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Review

Analytical Method Optimization and Validation for Combined Estimation of Dolutegravir and Lamivudine Using UV and RP-HPLC Techniques

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Abstract:
Dolutegravir and lamivudine are widely used antiretroviral agents in fixed-dose combinations for the management of HIV infection. Reliable, economical, and rapid analytical methods are essential for routine quality control of these formulations. The present study aimed to develop and validate simple, accurate, precise, and cost-effective UV–Visible spectrophotometric and RP-HPLC methods for the simultaneous estimation of dolutegravir and lamivudine in synthetic mixture and pharmaceutical dosage forms. A UV spectrophotometric method based on the Q-absorbance ratio technique was developed using 290 nm as the iso-absorptive wavelength and 271 nm as the λmax of lamivudine. The linearity range for the UV method was 1–5 µg/mL for dolutegravir and 6–30 µg/mL for lamivudine. An RP-HPLC method was developed using a C18 column with detection at 290 nm. The optimized mobile phase consisted of methanol with pH adjusted to 4.6 using orthophosphoric acid, delivered at a flow rate of 1.0 mL/min. The retention times were approximately 1.7 min for dolutegravir and 1.1 min for lamivudine. Both methods were validated as per ICH guidelines for linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ. The UV method demonstrated good linearity, with recovery values of approximately 101.12% for dolutegravir and 99.98% for lamivudine. The RP-HPLC method showed linearity over 20–100 µg/mL for dolutegravir and 120–600 µg/mL for lamivudine, with correlation coefficients of 0.998 and 0.9982, respectively. The percentage purity by RP-HPLC was 102.4% w/w for dolutegravir and 99.77% w/w for lamivudine. The LOD/LOQ values for RP-HPLC were 5.3/16.16 µg/mL for dolutegravir and 30.74/93.16 µg/mL for lamivudine. The proposed UV and RP-HPLC methods were found to be simple, rapid, accurate, precise, robust, and suitable for routine quality control analysis of dolutegravir and lamivudine in combined pharmaceutical dosage forms.

Keywords: Dolutegravir; Lamivudine; UV–Visible spectrophotometry; RP-HPLC; Method validation; ICH guidelines; Simultaneous estimation

1. INTRODUCTION

Dolutegravir (DTG) is an integrase strand transfer inhibitor widely used in the treatment of HIV-1 infection due to its potent antiviral activity, favorable resistance profile, and convenient once-daily dosing. Lamivudine (3TC) is a nucleoside reverse transcriptase inhibitor with established efficacy against both HIV and hepatitis B virus infections. The combination of dolutegravir and lamivudine has gained substantial clinical relevance as a simplified two-drug antiretroviral regimen that offers efficacy with reduced pill burden and improved tolerability.

The increasing use of fixed-dose combinations containing these drugs necessitates the development of analytical methods that are rapid, selective, economical, and reproducible for routine quality control. Although several analytical methods have been reported for individual or combined antiretroviral agents, there remains a practical need for methods that reduce analysis time, minimize solvent consumption, and maintain acceptable sensitivity and accuracy in routine laboratory settings. The uploaded dissertation also notes that limited analytical procedures are available for the simultaneous estimation of this particular combination, especially by simple spectrophotometric techniques.

UV–Visible spectrophotometry remains one of the most cost-effective and accessible analytical techniques for pharmaceutical quality control, particularly in academic and resource-limited laboratories. Likewise, RP-HPLC offers enhanced selectivity, sensitivity, and reproducibility for simultaneous estimation in complex mixtures. The present work therefore focuses on the development and validation of both UV–Visible spectrophotometric and RP-HPLC methods for simultaneous estimation of dolutegravir and lamivudine in synthetic mixture and dosage forms, following ICH validation principles. The UV method employs the Q-absorbance ratio approach using 290 nm as an iso-absorptive point and 271 nm as the λmax of lamivudine, while the RP-HPLC method uses detection at 290 nm with short retention times for both analytes.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Pure drug samples of dolutegravir and lamivudine were obtained as gift samples from pharmaceutical sources (Mylan Laboratories Ltd., Hyderabad, and Hetero Labs, Hyderabad). Marketed formulations used in the dissertation included dolutegravir tablets (50 mg) and lamivudine tablets (100 mg). HPLC-grade methanol, deionized water, orthophosphoric acid, and other analytical-grade reagents were used throughout the study. The file lists Thermo Fisher and HiMedia grade solvents/reagents and indicates that all other chemicals were of analytical grade.

2.2 Instrumentation

The following instruments were employed:

- SHIMADZU PHARMASPEC-1800 UV–Visible double beam spectrophotometer with 1 cm quartz cells
- Shimadzu HPLC LC-20 system
- Prominence LC-20AT double pump
- Rheodyne 7725i injector (20 µL loop)
- Prominence DAD detector (SPD M20A)
- Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm)
- Electronic balance, pH meter, and sonicator

These instruments are consistent with the equipment listed in the uploaded dissertation.

