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Development and Validation of Dual UV Spectrophotometric and RP-HPLC Methods for Simultaneous Quantification of Dolutegravir and Darunavir in a Synthetic Mixture

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ABSTRACT:

Objective: The present study reports the development and validation of efficient, sensitive, and reliable UV spectrophotometric and RP-HPLC methods for the simultaneous quantification of Dolutegravir and Darunavir in a synthetic mixture. **Method and Results:** The first-order derivative UV spectrophotometric technique was employed by measuring the amplitudes at 326 nm (zero-crossing point of Darunavir) for the quantification of Dolutegravir and at 258 nm (zero-crossing point of Dolutegravir) for Darunavir. The method demonstrated excellent linearity within the concentration ranges of 1–5 µg/mL for Dolutegravir and 16–80 µg/mL for Darunavir, yielding correlation coefficients (R^2) of 0.999 for both drugs. The percentage recovery values ranged from 99.67–99.94% for Dolutegravir and 99.96–99.97% for Darunavir, confirming the accuracy and precision of the method. For RP-HPLC analysis, chromatographic separation was performed on a Kromstar Vertex C_{18} column (250 × 4.6 mm, 5 µm) under isocratic conditions using a mobile phase composed of Acetonitrile: Phosphate Buffer (80:20 %v/v), adjusted to pH 2.8 with orthophosphoric acid. The flow rate was maintained at 1.0 mL/min, and detection was carried out at 270 nm. The retention times were found to be 5.8 min for Darunavir and 3.2 min for Dolutegravir. The calibration curves exhibited good linearity over the ranges of 1–5 µg/mL for Dolutegravir and 16–80 µg/mL for Darunavir, with recovery values between 99.76–99.98% and 99.93–99.97%, respectively. Validation results established that both the UV spectrophotometric and RP-HPLC methods are suitable, accurate, and reliable for the simultaneous quantitative determination of Dolutegravir and Darunavir in synthetic mixtures. **Conclusion:** Validation performed in accordance with ICH guidelines established the suitability and reliability of the developed methods for the simultaneous determination of Dolutegravir and Darunavir in synthetic mixtures.

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1. INTRODUCTION:

Dolutegravir (Figure 1), chemically known as (4R,12aS)-N-(2,4-difluorobenzyl)-7-hydroxy4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido [1′,2′:4,5] pyrazino [2,1-b][1,3]oxazine-9-carboxamide, is an HIV-1 integrase strand transfer inhibitor (INSTI). It exerts its antiviral activity by binding to the active site of HIV integrase and blocking the strand transfer step of retroviral DNA integration into the host cell genome. Dolutegravir has been demonstrated to be highly effective in the treatment of HIV-1 infection due to its potent antiviral activity, high genetic barrier to resistance, and favorable safety profile[1-4].

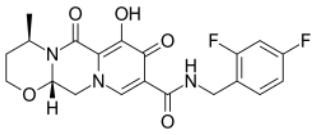

Figure 1: Structure of Dolutegravir

Darunavir (Figure 2), chemically described as (3R,3aS,6aR)-hexahydrofuro [2,3-b]furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)-4-aminobenzenesulfonamido]-1-phenylbutan-2-yl] carbamate, is a second-generation HIV protease inhibitor. Darunavir was specifically designed to form strong interactions with the HIV-1 protease enzyme, enabling effective inhibition of viral maturation even in strains exhibiting resistance to earlier protease inhibitors [9]. It exhibits robust antiviral activity and improved resistance profiles due to its multiple hydrogen bond interactions within the protease active site[1-4].

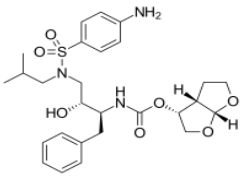

Figure 2: Structure of Darunavir

The combination of Dolutegravir and Darunavir has been extensively evaluated in clinical studies and has demonstrated high efficacy, strong resistance barriers, and good tolerability, particularly in treatment-experienced and virologically suppressed HIV-1 patients. Dual therapy with Dolutegravir and boosted Darunavir has been shown to be non-inferior to conventional triple-drug regimens, with additional clinical benefits including improved lipid profiles and renal safety. Long-term studies further confirm the safety and reliability of this potent nucleoside reverse transcriptase inhibitor (NRTI)-free regimen, making it a promising therapeutic strategy in salvage and switch therapy settings[5,6].

Indian Pharmacopoeia includes monographs for both Dolutegravir and Darunavir. A comprehensive review of the literature indicates that several analytical techniques—such as UV spectrophotometry, HPLC, HPTLC, GC, LC–MS, and LC–MS/MS[7-19]—have been reported for the individual determination of these drugs or in combination with other antiretroviral agents. However, no validated method has yet been described for their simultaneous estimation. Accordingly, the present investigation was undertaken to develop and validate a simple, accurate, and precise RP-HPLC method for the concurrent quantification of Dolutegravir and Darunavir in a synthetic mixture.

