



Validated stability indicating HPLC method to quantify *lamivudine* and *dolutegravir* in tablets

Rama Kumar Kandula*, Raja Sundararajan

Institute of Pharmacy, GITAM Deemed to be University, Visakhapatnam-530045, Andhra Pradesh, India



Article History:

Received on: 24 Apr 2020
Revised on: 24 May 2020
Accepted on: 26 May 2020

Keywords:

Acquired immunodeficiency syndrome, human immunodeficiency virus, Dovato tablets, Lamivudine, Dolutegravir, Analysis

ABSTRACT

Dovato tablets (lamivudine and dolutegravir combination) are full therapy regimen for the Type 1 human immunodeficiency virus (HIV-1) infection in adults without history of antiretroviral therapy. The aim of present research focused on development of validated stability indicating method for the quantification of lamivudine and dolutegravir in combined dosage form. The separation was achieved on a Cosmicsil C18 column. The mobile phase consisted of 0.1% orthophosphoric acid (pH 3.5) – acetonitrile (50:50, v/v). Photodiode array detector was used to detect the analytes at 258 nm. The method performance was validated in compliance with the recommendations of the International Conference on Harmonization. The method was validated with selectivity, limit of detection (0.262 $\mu\text{g/ml}$ for lamivudine and 0.238 $\mu\text{g/ml}$ for dolutegravir), linearity (150-450 $\mu\text{g/ml}$ for lamivudine and 25-75 $\mu\text{g/ml}$ for dolutegravir), limit of quantification (0.874 $\mu\text{g/ml}$ for lamivudine and 0.793 $\mu\text{g/ml}$ for dolutegravir), accuracy (percent recovery was nearer to 100%), precision (percent relative standard deviation was less than 2.0%) and robustness (system suitability values are within limits). The stability indicating method was performed by under the various stress conditions. Degradants did not interfere with lamivudine and dolutegravir detection. The developed method can suggest that quantification of lamivudine and dolutegravir in quality control of analytical laboratories.

*Corresponding Author

Name: Rama Kumar Kandula
Phone: 9885995823
Email: ramakumarkandula@gmail.com

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i4.3105>

Production and Hosted by

IJRPS | www.ijrps.com

© 2020 | All rights reserved.

INTRODUCTION

Acquired immunodeficiency syndrome is a human immunodeficiency virus (HIV)-induced medical condition. Presently, HIV infection is very danger

and can readily be called a curse on mankind (Sauter and Kirchhoff, 2019). Shortly after the official recognition of HIV patients in the USA, HIV-1 was recognized as the causative organism (Sharp and Hahn, 2011). In Africa, HIV-2 was first recorded in 1985 and is significantly distinct from HIV-1 (Sharp and Hahn, 2010). In developing nations, mortality and morbidity rates associated with HIV infections were high.

In April 2019, the U.S. Food and Drug Administration endorsed Dovato tablets as a full therapy regimen for Type 1 human immunodeficiency virus (HIV-1) infection in adults without history of antiretroviral therapy (U.S. Food and Drug Administration, 2019). Davato tablets were labeling with to300 mg of lamivudine (LMD) and 50 mg of dolutegravir (DTG). DTG is an integrase inhibitor group of human immunodeficiency viral drug (Min et al.,

2010), (Dow and Bartlett, 2014). Integrase inhibitor blocks an enzyme called integrase present in human immunodeficiency virus and stop multiplication of human immunodeficiency virus (Snedecor *et al.*, 2019). It operates by lowering blood levels of human immunodeficiency virus and hepatitis B (Quercia *et al.*, 2018). LMD does not cure HIV, but the possibility of developing acquired immunodeficiency syndrome and human immunodeficiency virus related diseases such as severe infections or cancer may be reduced (Budamakuntla *et al.*, 2014).

From the literature reveals that few HPLC methods (Devanna *et al.*, 2017), (Dhanwate *et al.*, 2019) and only one HPTLC (Dhanve *et al.*, 2018) method was developed for the analysis of pharmaceutical formulations containing LMD and DTG. There is no stability indicating HPLC method was found for the quantification of LMD and DTG simultaneously. The International Conference on Harmonization guideline says that forced degradation study was designed to recognize the probable degradation products that further assist in determining the molecule's inherent stability and developing pathways for the degradation (Mistry and Mishra, 2018). Based on the importance of stress testing, a stability indicating method using HPLC technique was developed and validated to quantify LMD and DTG simultaneously in bulk and marketed formulation.

