovirals.\(^12\) A literature survey was performed, and very few stability-indicating reverse-phase high performance liquid chromatographic (HPLC) isocratic elution methods to estimate the drugs of interest are reported.

MATERIALS AND METHODS

Experiment

Collection of drugs

Bictegravir of purity 99% w/w, emtricitabine of purity 99% w/w, and tenofovir AF of purity 99% w/w were procured from Hetero Labs, Hyderabad.

Chemicals and reagents

HPLC grade methanol (Rankem), Milli-Q grade water for HPLC (Merck), and HPLC grade triethylamine (Fine Chem Industries Research Laboratory) were used.

Apparatus

The HPLC WATERS system (2695 separation module 7 auto sampler) used in this method was equipped with a photodiode array (PDA) detector. Empower chromatography software (EMPOWER-2) was used for liquid chromatogram peak integration. Empower-2 software was used in data acquisition and processing.
a- The inertsil octadecylsilica (ODS) C\textsubscript{18} (4.6×250 mm, 5 μm) column was found to be ideal for analyzing the selected drugs.
b- A rheodyne injector (20 μL loop) was used to inject the samples.
c- A ultraviolet (UV)-visible spectrophotometer (LABINDIA UV 300\textsuperscript{+}) with UV Win software was used to establish the analytical wavelength.
d- Other instruments included an afcoset ER-200A electronic weighing balance, micropipettes, pipettes, burettes, micro-pore filtration assembly, ultra-sonic water bath for sonication of the mobile phase, and pH meter (Adwa-AD 1020).

**Optimized chromatographic conditions**

Once several trials had been conducted for optimization, the appropriate conditions were selected for the study, the details of which are as follows:

- **Instrument:** HPLC (waters) with auto sampler
- **Detector:** PDA detector
- **Temperature:** Ambient
- **Column:** ODS C\textsubscript{18} (4.6×250 mm, 5 μm)
- **Mobile phase:** 0.2% triethyl amine (TEA), buffer: Methanol (40:60 v/v)
- **Flow rate:** 1.2 mL/min
- **Run time:** 15 min
- **Wavelength:** 260 nanometers (nm)

**Preparation of 0.2% TEA buffer solution**

TEA (2 mL) was measured accurately by pipetting into 1000 mL HPLC grade water and dissolved. The pH was adjusted to 3.5 with dilute formic acid.

**Preparation of the mobile phase**

Four hundred milliliters (40%) of the above-prepared buffer and 600 mL (60%) of methanol were measured accurately and mixed well.

**Standard and sample preparation (emtricitabine, tenofovir AF, and bictegravir)**

**Standard preparation**

Emtricitabine (100 mg), tenofovir AF (12.5 mg), and bictegravir (25 mg) working standards were weighed into a volumetric flask and added to 100 mL of diluent to makeup the volume. From the prepared stock solution, 3 mL was diluted to 10 mL. The resulting solution contained each of 300 ppm of emtricitabine, 37.5 ppm, of tenofovir AF, and 75 ppm of bictegravir.

**Sample preparation**

Ten tablets [prepared in-house by weighing the quantities as stated in the marketed formulation of emtricitabine (200 mg), tenofovir AF (25 mg), and bictegravir (50 mg)] were weighed accurately, and quantities equal to emtricitabine (100 mg), tenofovir AF (12.5 mg), and bictegravir (25 mg) samples were diluted to 100 mL. Three milliliters of each stock solution was diluted to 10 mL containing 300 ppm of emtricitabine, 37.5 ppm, of tenofovir AF, and 75 ppm of bictegravir.

**Procedure**

The % assay was estimated from the obtained peak areas of standard and sample using the formula:

\[
\text{% Assay} = \frac{\overline{AT}}{\overline{AS}} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{Average \ weight}{Label \ Claim} \times P \times \frac{1}{100}
\]

Where;

- AT: Average area counts of test (sample) preparation.
- AS: Average area counts of standard preparation.
- WS: Weight of working standard taken in mg.
- DS: Dilution of working standard in mL.
- DT: Dilution of test (sample) in mL.
- WT: Weight of test (sample) taken in mg.
- P: Percentage purity of working standard.
**Method validation**

The analytical method validation for the developed method was implemented to ensure that the method meet the intended requirements as stated in the respective guidelines. The results obtained for the method validation can be considered to determine the reliability and consistency of the developed method. The proposed method was validated according to the ICH guidelines with respect to the following parameters.