MATERIALS AND METHODS:

Chemicals and Reagents:

Dolutegravir was received as a bulk drug sample from Torrent Pharmaceuticals Limited, whereas Darunavir was kindly provided as a gift sample by Cadila Pharmaceuticals Limited. All solvents used in the study were procured from Finar Chemicals, and AR-grade potassium dihydrogen phosphate was obtained from Astron Chemical Ltd. Freshly prepared solutions were used throughout the analysis.

Spectrophotometric and Chromatographic Condition:

UV spectrophotometric measurements were conducted using a Shimadzu UV-1800 equipped with UV-Probe software, with methanol employed as the diluent. Chromatographic separation was performed on a Systronics LC-138 system comprising a photodiode array detector, manual injector, and a Kromstar Vertex C_{18} column (250 × 4.6 mm, 5 µm)[20,21]. Data acquisition and processing were carried out using Clarify software. The analysis

was executed under isocratic conditions using a mobile phase of Acetonitrile: Phosphate Buffer (80:20 %v/v), adjusted to pH 2.8 with orthophosphoric acid. The flow rate was maintained appropriately, detection was set at 270 nm, and the total run time was 10 minutes. Under the optimized chromatographic conditions, the retention times were approximately 5.8 minutes for Darunavir and 3.2 minutes for Dolutegravir.

Method for Preparation of Analytical Solutions:

Stock and standard Solution:

Precisely weigh 10 mg each of Darunavir and Dolutegravir and transfer them into separate volumetric flasks. Add an adequate volume of methanol and sonicate for approximately 30 minutes to ensure complete dissolution. Dilute to volume with methanol to obtain standard stock solutions having a concentration of 100 µg/mL, and label them appropriately as standard stock solutions.

Preparation of Sample Solution:

An accurately weighed quantity of Darunavir (80 mg) and Dolutegravir (5 mg) was transferred into a 100 mL volumetric flask. Methanol was added up to approximately half the volume, and the mixture was sonicated to ensure complete dissolution of the drugs. The solution was then diluted to the mark with methanol and filtered through Whatman filter paper to obtain stock concentrations of 800 µg/mL for Darunavir and 50 µg/mL for Dolutegravir.

Subsequently, 0.4 mL of this solution was transferred into a 10 mL volumetric flask and diluted to volume with methanol to obtain final concentrations of 32 µg/mL for Darunavir and 2 µg/mL for Dolutegravir.

Preparation of Mobile phase:

A mixture of Acetonitrile: Phosphate Buffer in the ratio of 80:20%v/v, was prepared, thoroughly mixed, and the pH was adjusted to 2.8 using 10% orthophosphoric acid.

Selection of Suitable Analytical Wavelength:

The blank solution was scanned for absorbance over the range of 200–400 nm. The analytes were detected at a wavelength of 270 nm, at which both drugs exhibited satisfactory absorbance characteristics, as shown in Figure 3.

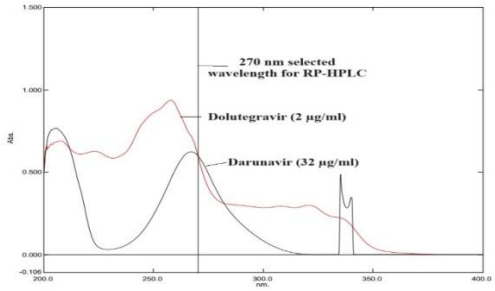

Figure 3: Overlain UV Spectrum of Dolutegravir (2 µg/ml) and Darunavir (32 µg/ml) in Methanol

UV SPECTROPHOTOMETRIC METHOD

SELECTION OF WAVELENGTH FOR DARUNAVIR AND DOLUTEGRAVIR

For the selection of analytical wavelengths, solutions of Darunavir (32 µg/mL) and Dolutegravir (2 µg/mL) were scanned within the spectral range of 200–400 nm. Darunavir and Dolutegravir showed maximum absorbance (λmax) at 258 nm and 326 nm, respectively, as illustrated in Figure 4.

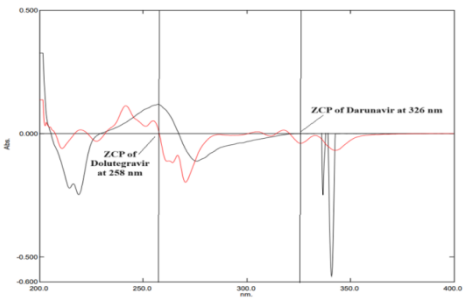

Figure 4: Overlay spectra of Darunavir (32 µg/ml) and Dolutegravir (2 µg/ml) in Methanol (First Order)

Linearity:
Precisely measured aliquots of Darunavir standard stock solution (100 µg/mL) (1.6, 3.2, 4.8, 6.4, and 8.0 mL) and Dolutegravir standard stock solution (100 µg/mL) (0.1, 0.2, 0.3, 0.4, and 0.5 mL) were separately transferred into five individual 10 mL volumetric flasks. Each flask was diluted to volume with methanol to obtain final concentrations of 16, 32, 48, 64, and 80 µg/mL for Darunavir and 1, 2, 3, 4, and 5 µg/mL for Dolutegravir.