MATERIALS AND METHODS

Standards and tablet dosage forms of LMD and DTG

Standards of LMD and DTG were obtained from Rainbow Pharma Training Labs, Hyderabad, India. Dovato tablets (ViiV Healthcare, US) labeled with 300 mg of LMD and 50 mg of DTG were bought from the local pharmacy market.

Chemicals and solvents

Analytical grade chemicals and HPLC grade solvents have been used. Hydrochloric acid, peroxide, sodium hydroxide and orthophosphoric acid were purchased from Sd Fine Chemicals Ltd., Mumbai, India. Methanol and water are from Merck India Ltd (Mumbai, India) and Milli-Q[®] system (Millipore, USA), respectively.

Instrumentation and conditions

Liquid chromatographic simultaneous assay of LMD and DTG were performed with a Waters alliance liquid chromatography system equipped with an autosampler and Waters photodiode array detector. Analysis of chromatography data was done using the Empower2 version software. Chromatography separations of LMD and DTG were performed in a

Cosmicsil C18 column (5 μm , 4.6 \times 250 mm) with using 50% of 0.1% phosphoric acid (pH 3.5) and 50% of acetonitrile as components of mobile phase. The flow rate was fixed at 1.0 ml/min, the volume of injection was 10 μl , the analytes (LMD and DTG) were monitored at 258 nm, and analysis was completed to 6 min.

Standard solutions of LMD and DTG

LMD (3000 $\mu\text{g/ml}$) and DTG (500 $\mu\text{g/ml}$) standard solution was prepared with mobile phase by using 25 ml standard flask. To construct calibration graphs, LMD and DTG calibration solutions in the range of 150-450 $\mu\text{g/ml}$ and 25-75 $\mu\text{g/ml}$ concentration, respectively were individually prepared in a set of 10 ml standard flasks using the stock solution (LMD 3000 $\mu\text{g/ml}$ and DTG 500 $\mu\text{g/ml}$). To validate the method, 300 $\mu\text{g/ml}$ of LMD and 50 $\mu\text{g/ml}$ of DTG working standards were prepared from the standard stock solution.

LMD and DTG calibration graphs

10 μl of each standard dilution was injected to the HPLC system under specified chromatographic conditions. To plot the calibration curves were peak area Vs concentration and then calculate the correlation coefficient.

Assay of LMD and DTG in tablets

Ten tablets were triturate to the fine powder. Appropriately weighed tablet powder was equivalent to 300 mg of LMD and 50 mg of DTG was transferred into a 100 ml volumetric flask, and dissolved in mobile phase, the solution was make up with diluent up to the mark. The solution was filtered via a 0.45 μm size membrane filter. Further respected dilutions were prepared from the above standard solution. Chromatography development was performed using conditions indicated in the "Instrumentation and Conditions" section. The peak areas of LMD and DTG were calculated and then used to analyze the content of LMD and DTG in the samples using the earlier computed calibration graphs or regression equations.

Stress testing

As indicated by International Conference on Harmonization guideline, stress test includes studying the susceptibility of LMD and DTG (Thota *et al.*, 2018), (Ravichandran *et al.*, 2010) standard solutions were placed under various stress conditions such as acid, base, peroxide, thermal and photolytic conditions.

Stress test was carried out by adding 10 ml of reagent to 10 ml of tablet sample (3000 $\mu\text{g/ml}$ for LMD and 500 $\mu\text{g/ml}$ for DTG). 0.1 N sodium

Table 1: Linearity, regression and limits data for LMD and DTG

| Parameter | LMD | DTG |
|--|---------|--------|
| Linearity ($\mu\text{g/ml}$) | 150-450 | 25-75 |
| Regression coefficient (R ²) | 0.9998 | 0.9996 |
| Intercept (c) | -2566 | -1374 |
| Slope (m) | 55323 | 15438 |
| LQ ($\mu\text{g/ml}$) | 0.874 | 0.793 |
| LD ($\mu\text{g/ml}$) | 0.262 | 0.238 |

