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SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR DOLUTEGRAVIR SULPHATE BY ULTRAVIOLET SPECTROSCOPY (UV) AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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1. ABSTRACT

This study focuses on the development and validation of simple, precise, and accurate analytical methods for the estimation of Dolutegravir sulphate in bulk and tablet dosage form using UV spectrophotometric and RP-HPLC techniques. The UV method was performed at a wavelength of 259.80 nm, while the RP-HPLC method utilized a C-18 column with a mobile phase consisting of Acetonitrile and phosphate buffer (75:25 v/v) at a flow rate of 0.8 ml/min. The developed methods were validated as per ICH guidelines for parameters such as linearity, precision, accuracy, robustness, ruggedness, limit of detection (LOD), and limit of quantitation (LOQ). The linearity range for UV method was found to be 5–40 µg/ml and for HPLC method 10–50 µg/ml with correlation coefficients close to 0.999, indicating excellent linearity. The %RSD values for precision studies were found to be less than 2%, confirming high precision of the methods. Accuracy studies showed % recovery in the range of 99–101%, indicating the reliability of the methods. The developed methods were found to be robust and rugged under varying experimental conditions. The assay of marketed formulation showed drug content within acceptable limits. In conclusion, the proposed UV and RP-HPLC methods are simple, accurate, precise, and suitable for routine quality control analysis of Dolutegravir sulphate in pharmaceutical dosage forms.

2. **KEYWORDS:** Dolutegravir sulphate, UV spectrophotometry, RP-HPLC, Method validation, ICH guidelines.

3. INTRODUCTION

Analytical chemistry is fundamental to pharmaceutical research, ensuring the quality, safety, and efficacy of drug substances. It is broadly categorized into classical methods (qualitative and quantitative) and modern instrumental techniques.

1.1 UV-Visible Spectrophotometry

Spectroscopy involves the interaction of electromagnetic radiation with matter. UV-Visible spectrophotometry is a primary tool for drug analysis due to its simplicity and cost-effectiveness. The technique is governed by the **Beer-Lambert Law**:

$$A = \log_{10} (I_0 / I) = \varepsilon CL$$

Where **A** is absorbance, **ε** is molar absorptivity, **C** is concentration, and **L** is the path length. Absorption in the UV range (200–400 nm) triggers electronic transitions ($n \to \pi^*$, $\pi \to \pi^*$), which are characteristic of the analyte's molecular structure.

1.2 High-Performance Liquid Chromatography (HPLC)

HPLC is an advanced chromatographic technique used for the separation, identification, and quantification of drugs in complex matrices. It offers superior resolution and sensitivity compared to classical methods.

- **Instrumentation:** A standard HPLC system consists of a high-pressure pump, an injector, a stationary phase (column), and a detector (UV or PDA).
- **Reverse Phase Chromatography (RP-HPLC):** This is the most common mode used in pharmaceutical analysis, utilizing a non-polar stationary phase (e.g., C18) and a polar mobile phase.

2. METHOD DEVELOPMENT AND OPTIMIZATION

The development of a robust HPLC method follows a systematic approach:

1. **Initial Studies:** Reviewing physicochemical properties like pKa, solubility, and λ_{max}.
2. **Selectivity Optimization:** Adjusting the mobile phase composition and pH to achieve peak separation.

3. **System Optimization:** Balancing resolution and analysis time by adjusting flow rate and column dimensions.

2.1 System Suitability Parameters

To ensure the analytical system is performing adequately, the following parameters are monitored:

Parameter	Recommended Limit (ICH/USP)
Theoretical Plates (N)	> 2000
Tailing Factor (T)	≤ 2.0
Resolution (R_s)	> 2.0
Capacity Factor (k')	> 2.0

3. ANALYTICAL METHOD VALIDATION

As per **ICH (Q2R1)** and **USP** guidelines, every new analytical method must be validated to demonstrate it is fit for its intended purpose.

- **Accuracy:** Measured as % recovery of known added amounts of analyte.
- **Precision:** Evaluated through repeatability (intra-day) and intermediate precision (inter-day).
- **Linearity & Range:** The ability of the method to elicit test results proportional to the concentration.
- **Sensitivity:** Defined by the **Limit of Detection (LOD)** and **Limit of Quantitation (LOQ)**:

$$LOD = \frac{3.3 \sigma}{S}$$

$$LOQ = \frac{10 \sigma}{S}$$

(Where σ is the standard deviation of the response and S is the slope of the calibration curve).