The absorbance of the prepared solutions was recorded at 258 nm for Darunavir and 326 nm for Dolutegravir, using methanol as the blank. The corresponding calibration curves are presented in Figures 5 and 6.[22]

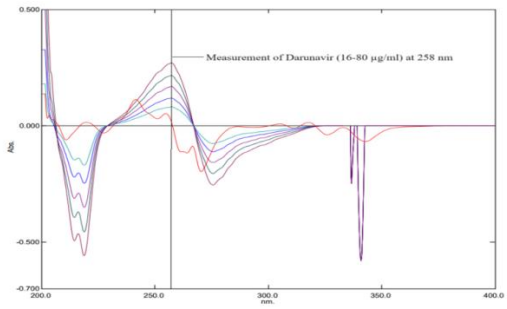

Figure 5: Linearity of 1st Derivative Spectra of Darunavir (16-80 µg/ml) at 258 nm

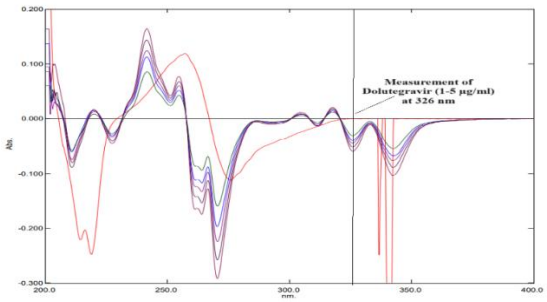

Figure 6: Linearity of 1st Derivative Spectra of Dolutegravir (1-5 µg/ml) at 326 nm

Precision:
Method precision was assessed through intraday, interday, and repeatability studies. For intraday precision, Darunavir solutions at concentrations of 16, 32, and 48 µg mL^{-1} and Dolutegravir solutions at 1, 2, and 3 µg mL^{-1} were analyzed in triplicate within the same day. Interday precision was evaluated by examining the same concentration levels on three consecutive days. Repeatability was determined by analyzing Darunavir (32 µg mL^{-1}) and Dolutegravir (2 µg mL^{-1}) six times under identical conditions. The precision of the method was expressed in terms of percentage relative standard deviation (%RSD).[22]

Accuracy:
The pre-analyzed solution was spiked with known quantities of Darunavir and Dolutegravir at three concentration levels (50%, 100%, and 150%), and the mean percentage recovery for both drugs was calculated.[22]

Detection Limit and Quantification Limit:
In accordance with ICH guidelines, the Limits of Detection (LOD) and Quantification (LOQ) were calculated using standard equations.[22]

RP-HPLC METHOD DEVELOPMENT AND VALIDATION:
The objective of the present investigation was to develop a simple, robust, precise, and economical RP-HPLC method for the simultaneous determination of both drugs in a synthetic mixture. The developed method was validated in accordance with ICH guidelines, evaluating parameters such as system suitability, linearity, precision, limits of detection and quantification (LOD and LOQ), accuracy, assay, and robustness.

System Suitability

System suitability was assessed by performing six replicate injections of freshly prepared standard solutions of Darunavir and Dolutegravir. Chromatographic parameters such as retention time, number of theoretical plates, and tailing factor were determined from the resulting standard chromatograms, and the findings are presented in Table 1.

Table 1: System Suitability Parameter

Name of drugs	Area	Retention time (min)	Tailing factor	No. of Theoretical Plate
Darunavir	767.35	5.8	0.74	7262.30
Dolutegravir	947.80	3.2	1.01	8142.50

Specificity:

To evaluate degradation behavior and potential analytical interferences, sample solutions containing Darunavir (32 µg mL^{-1}) and Dolutegravir (2 µg mL^{-1}) were prepared and injected into the system. Method specificity was established by examining any interference through analysis of the blank chromatogram, as well as the individual and combined chromatograms of Darunavir and Dolutegravir, as illustrated in Figures 7–10.