Table 2: Recovery values of LMD and DTG

| Spiked ($\mu\text{g/ml}$) | LMD | | Spiked ($\mu\text{g/ml}$) | DTG | |
|--|----------------------------|--------------|-----------------------------|----------------------------|--------------|
| | Found ($\mu\text{g/ml}$) | Recovery (%) | | Found ($\mu\text{g/ml}$) | Recovery (%) |
| 50% spiked concentration level | | | | | |
| 150 | 148.37 | 98.91 | 25 | 25.04 | 100.14 |
| 150 | 149.03 | 99.35 | 25 | 25.06 | 100.25 |
| 150 | 147.59 | 98.39 | 25 | 25.12 | 100.46 |
| 100% spiked concentration level | | | | | |
| 300 | 299.04 | 99.68 | 50 | 49.23 | 98.46 |
| 300 | 299.76 | 99.92 | 50 | 49.74 | 99.47 |
| 300 | 300.33 | 100.11 | 50 | 49.58 | 99.15 |
| 150% spiked concentration level | | | | | |
| 450 | 449.46 | 99.88 | 75 | 74.61 | 99.48 |
| 450 | 449.10 | 99.8 | 75 | 75.13 | 100.17 |
| 450 | 448.88 | 99.75 | 75 | 74.81 | 99.74 |

* values for three determinations

Table 3: Robustness values of LMD and DTG

| Parameter | Plate count (N) | | Tailing factor (Tf) | | Resolution (R _s) | |
|-------------------------|-----------------|------|---------------------|------|------------------------------|-------|
| | LMD | DTG | LMD | DTG | LMD | DTG |
| Flow condition 1 | 4336 | 3959 | 1.38 | 1.00 | - | 9.58 |
| Flow condition 2 | 4090 | 5092 | 1.31 | 0.98 | - | 10.41 |
| Temperature value 1 | 4405 | 5364 | 1.34 | 0.98 | - | 10.73 |
| Temperature value 2 | 4559 | 5722 | 1.35 | 0.97 | - | 11.10 |
| Ratio of acetonitrile 1 | 4090 | 5092 | 1.31 | 0.98 | - | 10.41 |
| Ratio of acetonitrile 2 | 4405 | 5364 | 1.34 | 0.98 | - | 10.73 |
| pH 1 | 5056 | 5621 | 1.41 | 0.99 | - | 9.15 |
| pH 2 | 5027 | 5603 | 1.42 | 0.98 | - | 9.10 |
| Wavelength 1 | 4906 | 5695 | 1.41 | 0.99 | - | 9.13 |
| Wavelength 2 | 4903 | 5620 | 1.40 | 1.00 | - | 90.6 |
| Recommend limits | > 2000 | | ≤ 2.0 | | ≤ 2.0 | |

Flow condition 1 - 0.9 ml/min; Flow condition 2 - 1.1 ml/min; Temperature value 1 - 23°C; Temperature value 2 - 27°C; Ratio of acetonitrile 1 - 45%; Ratio of acetonitrile 2 - 50%; pH 1 - 3.4 units; pH 2 - 3.6 units; Wavelength 1 - 256nm; Wavelength 2 - wavelength 260 nm

hydroxide, 0.1N hydrochloric acid, 30 % peroxide and deionized water were employed as reagents for base, acid, oxidative and neutral stress, respectively. At room temperature, the solutions were sonicated for 30 min, followed by diluted to 100 ml of mobile phase.

The thermal and photolytic stress were carried out by subjecting the tablet powder (proportional to 300 mg of LMD and 50 mg of DTG) to 105°C for 6 *hand* to sun light for 24 h, respectively. After the stress time, thermal and photolytic stressed samples were transferred to the 100 ml volumetric flask, dissolved in mobile phase and diluted with the same solvent. The solution was filtered via a 0.45 μm size membrane filter. 300 $\mu\text{g}/\text{ml}$ LMD and 50 $\mu\text{g}/\text{ml}$ DTG standard concentrations were prepared from the stock solution by using the mobile phase.

10 μl of the stressed sample was injected to the HPLC system. Chromatography development was performed using conditions indicated in the "Instrumentation and Conditions" section. The peak areas of both analytes were calculated and then used to analyze the percent assay and percent degradation of studied analytes.