- **Robustness:** The capacity of the method to remain unaffected by small, deliberate variations in parameters like flow rate or mobile phase ratio.

4. MATERIALS AND METHODS

4.1 Materials

Dolutegravir sulphate (API)

Methanol

Acetonitrile

Phosphate buffer

Distilled water

4.2 Instruments

UV Spectrophotometer

HPLC system with C-18 column

Analytical balance

Sonicator

4.3 UV Spectrophotometric Method

Wavelength selected: 259.80 nm

Standard solutions prepared in suitable solvent

Calibration curve plotted (5–40 µg/ml)

2.4 HPLC Method

Column: C-18

Mobile phase: Acetonitrile: Phosphate buffer (75:25 v/v)

Flow rate: 0.8 ml/min

Detection: UV detector

Linearity range: 10–50 µg/ml

5. METHOD VALIDATION

Validation was performed as per ICH guidelines for:

Linearity

Precision

Accuracy

Robustness

Ruggedness

LOD & LOQ

6. RESULTS AND DISCUSSION

The developed UV spectrophotometric and RP-HPLC methods for estimation of Dolutegravir sulphate were validated as per ICH guidelines.

1. Linearity

Both methods showed excellent linearity:

UV method: 5–40 µg/ml ($R^2 \approx 0.999$)

HPLC method: 10–50 µg/ml ($R^2 = 0.9995$)

HPLC: 99.25% – 100.50%

This confirms high accuracy of the methods.

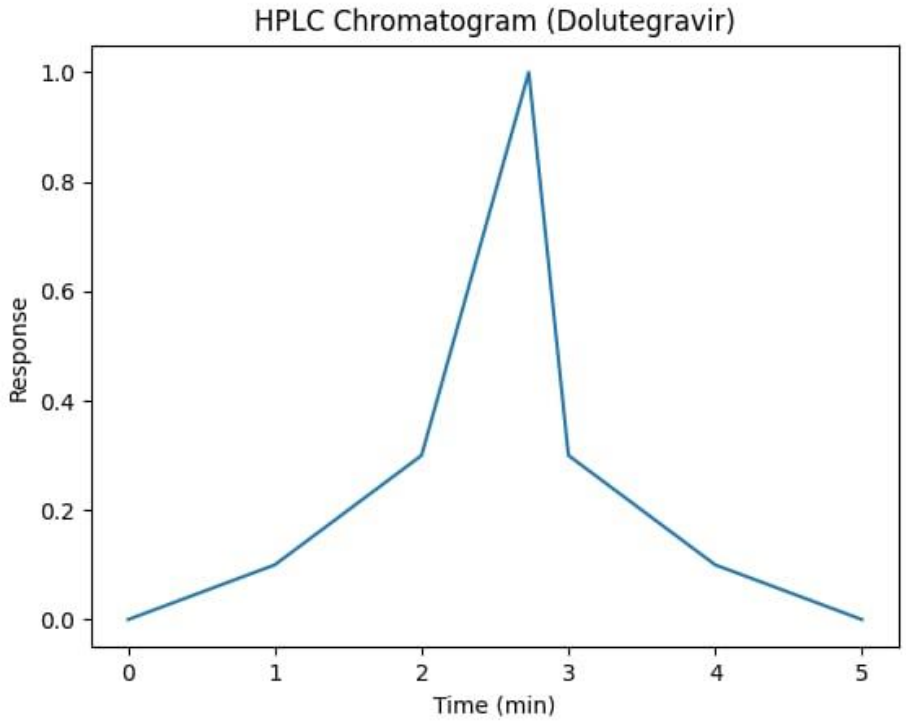

Fig 1: Calibration Curve of Dolutegravir sulphate (HPLC method).

This indicates a strong correlation between concentration and response.

2. Precision

The method was found to be precise:

UV Method:

Intraday %RSD: 0.2323

Interday %RSD: 0.2012

HPLC Method:

Intraday %RSD: 0.2779

Interday %RSD: 0.4386

All values were below 2%, indicating good precision.