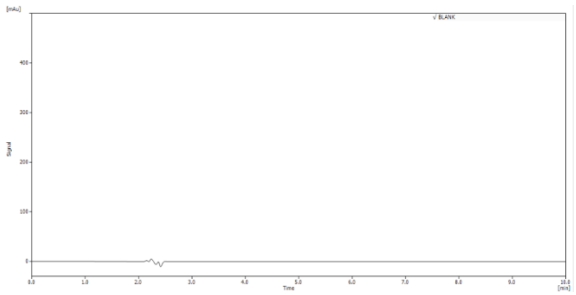

Figure 7: RP-HPLC Chromatogram of Blank in Acetonitrile: Phosphate Buffer (pH= 2.8) (80:20 %v/v) Flow rate: 1 ml/min at 270 nm

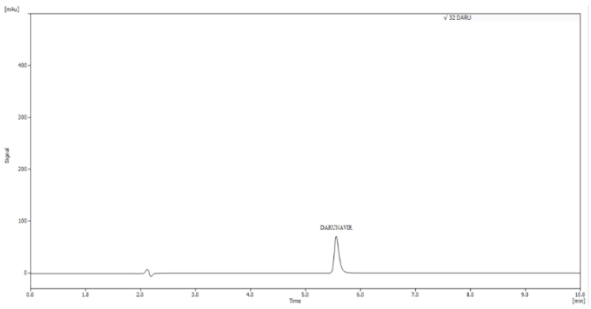

Figure 8: RP-HPLC Chromatogram of Darunavir (32 µg/ml) in Acetonitrile: Phosphate Buffer (pH= 2.8) (80:20 %v/v) Flow rate: 1 ml/min at 270 nm

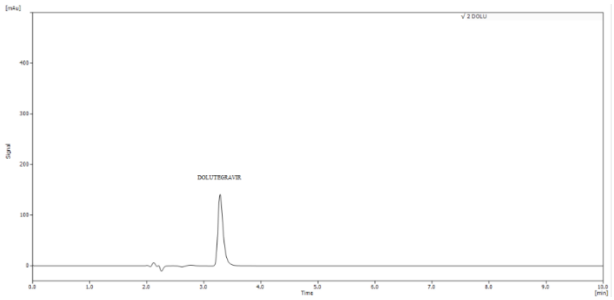

Figure 9: RP-HPLC Chromatogram of Dolutegravir (2 µg/ml) in Acetonitrile: Phosphate Buffer (pH= 2.8) (80:20 %v/v) Flow rate: 1 ml/min at 270 nm

Chinese Journal of Health Management
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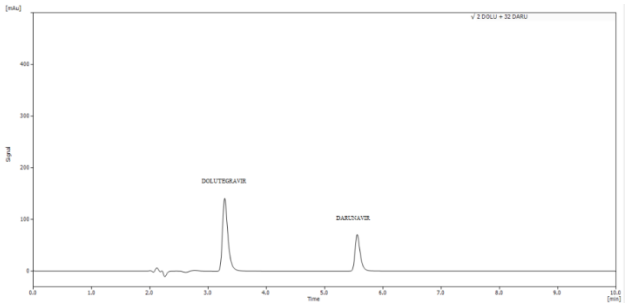

Figure 10: RP-HPLC Chromatogram of Darunavir (32 µg/ml) and Dolutegravir (2 µg/ml) in Acetonitrile: Phosphate Buffer (pH= 2.8) (80:20 %v/v) Flow rate: 1 ml/min at 270 nm

Linearity:
Accurately measured aliquots of Darunavir stock solution (100 µg mL^{-1}), specifically 1.6, 3.2, 4.8, 6.4, and 8.0 mL, and Dolutegravir stock solution (100 µg mL^{-1}), namely 0.1, 0.2, 0.3, 0.4, and 0.5 mL, were transferred into five separate 10 mL volumetric flasks. Each flask was diluted to volume with the mobile phase consisting of Acetonitrile: Phosphate Buffer (pH 2.8) in the ratio of 80:20 (v/v), to obtain final concentrations of 16, 32, 48, 64, and 80 µg mL^{-1} for Darunavir and 1, 2, 3, 4, and 5 µg mL^{-1} for Dolutegravir.

Subsequently, 20 µL of each prepared solution was injected into the RP-HPLC system using a Hamilton syringe, and the chromatographic analysis was performed. The corresponding chromatograms are presented in Figure 11.[23]

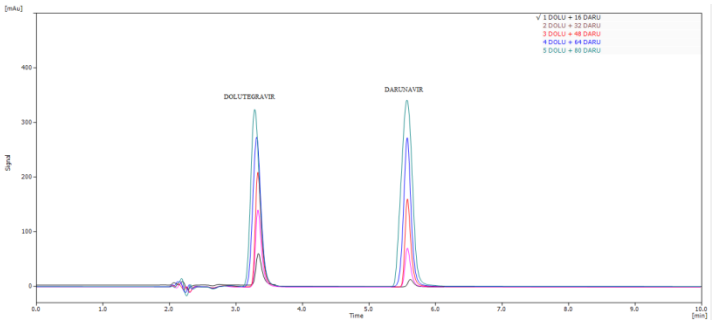

Figure 11: Overlay Chromatogram of Darunavir (16 – 80 µg/ml) and Dolutegravir (1 – 5 µg/ml) in Acetonitrile: Phosphate Buffer (pH= 2.8) (80:20 %v/v) Flow rate: 1 ml/min at 270 nm