RESULTS AND DISCUSSION

Development of method

Different reversed phase columns such as Supelco C18, Sunsil C8, Inertsil C18 and Cosmicsil C18 were evaluated. Two solvent combinations such as phosphate buffer: methanol and orthophosphoric acid: acetonitrile of different pH, flow rate and proportions were evaluated to optimize the conditions such as tailing factor, theoretical plates, resolution and retention times of LMD and DTG. Finally Cosmicsil C18 reverse phase column with mobile phase consisting of orthophosphoric acid (pH 3.5): acetonitrile (50:50, v/v). 1.0 ml/min flow rate was provided the good separation of LMD and DTG. The analytes showed retention times were 1.799 min (LMD) and 3.035 min (DTG). The optimized chromatogram was shown in Figure 1.

Validation

Method validation was performed to the according to International Conference on Harmonization guideline to guarantee that the method is appropriate for intended use (Sharma *et al.*, 2018).

Selectivity

Analysis of mobile phase blank and placebo solution was done to evaluate the selectivity of the proposed method. The chromatograms of mobile phase blank and placebo solution showed no peak (Figure 2).

The proposed method is therefore selective for analyzing the studied drug combination.

Linearity

Linearity was determined for LMD and DTG using standard solutions with concentrations 150-450 $\mu\text{g}/\text{ml}$ of LMD and 25-75 $\mu\text{g}/\text{ml}$ of DTG, injected to the HPLC system to construct calibration curves.

Limit of detection (LD) and quantification (LQ)

Based on the calibration curves, LD and LQ were determined as 3.3 SD/m and 10 SD/m respectively. SD is standard deviation of intercept and m is slope of regression line. Lower LD and LQ values (Table 1) confirm the adequate sensitivity of the method developed.

Precision

Precision was inspected by injecting the standard solution of LMD (300 $\mu\text{g}/\text{ml}$) and DTG (50 $\mu\text{g}/\text{ml}$) six times in to the HPLC system on the same day. The mean peak area was 5525181 and 1543574 for LMD and DTG, respectively. The results, percent relative standard deviation of peak areas, were within acceptable conditions (%RSD <2).

Accuracy

For accuracy, the tablet formulation was spiked with known quantities of LMD and DTG at 50%, 100% and 150% concentration levels and evaluated using the proposed method. Percentage recoveries of added LMD and DTG have been evaluated thrice and the findings are given as mean recovery. Recovery values (Table 2) for LMD and DTG ranged from 98.88-99.81% and 99.03-100.28%, respectively. The results confirm the adequate accuracy of the method developed.

Robustness

By changing of few chromatographic conditions, then observe the system suitability parameters such as tailing factor, resolution and theoretical plates were evaluated and verified the method. The conditions verified are Flow rate (1.0 ± 0.1 ml/min), pH (3.5 ± 0.1), Ratio of acetonitrile in mobile phase ($50 \pm 5\%$), Column temperature (25 ± 2 °C), Wavelength (258 ± 2 nm). The robustness data was shown in (Table 3).

Specificity / stability indicating nature / degradation study

Different stress conditions were applied such as acid hydrolysis, neutral hydrolysis, base hydrolysis, oxidative degradation, thermal degradation and photolytic degradation. The percent degradation and percent assay of LMD and DTG were depicted in Table 4. In all cases, the method was capable of

Table 4: Stability data of LMD and DTG

| Condition | Assay (%) | Degradation (%) | Purity angle | Purity threshold | Assay (%) | Degradation (%) | Purity angle | Purity threshold |
|------------|-----------|-----------------|--------------|------------------|-----------|-----------------|--------------|------------------|
| | | | | | | | | |
| Acid | 92.28 | 7.72 | 0.627 | 1.376 | 86.06 | 13.94 | 0.273 | 0.935 |
| Base | 87.21 | 12.79 | 0.748 | 1.151 | 88.67 | 11.33 | 0.377 | 0.831 |
| Peroxide | 94.03 | 5.97 | 0.548 | 1.059 | 92.27 | 7.73 | 0.468 | 0.937 |
| Thermal | 87.70 | 12.30 | 0.893 | 1.489 | 89.35 | 10.65 | 0.401 | 0.881 |
| Photolytic | 91.62 | 8.38 | 0.707 | 1.012 | 84.04 | 15.96 | 0.399 | 0.701 |
| Neutral | 98.66 | 1.34 | 0.694 | 1.158 | 99.22 | 0.78 | 0.507 | 0.941 |