Calibration curves were obtained at concentrations of 25-125 μg/mL for bictegravir, 100-500 μg/mL for Emtricitabine, and 12.5-62.5 μg/mL for tenofovir AF.

**Linearity**

Linearity can be illustrated by examining different concentrations of active pharmaceutical ingredients. The linearity of a method can be evaluated from the calibration plots of bictegravir, emtricitabine, and tenofovir AF constructed from peak response vs. concentration, which approaches a straight line.

Emtricitabine (100 mg), tenofovir AF (12.5 mg), and bictegravir (25 mg) were diluted to 100 mL. From this stock solution, 1-5 mL was pipetted into five different 10 mL volumetric flasks, and a series of aliquots was prepared and analyzed.

**Accuracy**

Accuracy was illustrated from the % recovery of standard containing known concentrations of active pharmaceutical ingredients.

Emtricitabine (100 mg), tenofovir AF (12.5 mg), and bictegravir (25 mg) working standards were diluted to 100 mL.

Three milliliters of the resulting stock solution was diluted to 10 mL. This solution thus contained emtricitabine (300 ppm), tenofovir AF (37.5 ppm), and bictegravir (75 ppm). The standard solutions for accuracy determination, 50%, 100%, and 150%, were prepared and injected, and the recovery values for emtricitabine, tenofovir AF, and bictegravir were calculated.

**Precision**

Precision was evaluated on the basis of the closeness between the obtained results.

Emtricitabine (100 mg), tenofovir AF (12.5 mg), and bictegravir (25 mg) working standards were diluted to 100 mL. Three milliliters of this stock solution was diluted to 10 mL.

**Specificity**

Specificity can be illustrated by ensuring that the peaks are free from interference.

It is determined by injecting a blank and a standard into the chromatographic system and corroborating that no interference exists.

**Detection limit (DL) and quantification limit (QL)**

DL and QL values deal with the method’s sensitivity. DL is the analyte’s lowest detectable concentration, while QL is the lowest quantifiable concentration.

**RESULTS AND DISCUSSION**

**Optimization of the method**

For the selection of a suitable mobile phase for simultaneous estimation of the selected drugs, various solvents such as water, ACN, TEA, and methanol varying in polarity were used in different combinations of concentrations to obtain high peak resolutions within a shorter runtime. Among all the different
mobile phase combinations employed, the mobile phase comprising 0.2% TEA buffer and methanol in the ratio of 40:60 v/v exhibited well-defined peaks.

Different flow rates from 0.5 to 1.2 mL/min have been studied to achieve a good peak resolution. Among all the flow rates employed, 1.2 mL/min was selected as optimal for the study. The column temperature was set at 25, 30, and 35°C for optimization, according to its effect on peak resolutions and RT of the drug samples.

During the method optimization, the selected combinations of three drugs were analyzed using different columns, the column [ODS C\(_{18}\) (4.6×250 mm, 5 \(\mu\)m)] that exhibited good peak shape and resolution was selected for current study. The details are specified in Table 2.

Also, based on the UV-absorption spectra of the three drugs scanned over the range of 200-400 nm, the wavelength of 260 nm was selected as the ideal wavelength for the study.

**System suitability**

According to the optimized experimental conditions, the retention times obtained for bictegravir, emtricitabine, and tenofovir AF are 5.998 min, 2.805 min, and 4.537 min. The optimized chromatogram with tailing factor (<2), theoretical plates (>2000), resolution (>2), capacitance factor (>1) is shown in (Figure 4). Hence, the proposed method proved “selective” to determine the drugs (bictegravir, emtricitabine, and tenofovir AF). The system suitability results of the standard injections are tabulated in Table 3.

**Assay of marketed formulation**

The assay results obtained for the three drugs (bictegravir, emtricitabine, and tenofovir AF) are detailed in Table 4. No interference of the excipients was noticed in the current method; hence, the method is “specific”. The typical chromatogram for assay of the commercial formulation (in-house preparation) is shown in Figure 5.