Precision:
Method precision was assessed through intraday, interday, and repeatability studies. For intraday precision, Darunavir solutions at concentrations of 16, 32, and 48 µg mL^{-1} and Dolutegravir solutions at 1, 2, and 3 µg mL^{-1} were analyzed in triplicate within the same day. Interday precision was evaluated by examining the same concentration levels on three consecutive days. Repeatability was determined by analyzing Darunavir (32 µg mL^{-1}) and Dolutegravir (2 µg mL^{-1}) six times under identical conditions. The precision of the method was expressed in terms of percentage relative standard deviation (%RSD).[23]

Accuracy:
The pre-analyzed solution was spiked with known amounts of Darunavir and Dolutegravir at three concentration levels 50%, 100%, and 150%. Each level was injected in triplicate into the HPLC system, and the mean percentage recovery for both drugs was calculated.[23]

Detection Limit and Quantification Limit:
According to ICH guidelines, the Detection Limit and Quantification Limit are calculated using standardized equations.[23]

Robustness:
Robustness was evaluated by deliberately introducing small, controlled variations in analytical parameters such as detection wavelength and flow rate, and verifying that the system suitability criteria were consistently met.

Method robustness was further confirmed through repeated analysis under these modified conditions.[23]

RESULTS:

UV SPECTROPHOTOMETRIC METHHOD:

A reliable first order derivative Spectrophotometric method was developed for simultaneous estimation of Darunavir and Dolutegravir in synthetic mixture by UV Spectrophotometric.

Linearity:

The method demonstrated excellent linearity over the concentration ranges of 16–80 µg mL^{-1} for Darunavir and 1–5 µg mL^{-1} for Dolutegravir. The correlation coefficients were found to be 0.999 for Darunavir and 0.999 for Dolutegravir. The corresponding calibration curves are shown in Figures 12 and 13, and the detailed results are summarized in Table 2.

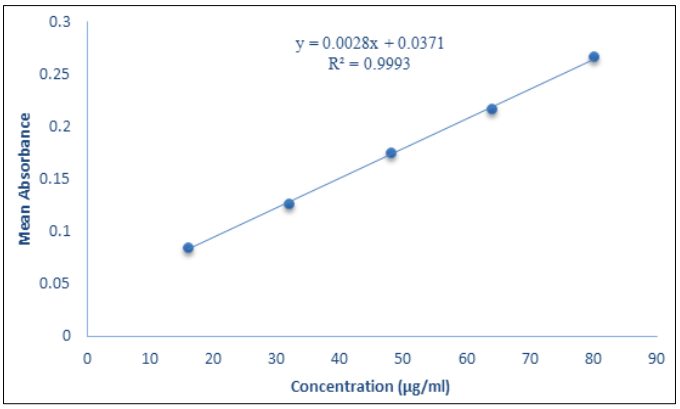

Figure 12: Calibration curve of Darunavir (16 – 80 µg/ml) at 258 nm

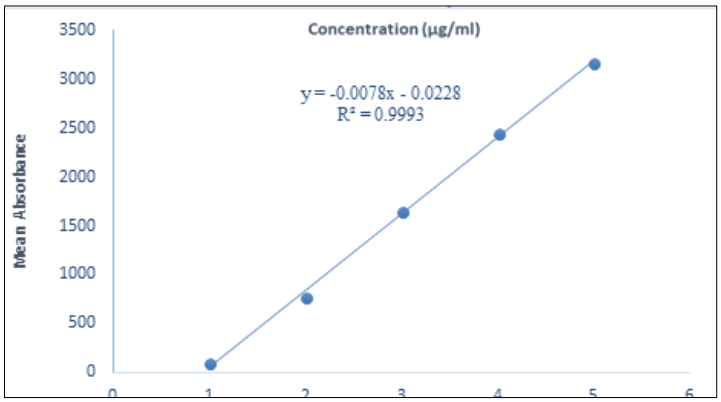

Figure 13: Calibration curve of Dolutegravir (1 – 5 µg/ml) at 326 nm

Table 2: Linearity of Darunavir and Dolutegravir

Concentration (µg/ml)		Mean Absorbance ± SD (n=6)		% RSD	
Darunavir	**Dolutegravir**	**Darunavir**	**Dolutegravir**	**Darunavir**	**Dolutegravir**
16	1	0.084 ± 0.001472	|-0.031| ± 0.00055	1.75	1.77
32	2	0.126 ± 0.001472	|-0.038| ± 0.00056	1.16	1.47
48	3	0.175 ± 0.001414	|-0.046| ± 0.00037	0.80	0.80
64	4	0.217 ± 0.001472	|-0.054| ± 0.00040	0.67	0.74
80	5	0.266 ± 0.001673	|-0.062| ± 0.00016	0.62	0.26

Precision:

Precision refers to the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogeneous sample. Method precision was evaluated through intraday, interday, and repeatability studies. The %RSD values for system precision are presented in Tables 3 and 4 for Darunavir and Dolutegravir, respectively. Since all %RSD values were below 2%, the method was confirmed to be precise, reproducible, and repeatable.