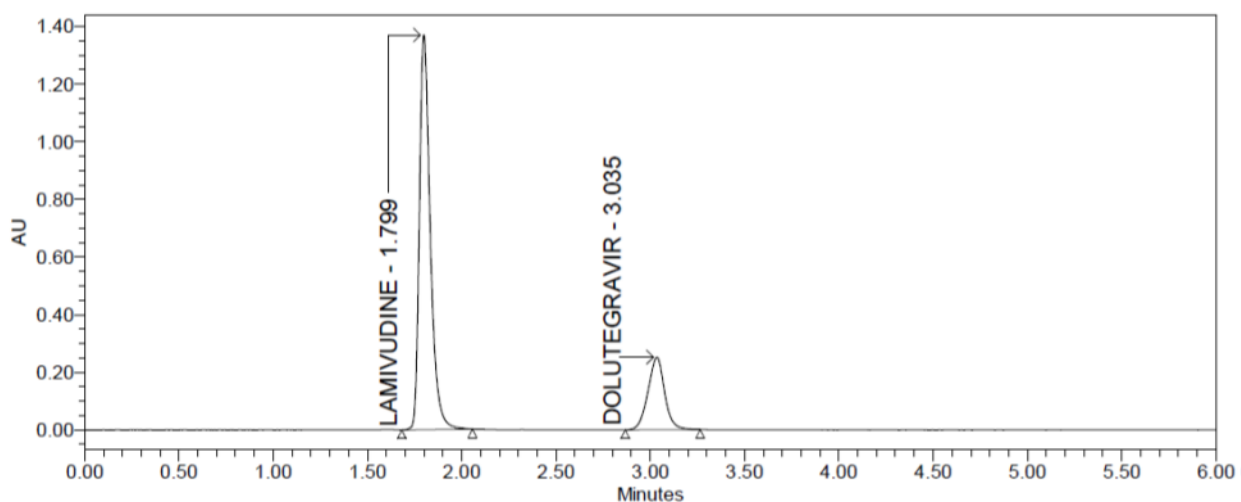


Figure 1: Chromatogram obtained under finalized conditions

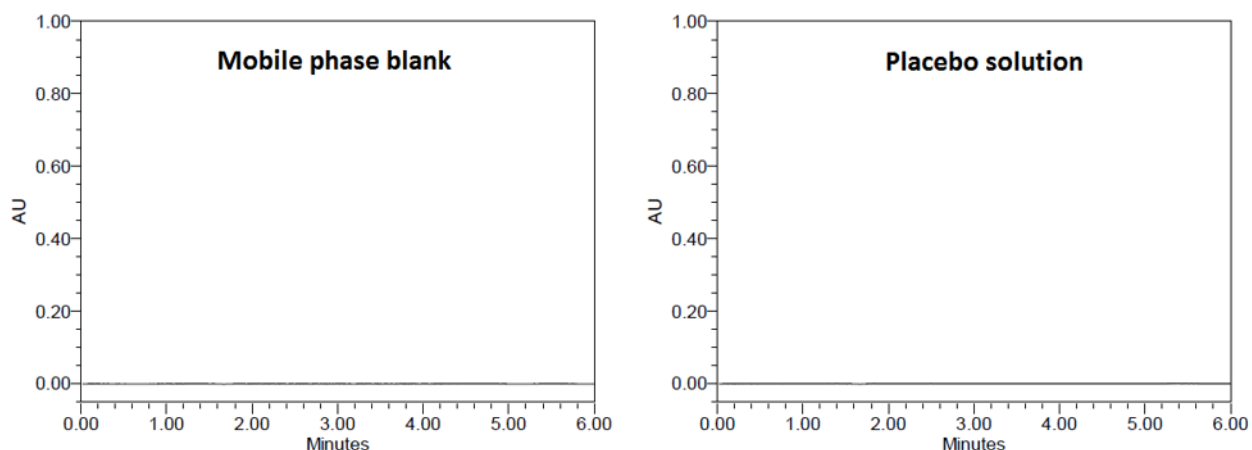


Figure 2: Chromatograms of mobile phase blank and placebo blank