**Linearity**

To construct the calibration curve, different concentration ranges of bictegravir (25-125 \(\mu\)g/mL), emtricitabine (100-500 \(\mu\)g/mL), and tenofovir AF (12.5-62.5 \(\mu\)g/mL) were considered. The correlation coefficient \((r^2)\) values obtained were found satisfactory. The results obtained are summarized in Table 5. The calibration plots of three drugs are as shown in (Figure 6-8).

### Accuracy

Accuracy was determined at 50%, 100%, and 150% of the test concentrations by calculating the individual recovery and mean recovery values of emtricitabine, tenofovir AF, and bictegravir. The recoveries ranged from 99.26% to 100.30% for bictegravir, 99.06% to 100.79% for emtricitabine, and 99.66% to 100.06% for tenofovir AF. The recovery values obtained were found to meet the acceptance criteria (not less than 98.0% and not more than 102.0%). The RSD values obtained were <2 with respect to three drugs. The accuracy results are outlined in Table 6.

### Precision

The precision for the developed method is estimated as follows:

- System precision
- Intermediate precision
- Method precision

**System precision**

The % RSD of six standard injection areas were found to be less than 2% (acceptance criteria: Not more than 2%), hence the method is “precise”. The results for emtricitabine, tenofovir AF, and bictegravir are summarized in Table 7.

**Intermediate precision/ruggedness**

No significant effect was observed in the recoveries, the peak area responses of all the three drugs, thus indicating that the proposed and developed method is rugged.

The results are summarized for emtricitabine, tenofovir AF, and bictegravir in Table 8.

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**Table 2. Comparison of optimum conditions**

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Column used</th>
<th>Specification</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hypersil</td>
<td>5.0×250 mm, 10 (\mu)m</td>
<td>Deformed peak shape was observed</td>
</tr>
<tr>
<td>2</td>
<td>Lichrosorb</td>
<td>4.6×250 mm, 5 (\mu)m</td>
<td>Low resolution observed</td>
</tr>
<tr>
<td>3</td>
<td>Inertsil (\text{ODS C}_{18})</td>
<td>4.6×250 mm, 5 (\mu)m</td>
<td>Peak shape is sharp and free from Tailing with high resolution</td>
</tr>
</tbody>
</table>

ODS: Octyldecsylsilyl
Method precision
To evaluate the method precision, the % assay was calculated from six individual samples solutions analyzed on same day. The % RSD obtained with respect to the results of the method precision met the acceptance criteria (not more than 2%), and the details of peak areas and % RSD values are summarized in Table 9.

Robustness
Robustness is defined as how the method can resist (less impact) small and deliberate changes in analytical procedure parameters such as the flow rate (±10%) and the organic phase composition (±10%). Minor changes did not affect the peak area responses of the method significantly; hence, the proposed method is robust.

The flow rate (1.08 mL/min and 1.32 mL/min) and organic phase composition (lesser to more organic) were altered, and there was no significant variation in the results obtained when deliberate changes were made to the developed method. The results obtained for the parameter robustness are summarized in Table 10-15.
DL and QL
DL and QL values were estimated using the formulas:

\[ DL = 3.3 \times \left( \frac{\sigma}{S} \right) \]
\[ QL = 10 \times \left( \frac{\sigma}{S} \right) \]

where:
\( \sigma \) = standard deviation;
\( S \) = slope.

The DL values for bictegravir, emtricitabine, and tenofovir AF obtained were 2.7, 1.05, and 1.35 \( \mu \)g/mL, with signal to noise ratio of 3:1, and the QL values for bictegravir, emtricitabine, and tenofovir AF obtained were 8.78, 3.30, and 4.61 \( \mu \)g/mL, with a signal to noise ratio of 10:1, which indicates that the “sensitivity” of the method is adequate. The results are summarized in Table 16.

Hydrolytic degradation under acidic conditions
To 3.0 mL of the stock solution, 3 mL of 1N HCl was added, diluted to 10 mL, and incubated at 60°C for 6 hours. The resulting solution was neutralized with 1N NaOH and adjusted to the mark with the diluent. There was no remarkable acid degradation with respect to the subject drugs, and the chromatogram is shown in Figure 9.