Table 3: Precision Study of Darunavir

Intraday Precision of Darunavir		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
16	0.083 ± 0.001269	1.53
32	0.125 ± 0.001462	1.17
48	0.174 ± 0.001705	0.98
Interday Precision of Darunavir		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
16	0.086 ± 0.001591	1.85
32	0.128 ± 0.001945	1.52
48	0.176 ± 0.002076	1.18
Repeatability of Darunavir		
Conc. (µg/ml)	**Mean Area ± SD (n=6)**	**% RSD**
32	0.126 ± 0.001335	1.06

Table 4: Precision Study of Dolutegravir

Intraday Precision of Dolutegravir		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
1	|-0.031| ± 0.00051	1.64
2	|-0.038| ± 0.00054	1.42
3	|-0.046| ± 0.00034	0.73
Interday Precision of Dolutegravir		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
1	|-0.032| ± 0.00057	1.78
2	|-0.039| ± 0.00062	1.58
3	|-0.048| ± 0.00061	1.27
Repeatability of Dolutegravir		
Conc. (µg/ml)	**Mean Area ± SD (n=6)**	**% RSD**
2	|-0.038| ± 0.00037	0.97

Accuracy:

Recovery studies were conducted at three concentration levels (50%, 100%, and 150%). Three replicates at each level were analyzed, and the mean percentage recoveries were calculated. As shown in Table 5, the recovery values for Darunavir and Dolutegravir ranged from 99.97% to 99.96% and 99.67% to 99.94%, respectively. Since all recovery values were within the acceptable range of 98.0%–102%, the method was confirmed to be accurate. These satisfactory recovery results further demonstrate the suitability of the method for routine quality control analysis.

Table 5: Recovery of Darunavir and Dolutegravir

Name of Drug	**%Level Of Recovery**	**Test Amount (µg/ml)**	**Amount of drug taken (µg/ml)**	**Spiked Std Amount (µg/ml)**	**Total amount Recovered (µg/ml)**	**% Recovery ±S.D (n=3)**
Darunavir	50	32	16	48	47.89	99.77±2.1153
	100	32	32	64	63.92	99.87±1.3487
	150	32	48	80	83.97	99.96±1.0712
Dolutegravir	50	2	1	3	2.99	99.67±1.1232
	100	2	2	4	3.99	99.75±1.2828
	150	2	3	5	4.997	99.94±1.1421

Detection Limit and Quantitation Limit:

The Limit of Detection (LOD) indicates the lowest concentration of analyte that can be detected, while the Limit of Quantification (LOQ) represents the lowest concentration that can be quantified with acceptable accuracy and precision, making it useful for the assessment of impurities or degradation products. As shown in Table 6, the LOD and LOQ values were 0.23 µg mL^{-1} and 0.70 µg mL^{-1} for Dolutegravir, and 1.73 µg mL^{-1} and 5.25 µg mL^{-1} for Darunavir, respectively.

Table 6: LOD and LOQ for Darunavir and Dolutegravir

Parameter	**Darunavir**	**Dolutegravir**
LOD(µg/ml)	1.73	0.23
LOQ(µg/ml)	5.25	0.70

Assay:
Three replicate injections of the same sample solution were analyzed, and the resulting chromatograms were recorded. Darunavir and Dolutegravir showed mean recoveries of 99.96% and 99.65%, respectively, as presented in Table 7.

Table 7: Analysis of Pharmaceutical Dosage form

Name of Drug	Amount taken (µg/ml)	amount Found(µg/ml)	%Assay± S.D (n=3)
Darunavir	32	31.99	99.96±2.127
Dolutegravir	2	1.993	99.65±3.013

RP-HPLC METHOD"
A simple and reliable isocratic RP-HPLC method was developed and validated for the simultaneous quantification of Darunavir and Dolutegravir in a synthetic mixture. The proposed method demonstrated satisfactory accuracy, precision, and rapid analysis. Both analytes exhibited significant absorbance at 270 nm, which was selected as the optimal detection wavelength.