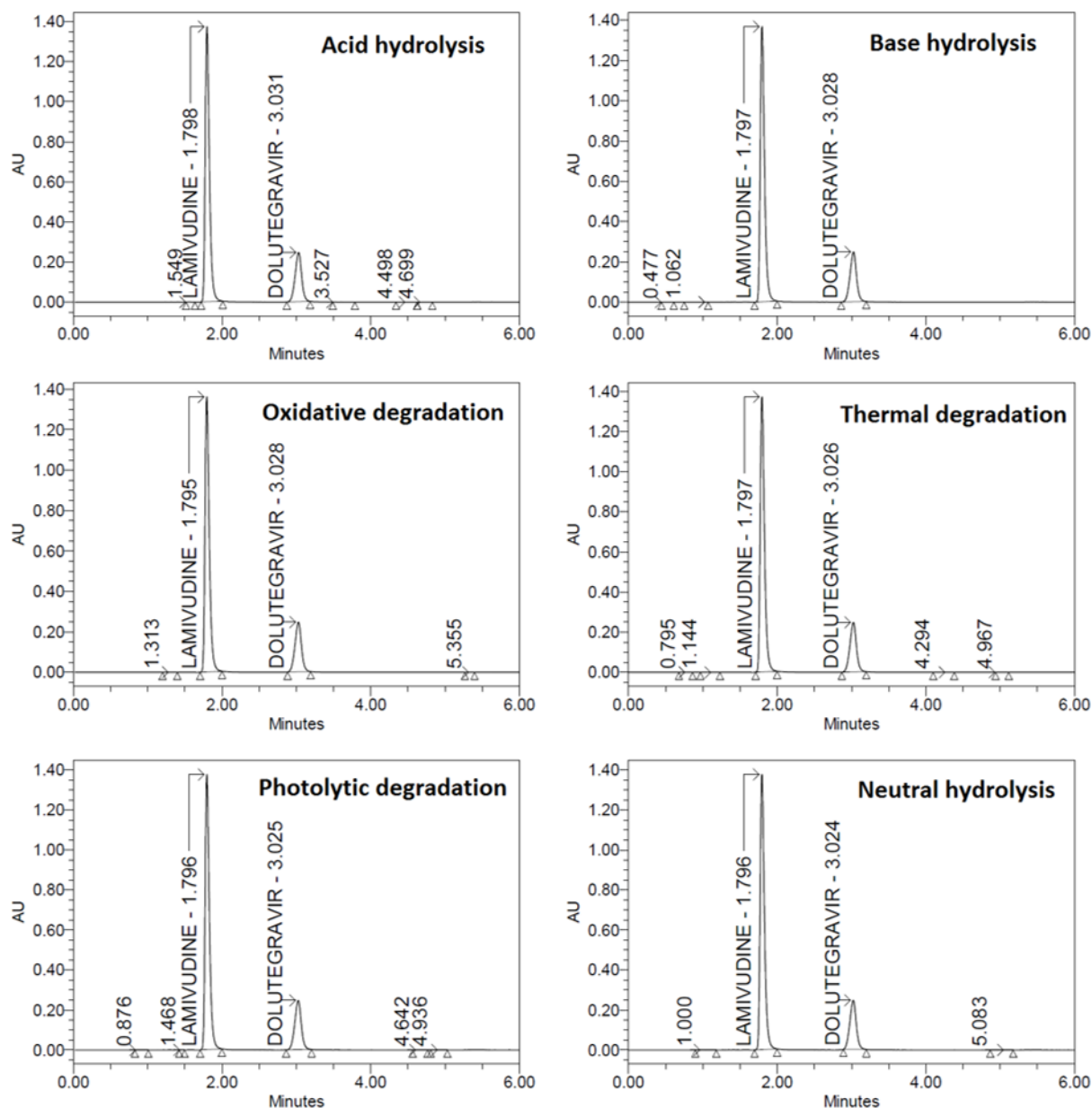


Figure 3: Chromatograms of degradation studies

separating degradation products from the LMD and DTG (Figure 3). The peak purity of LMD and DTG stressed samples were also determined (Table 4).