3. Accuracy

Recovery studies showed:

UV: 99.88% – 100.60%

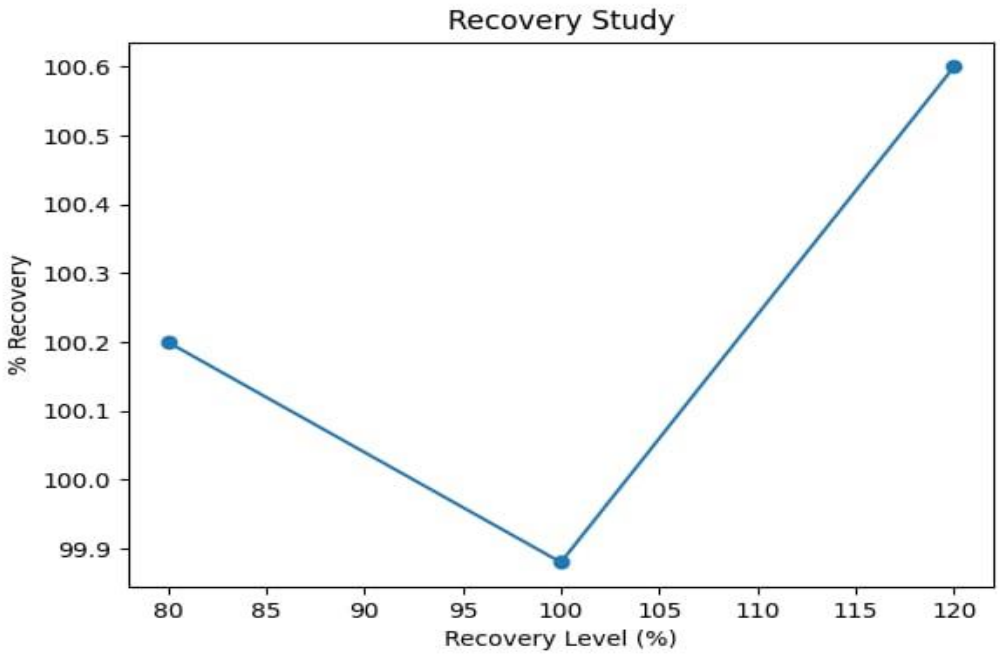

Fig 2: Recovery Study of Dolutegravir sulphate.

4. LOD and LOQ

UV Method:

LOD: 1.86 µg/ml

LOQ: 5.64 µg/ml

HPLC Method:

LOD: 15.45 µg/ml

LOQ: 46.82 µg/ml

5. Robustness

Small changes in wavelength, flow rate, mobile phase, and pH did not significantly affect results. %RSD values were within acceptable limits.

6. Ruggedness

The method showed good reproducibility between different analysts:

%RSD < 2%

7. Assay of Marketed Formulation

% Drug content: 99.96%

Within acceptable limit (80–120%)

8. System Suitability (HPLC)

Retention time: 2.73 min

Theoretical plates: 2940

Tailing factor: 1.33

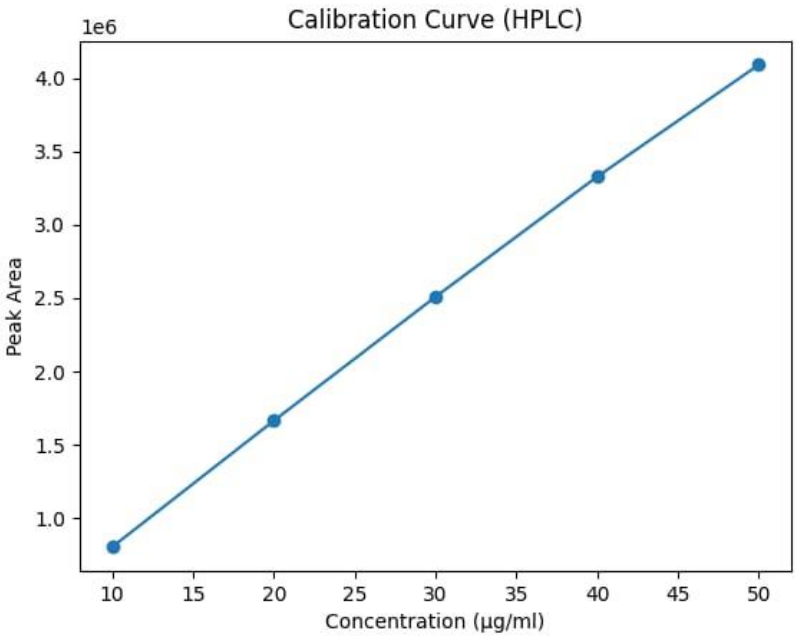

Fig 3: HPLC Chromatogram of Dolutegravir sulphate.

7. CONCLUSION

The developed UV spectrophotometric and RP-HPLC methods for Dolutegravir sulphate are simple, precise, accurate, and economical. These methods can be successfully applied for routine quality control analysis in pharmaceutical industries.

8. ACKNOWLEDGEMENT

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