Efficient chromatographic separation with well-resolved and symmetrical peaks was achieved using a mobile phase composed of Acetonitrile: Phosphate Buffer (pH 2.8) in the ratio of 80:20 % v/v, delivered at a flow rate of 1.0 mL min^{-1}. The separation was carried out on a Kromstar Vertex C18 column (250 × 4.6 mm, 5 µm) maintained at ambient temperature, with an injection volume of 20 µL, providing consistent reproducibility and repeatability.

Linearity:
The method demonstrated excellent linearity over the concentration ranges of 16–80 µg mL^{-1} for Darunavir and 1–5 µg mL^{-1} for Dolutegravir. The correlation coefficients were found to be 0.999 for Darunavir and 0.997 for Dolutegravir. The corresponding calibration curves are shown in Figures 14 and 15, and the detailed results are summarized in Table 8.

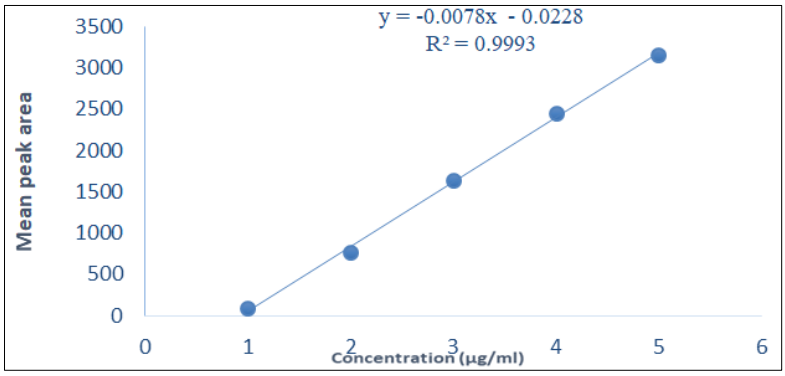

Figure 14: Calibration curve of Darunavir (16 – 80 µg/ml) at 270 nm

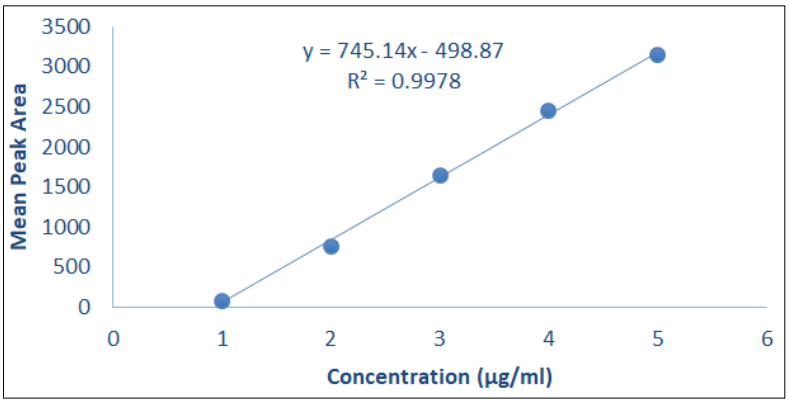

Figure 15: Calibration curve of Dolutegravir (1 – 5 µg/ml) at 270 nm

Table 8: Linearity of Darunavir and Dolutegravir

Concentration (µg/ml)		Area ± SD (n=6)		% RSD	
Darunavir	**Dolutegravir**	**Darunavir**	**Dolutegravir**	**Darunavir**	**Dolutegravir**
16	1	88.1±1.1089	308.85±4.2836	1.25	1.38
32	2	767.35± 2.4168	947.80±2.9220	0.31	0.30
48	3	1644.18±3.6834	1665.06±3.0865	0.22	0.18
64	4	2449.05±4.9289	2505.36±4.0207	0.20	0.16
80	5	3158.33±3.2282	3255.86±3.7638	0.10	0.11

Precision:

Precision refers to the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogeneous sample. Method precision was evaluated through intraday, interday, and repeatability studies. The %RSD values for system precision are presented in Tables 9 and 10 for Darunavir and Dolutegravir, respectively. Since all %RSD values were below 2%, the method was confirmed to be precise, reproducible, and repeatable.

Table 9: Precision Study of Darunavir

Intraday Precision of Darunavir		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
16	88.11±1.1189	1.27
32	767.35± 2.9159	0.38
48	1644.18±3.6171	0.22
Interday Precision of Darunavir		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
16	88.21±1.2790	1.45
32	767.35± 9.2849	1.21
48	1644.18±19.565	1.19
Repeatability of Darunavir		
Conc. (µg/ml)	**Mean Area ± SD (n=6)**	**% RSD**
32	767.36± 5.2180	0.68

Table 10: Precision Study of Dolutegravir

Intraday Precision of Dolutegravir		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
1	308.85±3.9532	1.28
2	947.80±3.7912	0.40
3	1665.06±2.6640	0.16
Interday Precision of Dolutegravir		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
1	308.85±4.6018	1.49
2	947.81±10.899	1.15
3	1665.06±9.4908	0.57
Repeatability of Dolutegravir		
Conc. (µg/ml)	**Mean Area ± SD (n=6)**	**% RSD**
2	947.82±5.1182	0.54