CONCLUSIONS

Stability indicating HPLC method was developed to evaluate LMD and DTG in combined dosage form. The chromatographic separation was achieved by using the column Cosmicsil C18. The complete analytical run time was 6 minutes. The method suggested showed good recovery in the presence of tablet excipients. The method was also precise, sensitive, selective and robust. Degradation data was suggested to develop HPLC method was specific and stability indicating. This method can be employed

to quantify LMD and DTG in quality control test for analytical laboratories.

ACKNOWLEDGEMENT

The authors are thankful to the management of GITAM Deemed to be University, Visakhapatnam, Andhra Pradesh, India, for providing necessary facilities to carry out the research work.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Funding Support

The authors declare that they have no funding support for this study.

REFERENCES

- Budamakuntla, L., Basappa, P., Shankar, V., Nirmala, Kumar, R., Suryanarayana, S. 2014. Lamivudine sensitivity - a case report. *BMC Infectious Diseases*, 14(S3):39.
- Devanna, N., Benzil, D., Ramachandraiah, C. 2017. Development and validation for the simultaneous estimation of dolutegravir and lamivudine in drug product by RP-HPLC. *Int J Res Pharma Nano Sci*, 6:173-80.
- Dhanve, A., Rao, J. R., Dhale, C. 2018. Development and validation of stability indicating HPTLC method for simultaneous estimation of lamivudine and dolutegravir sodium in bulk and pharmaceutical dosage formulation. *Int J Pharm Sci & Res*, 9:4701-4709.
- Dhanwate, S. S., Pachauri, A. D., Ghode, P. D., Khandelwal, K. R. 2019. Development and validation of analytical method for the estimation of lamivudine and dolutegravir sodium in dosage form. *J Pharm Sci & Res*, 11:2886-90.
- Dow, D. E., Bartlett, J. A. 2014. Dolutegravir, the Second-Generation of Integrase Strand Transfer Inhibitors (INSTIs) for the Treatment of HIV. *Infectious Diseases and Therapy*, 3(2):83-102.
- Min, S., Song, I., Borland, J., Chen, S., Lou, Y., Fujiwara, T., Piscitelli, S. C. 2010. Pharmacokinetics and Safety of S/GSK1349572, a Next-Generation HIV Integrase Inhibitor, in Healthy Volunteers. *Antimicrobial Agents and Chemotherapy*, 54(1):254-258.
- Mistry, V., Mishra, R. 2018. Simultaneous estimation, validation, and forced degradation studies of betahistine dihydrochloride and domperidone in a pharmaceutical dosage form using RP-HPLC method. *Asian Journal of Pharmaceutical and Clinical Research*, 11(10):125.
- Quercia, R., Perno, C. F., Koteff, J., Moore, K., Mccoig, C., Clair, M. S. 2018. Twenty-five years of lamivudine: Current and future use for the treatment of HIV-1 infection. *J Acquir Immune Defic Syndr*, 78:125-160.
- Ravichandran, V., Shalini, S., Sundaram, K. M., Rajak, H. 2010. Validation of analytical methods-strategies and importance. *Int J Pharm Pharm Sci*, 2:18-22.
- Sauter, D., Kirchhoff, F. 2019. Key Viral Adaptations Preceding the AIDS Pandemic. *Cell Host and Microbe*, 25(1):27-38.
- Sharma, S., Goyal, S., Chauhan, K. 2018. A review on analytical method development and validation. *International Journal of Applied Pharmaceutics*, 10(6):8.
- Sharp, P. M., Hahn, B. H. 2010. The evolution of HIV-1 and the origin of AIDS. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1552):2487-2494.
- Sharp, P. M., Hahn, B. H. 2011. Origins of HIV and the AIDS Pandemic. *Cold Spring Harbor Perspectives in Medicine*, 1(1):a006841.
- Snedecor, S. J., Radford, M., Kratochvil, D., Grove, R., Puneekar, Y. S. 2019. Comparative efficacy and safety of dolutegravir relative to common core agents in treatment-naïve patients infected with HIV-1: a systematic review and network meta-analysis. *BMC Infectious Diseases*, 19(1):484.
- Thota, B., Adepu, G. S., Galla, R. 2018. Method development, validation and stability studies for the determination of lurasidone hydrochloride in bulk and tablet dosage form by RP-HPLC. *International Journal of Pharmacy and Pharmaceutical Sciences*, 10(12):58.