Hydrolytic degradation under alkaline conditions
To 3.0 mL of the stock solution, 1N NaOH (3 mL) was added, diluted to 10 mL, and incubated at 60°C for 6 hours. Later, the solution was neutralized with 1N HCl. There was no significant degradation with respect to the three drugs, and the chromatogram obtained for alkali degradation is shown in Figure 10.

Thermal-induced degradation
The subject samples were placed separately in Petri dishes and remained in an oven at 110°C for a period of 24 hours. There was a minimal effect of thermal degradation.

Table 7. Results of system precision

<table>
<thead>
<tr>
<th>Injection</th>
<th>Emtricitabine</th>
<th>Tenofovir AF</th>
<th>Bictegravir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection-1</td>
<td>4,74,652</td>
<td>50,304</td>
<td>97,274</td>
</tr>
<tr>
<td>Injection-2</td>
<td>4,70,806</td>
<td>50,532</td>
<td>96,658</td>
</tr>
<tr>
<td>Injection-3</td>
<td>4,79,900</td>
<td>50,680</td>
<td>97,574</td>
</tr>
<tr>
<td>Injection-4</td>
<td>4,73,621</td>
<td>50,727</td>
<td>97,021</td>
</tr>
<tr>
<td>Injection-5</td>
<td>4,75,167</td>
<td>50,255</td>
<td>98,232</td>
</tr>
<tr>
<td>Injection-6</td>
<td>4,76,538</td>
<td>50,235</td>
<td>97,987</td>
</tr>
<tr>
<td>Average</td>
<td>4,75,114.0</td>
<td>50,455.5</td>
<td>97,457.7</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>3,031.1</td>
<td>219.9</td>
<td>592.8</td>
</tr>
<tr>
<td>% RSD (n=6)</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 8. Results of intermediate precision/ruggedness

<table>
<thead>
<tr>
<th>Injection</th>
<th>Emtricitabine</th>
<th>Tenofovir AF</th>
<th>Bictegravir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection-1</td>
<td>4,77,752</td>
<td>49,821</td>
<td>97,234</td>
</tr>
<tr>
<td>Injection-2</td>
<td>4,74,159</td>
<td>50,388</td>
<td>96,991</td>
</tr>
<tr>
<td>Injection-3</td>
<td>4,69,272</td>
<td>50,289</td>
<td>95,433</td>
</tr>
<tr>
<td>Injection-4</td>
<td>4,69,317</td>
<td>50,176</td>
<td>96,414</td>
</tr>
<tr>
<td>Injection-5</td>
<td>4,77,171</td>
<td>50,337</td>
<td>97,491</td>
</tr>
<tr>
<td>Injection-6</td>
<td>4,73,102</td>
<td>50,073</td>
<td>97,166</td>
</tr>
<tr>
<td>Average</td>
<td>4,73,462.2</td>
<td>50,180.7</td>
<td>96,788.2</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>3,674.6</td>
<td>209.8</td>
<td>755.4</td>
</tr>
<tr>
<td>% RSD (n=6)</td>
<td>0.8</td>
<td>0.4</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 9. Results for method precision

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample weight (mg)</th>
<th>Emtricitabine</th>
<th>Tenofovir AF</th>
<th>Bictegravir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method precision-1</td>
<td>174.2</td>
<td>4,75,652</td>
<td>50,166</td>
<td>97,455</td>
</tr>
<tr>
<td>Method precision-2</td>
<td>174.5</td>
<td>4,76,888</td>
<td>50,425</td>
<td>97,563</td>
</tr>
<tr>
<td>Method precision-3</td>
<td>174.1</td>
<td>4,75,988</td>
<td>50,253</td>
<td>97,234</td>
</tr>
<tr>
<td>Method precision-4</td>
<td>174.3</td>
<td>4,75,377</td>
<td>50,497</td>
<td>97,331</td>
</tr>
<tr>
<td>Method precision-5</td>
<td>174.2</td>
<td>4,76,765</td>
<td>50,556</td>
<td>97,548</td>
</tr>
<tr>
<td>Method precision-6</td>
<td>174.3</td>
<td>4,76,653</td>
<td>50,335</td>
<td>97,397</td>
</tr>
<tr>
<td>Average</td>
<td>-</td>
<td>4,76,220.5</td>
<td>50,372.0</td>
<td>97,421.3</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>-</td>
<td>635.3</td>
<td>148.5</td>
<td>127.4</td>
</tr>
<tr>
<td>% RSD (n=6)</td>
<td>-</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