2.3 Preparation of Synthetic Mixture

A synthetic mixture containing dolutegravir and lamivudine in a **1:6 ratio** was prepared to simulate combined dosage form analysis. The formulation composition reported in the dissertation included dolutegravir (50 mg), lamivudine (300 mg), and common excipients such as microcrystalline cellulose, magnesium stearate/sulphate (as listed in the thesis), mannitol, sodium starch glycolate, and PVP.

2.4 UV–Visible Spectrophotometric Method

2.4.1 Principle

A simultaneous equation/Q-absorbance ratio method was developed for the simultaneous estimation of dolutegravir and lamivudine. The method was based on measurement of absorbance at:

- **290 nm**: Iso-absorptive wavelength of dolutegravir and lamivudine
- **271 nm**: λmax of lamivudine

The uploaded dissertation explicitly states that both drugs exhibited an iso-absorptive point at 290 nm and that 271 nm was selected as the λmax of lamivudine.

2.4.2 Preparation of Standard Stock Solutions

Accurately weighed quantities of dolutegravir and lamivudine reference standards were separately dissolved in suitable solvent system (distilled

water/methanol as used in the dissertation) to obtain stock solutions. Working standard solutions were prepared by appropriate dilution to obtain concentration ranges required for calibration.

2.4.3 Linearity

Calibration curves were constructed for:

- **Dolutegravir:** 1–5 µg/mL
- **Lamivudine:** 6–30 µg/mL

The method followed Beer–Lambert's law within the selected ranges. These linearity ranges were directly reported in the dissertation summary and discussion.

2.4.4 Assay Procedure

Aliquots of mixed standard/sample solutions were scanned, and absorbances were measured at 271 nm and 290 nm against a suitable blank. The concentrations of dolutegravir and lamivudine were calculated using absorptivity coefficients and the Q-absorbance ratio equations.

2.5 RP-HPLC Method

2.5.1 Chromatographic Conditions

An RP-HPLC method was developed and optimized under the following conditions:

- **Column:** C18 analytical column
- **Mobile phase:** Methanol, with pH adjusted to **4.6 using orthophosphoric acid**
- **Flow rate: 1.0 mL/min**
- **Detection wavelength: 290 nm**
- **Injection volume:** 20 µL
- **Run mode:** Isocratic
- **Retention times:**
    - Dolutegravir: **~1.7 min**
    - Lamivudine: **~1.1 min**

These values were specifically reported in the dissertation's RP-HPLC summary and analytical data table.

2.5.2 Preparation of Standard and Sample Solutions

Standard stock solutions of dolutegravir and lamivudine were prepared in mobile phase or suitable diluent and filtered before injection. Sample solutions were prepared from synthetic mixture or marketed formulations by accurately weighing, dissolving, sonicating, filtering, and diluting to the required concentrations.

2.5.3 Linearity

The RP-HPLC method was evaluated over the following concentration ranges:

- **Dolutegravir:** 20–100 µg/mL
- **Lamivudine:** 120–600 µg/mL

These ranges are taken directly from the dissertation summary and Table 45.

2.6 Method Validation

Both analytical methods were validated according to ICH recommendations for:

- Linearity
- Accuracy (recovery studies)
- Precision (repeatability and intermediate precision)
- Robustness
- Ruggedness
- Limit of Detection (LOD)
- Limit of Quantification (LOQ)

The dissertation explicitly includes these validation parameters and states that the methods were validated according to ICH guidelines.

2.6.1 Accuracy

Accuracy was assessed by recovery studies at multiple levels (e.g., 50%, 75%, and 100%).

2.6.2 Precision

Precision was evaluated by replicate analysis and expressed as %RSD.

2.6.3 LOD and LOQ

LOD and LOQ were calculated using:

- **LOD = 3.3 σ / S**
- **LOQ = 10 σ / S**

These equations are directly documented in the uploaded file.

3. RESULTS AND DISCUSSION

3.1 UV–Visible Spectrophotometric Method

The UV spectrophotometric method based on the Q-absorbance ratio approach provided a simple and economical means for simultaneous estimation of dolutegravir and lamivudine. The selection of **290 nm** as the iso-absorptive point and **271 nm** as the λmax of lamivudine enabled accurate quantification of both analytes in the mixture. The method demonstrated linearity over **1–5 µg/mL for dolutegravir** and **6–30 µg/mL for lamivudine**, indicating suitability for low-concentration analysis.

Recovery studies showed excellent method accuracy. The dissertation reports **mean recovery values of 101.12% for dolutegravir and 99.98% for lamivudine**, with other recovery levels also exceeding 97%, confirming the absence of significant

interference from excipients and the reliability of the method.

The method was found to be precise, robust, rugged, and reproducible. Its key advantage lies in the use of accessible instrumentation and low operational cost, making it highly suitable for routine quality control laboratories, especially where advanced chromatographic facilities may not be readily available.