Accuracy:

Recovery studies were conducted at three concentration levels (50%, 100%, and 150%). Three replicates at each level were analyzed, and the mean percentage recoveries were calculated. As shown in Table 11, the recovery values for Darunavir and Dolutegravir ranged from 99.93% to 99.97% and 99.76% to 99.98%, respectively. Since all recovery values were within the acceptable range of 98.0%–102%, the method was confirmed to be accurate. These satisfactory recovery results further demonstrate the suitability of the method for routine quality control analysis.

Table 11: Recovery of Darunavir and Dolutegravir

Name of Drug	%Level Of Recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Spiked Std Amount (µg/ml)	Total amount Recovered (µg/ml)	% Recovery± S.D (n=3)
Darunavir	50	32	16	48	47.97	99.93±3.3612
	100	32	32	64	63.98	99.96±2.3221
	150	32	48	80	79.98	99.97±1.4523
Dolutegravir	50	2	1	3	2.993	99.76±2.0122
	100	2	2	4	3.997	99.92±3.4013
	150	2	3	5	4.999	99.98±1.2135

Detection Limit and Quantitation Limit:
The Limit of Detection (LOD) indicates the lowest concentration of analyte that can be detected, while the Limit of Quantification (LOQ) represents the lowest concentration that can be quantified with acceptable accuracy and precision, making it useful for the assessment of impurities or degradation products. As shown in Table 12, the LOD and LOQ values were 0.074 µg mL^{-1} and 0.22 µg mL^{-1} for Darunavir, and 0.018 µg mL^{-1} and 0.057 µg mL^{-1} for Dolutegravir, respectively.

Table 12: LOD and LOQ for Darunavir and Dolutegravir

Parameter	Darunavir	Dolutegravir
LOD(µg/ml)	0.074	0.018
LOQ(µg/ml)	0.22	0.057

Robustness:
Deliberate variations in flow rate and detection wavelength were introduced, and the results are summarized in Table 13. The findings indicated that these minor changes did not significantly affect the analytical performance, thereby confirming the robustness of the method.

Table 13: Robustness data for Darunavir and Dolutegravir

Sr no.	Parameter	Variation	% Assay ± SD (n=3)	
			Darunavir	Dolutegravir
1	Flow rate (1 ml/min) (±0.2 ml/min)	0.8 ml/min	98.54±702.37	99.91±604.51
2		**1.0 ml/min**	99.16±750.55	99.28±699.42
3		1.2 ml/min	99.57±781.02	99.56±706.47
1	Detection Wavelength (270 nm) (± 2 nm)	268 nm	99.65±709.45	98.64±680.12
2		**270 nm**	99.35±750.55	99.39±699.42
3		272 nm	99.75±763.76	98.18±702.04

Assay:
Three replicate injections of the same sample solution were analyzed, and the resulting chromatograms were recorded. Darunavir and Dolutegravir showed mean recoveries of 99.96% and 99.50%, respectively, as presented in Table 14.

Table 14: Analysis of Pharmaceutical Dosage form

Name of Drug	Amount taken (µg/ml)	amount Found(µg/ml)	%Assay± S.D (n=3)
Darunavir	32	31.99	99.96±1.0547
Dolutegravir	2	1.99	99.50±1.0547

DISCUSSION:
The method was systematically optimized to enhance its sensitivity and selectivity. Validation performed in compliance with ICH guidelines confirmed acceptable linearity, precision, accuracy, and robustness. Further investigations may explore the applicability of the method to different pharmaceutical formulations and dosage forms.

CONCLUSION:
The findings of the present investigation demonstrate that the developed UV spectrophotometric and RP-HPLC methods are simple, rapid, accurate, and economical for the simultaneous determination of Darunavir and Dolutegravir in a synthetic mixture. Statistical analysis revealed excellent repeatability, precision, and selectivity, fulfilling all ICH validation criteria. The methods also showed consistent performance under deliberate variations in chromatographic conditions, confirming their robustness and suitability for routine analytical applications.

Moreover, the high percentage recovery values along with low %RSD further validate the reliability of the methods for quality control purposes and their capability to detect minor fluctuations in drug concentration. The satisfactory LOD and LOQ values indicate adequate sensitivity, supporting the applicability of the methods in impurity profiling and stability-indicating studies.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

ABBREVIATIONS:

ICH: International Council for Harmonization; **UV:** Ultraviolet, **RP-HPLC:** Reverse phase High Performance liquid chromatography; **API:** Active Pharmaceutical Ingredient; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **RSD:** Relative Standard deviation

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