n: Number of determinations, RSD: Relative standard deviation, AF: Alafenamide
with respect to the drug emtricitabine and no significant effect with respect to bictegravir and tenofovir AF. The chromatogram obtained for thermal degradation is shown in (Figure 11).

**Oxidative degradation**

To the above stock solution, 3 mL of 3% (w/v) hydrogen peroxide (1 mL) was added in a 10 mL, and the flask was retained at ambient temperature for 12 minutes. There was a minimal

| Table 10. System suitability results for emtricitabine at a flow rate variation of ±10% |
|----|-----|-----|
| S. no. | Flow rate (mL/min) | System suitability results |
| | | USP tailing (T<sub>f</sub>) | USP plate count (N) |
| 1 | 1.08 | 1.37 | 2371.09 |
| 2 | 1.2 | 1.30 | 2185.90 |
| 3 | 1.32 | 1.31 | 2231.87 |

T<sub>f</sub>: Tailing factor

| Table 11. System suitability results for tenofovir AF at a flow rate variation of ±10% |
|----|-----|-----|
| S. no. | Flow rate (mL/min) | System suitability results |
| | | USP resolution (R) | USP tailing (T<sub>f</sub>) | USP plate count (N) |
| 1 | 1.08 | 6.32 | 1.25 | 3223.82 |
| 2 | 1.2 | 6.05 | 1.13 | 2973.76 |
| 3 | 1.32 | 6.07 | 1.06 | 2863.39 |

AF: Alafenamide, T<sub>f</sub>: Tailing factor

| Table 12. System suitability results for bictegravir at a flow rate variation of ±10% |
|----|-----|-----|
| S. no. | Flow rate (mL/min) | System suitability results |
| | | USP resolution (R) | USP tailing (T<sub>f</sub>) | USP plate count (N) |
| 1 | 1.08 | 3.28 | 1.31 | 2143.54 |
| 2 | 1.2 | 3.14 | 1.33 | 2214.41 |
| 3 | 1.32 | 3.20 | 1.40 | 2183.37 |

T<sub>f</sub>: Tailing factor

Effect of thermal degradation on bictegravir and tenofovir AF and no significant effect noticed with respect to emtricitabine. The chromatogram obtained for the oxidative degradation is shown in (Figure 12).

**Photo degradation**

The sample solution was exposed to external sunlight. No significant degradation was noticed with respect to the subject drugs, and the chromatogram obtained for the photolytic degradation is shown in (Figure 13).

A stability study was conducted for the drugs emtricitabine, tenofovir AF, and bictegravir under the respective stress conditions. The peak areas obtained, the % assay calculated,
The newly developed method affirms good resolution between the three drugs bictegravir, emtricitabine, and tenofovir AF. The current method, method validation, and stability studies were found to be in line with the ICH guidelines and with official methods. The method requires no core extraction techniques; moreover, economical solvents are employed for the analysis, and good resolution is attained. No interference from any pharmaceutical dosage form or any remarkable impurities of degraded substance(s) was observed. Since the subject drugs of interest were analyzed by employing less expensive solvents and obtaining high resolution and shorter retention times with respect to the current method, the new proposed method is recommended for routine quality control analysis to provide simple, reliable, economical, and reproducible quantitative analysis for simultaneous estimation of the selected anti-retroviral fixed-dose regimen (bictegravir, emtricitabine, and tenofovir AF).
ACKNOWLEDGMENTS

Authors extend intense gratitude to M/S Pharma Train Labs, Hyderabad for providing the required research facilities and to Hetero Labs, Hyderabad for providing gift samples of bictegravir, emtricitabine and tenofovir AF.

Conflict of interest: No conflict of interest was declared by the authors. The authors are solely responsible for the content and writing of this paper.

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