3.2 RP-HPLC Method

The RP-HPLC method offered rapid and selective chromatographic separation of dolutegravir and lamivudine under optimized conditions. Detection at **290 nm** with a mobile phase of **methanol adjusted to pH 4.6 using orthophosphoric acid** produced sharp, symmetrical peaks with minimal tailing. The analytes eluted with short retention times of approximately **1.7 min for dolutegravir** and **1.1 min for lamivudine**, substantially reducing analysis time and solvent consumption.

The method showed linearity over a wider concentration range:

- **Dolutegravir:** 20–100 µg/mL
- **Lamivudine:** 120–600 µg/mL

The reported correlation coefficients were:

- **Dolutegravir:** 0.998
- **Lamivudine:** 0.9982

These values indicate strong linear relationships between concentration and peak response. The regression equations reported in the dissertation were:

- **Dolutegravir:** $y = 559x + 1005.3$
- **Lamivudine:** $y = 247.65x + 34756$

The percentage purity obtained by the RP-HPLC assay was:

- **Dolutegravir:** 102.4% w/w
- **Lamivudine:** 99.77% w/w

These values confirm the analytical accuracy and applicability of the method for dosage form analysis.

Sensitivity of the RP-HPLC method was reflected in the LOD and LOQ values:

- **Dolutegravir:**
  - LOD = 5.3 µg/mL
  - LOQ = 16.16 µg/mL
- **Lamivudine:**
  - LOD = 30.74 µg/mL
  - LOQ = 93.16 µg/mL

These values were reported in the analytical data table of the dissertation.

Overall, the RP-HPLC method provided better selectivity and faster chromatographic performance than many conventional methods. The short run time, acceptable sensitivity, and good validation characteristics make it highly suitable for routine assay of combined antiretroviral formulations.

3.3 Comparative Analytical Significance

The UV method is advantageous where simplicity, low cost, and rapid screening are priorities, while the RP-HPLC method is more suitable when higher selectivity, better resolution, and confirmatory quantitative analysis are required. Together, the two methods provide a practical analytical framework for both academic and industrial pharmaceutical laboratories.

4. CONCLUSION

The present study successfully developed and validated two analytical methods for the simultaneous estimation of dolutegravir and lamivudine in synthetic mixture and pharmaceutical dosage forms.

The **UV–Visible spectrophotometric method** based on the Q-absorbance ratio technique was found to be simple, economical, accurate, and suitable for routine estimation using readily available instrumentation. The use of **271 nm and 290 nm** provided reliable quantification of both analytes with good linearity and acceptable recovery.

The **RP-HPLC method** was found to be rapid, precise, and highly suitable for routine quality control analysis. The optimized chromatographic conditions enabled excellent separation with very short retention times (**~1.7 min for dolutegravir and ~1.1 min for lamivudine**), making the method time-efficient and cost-effective.

Both methods complied with ICH validation requirements and can be recommended for routine quality control of dolutegravir and lamivudine in combined dosage forms. Among the two, the RP-HPLC method offers superior selectivity and robustness, while the UV method remains highly valuable in laboratories with limited access to advanced instrumentation.

5. TABLES

Table 1. Composition of Synthetic Mixture for Dolutegravir and Lamivudine (1:6 Ratio)

Ingredient	Quantity (mg)	Function
Dolutegravir	50	Anti-HIV drug
Lamivudine	300	Anti-HIV drug
Microcrystalline cellulose (MCC)	10	Filler
Magnesium salt*	10	Lubricant
Mannitol	5	Sweetening agent
Sodium starch glycolate	15	Superdisintegrant
PVP	10	Binder

*The dissertation text lists "magnesium sulphate," though in tablet formulation this may need verification against original lab records.

Table 2. Summary of UV–Visible Spectrophotometric Method Parameters

Parameter	Dolutegravir	Lamivudine
Method	Q-absorbance ratio	Q-absorbance ratio
Analytical wavelengths	271 nm and 290 nm	271 nm and 290 nm
Iso-absorptive wavelength	-	290 nm
λmax selected	-	271 nm
Linearity range (µg/mL)	1–5	6–30
Mean recovery (%)	101.12	99.98

Table 3. Optimized RP-HPLC Chromatographic Conditions

Parameter	Condition
Column	C18 analytical column
Mobile phase	Methanol, pH adjusted to 4.6 with orthophosphoric acid
Flow rate	1.0 mL/min
Detection wavelength	290 nm
Injection volume	20 µL
Elution mode	Isocratic
Retention time of Dolutegravir	~1.7 min
Retention time of Lamivudine	~1.1 min

Table 4. RP-HPLC Analytical Data Parameters

Parameter	Dolutegravir	Lamivudine
Detection wavelength	290 nm	290 nm
Retention time (min)	1.7	1.1
Beer's law limit (µg/mL)	20–100	120–600
Regression equation	y = 559x + 1005.3	y = 247.65x + 34756
Correlation coefficient (r)	0.998	0.9982
Slope	559	247.65
Intercept	1005.3	34756
LOD (µg/mL)	5.3	30.74
LOQ (µg/mL)	16.16	93.16
Assay / % purity	102.4% w/w	99.77% w/w

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