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A new validated stability-indicating RP-HPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form

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Abstract

Background: A fresh selective, rapid, accurate, precise and RP-HPLC stability-indicating method was developed and validated for the quantitative simultaneous determination of dolutegravir and lamivudine in the bulk as well as pharmaceutical dosage form. A chromatographic separation was done by using Inertsil ODS 3V (250 × 4.6 mm, 5 μm) column and mobile phase composed of phosphate buffer, pH 3.0:acetonitrile:methanol (50:20:30% v/v/v) with flow rate of 1.0 mL/min, and the detection of eluents was carried out at a wavelength of 257 nm utilizing a PDA detector. The drugs, dolutegravir and lamivudine, were subjected to varied conditions like base hydrolysis, acid hydrolysis, oxidation, thermal, photochemical and UV. The suggested method was analysed statistically and validated to fulfil requirements of International Conference on Harmonisation (ICH) and the validation covered accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness, ruggedness and specificity.

Results: The retention time of dolutegravir and lamivudine were observed to be 6.36 and 2.16 min, respectively. The method was found to be linear within the range of 14.98 to 91.25 μg/mL for lamivudine and 2.54 to 15.35 μg/mL for dolutegravir. The percentage recoveries (accuracy) for dolutegravir and lamivudine were in the range of 98.35 to 102.14% and 98.01 to 101.5%. The computed relative variance (%RSD) was within the suitable criterion of less than 2.0.

Conclusion: The suggested method was set to be precise as well as stability-indicating since no interfering degradant peaks and excipients were evident. All the peaks of degradation were successfully resolved by the use of the developed analytical method with altered retention times. Results obtained were analysed statistically and found to be acceptable in line with the ICH guidelines. Hence, such method is often employed successfully for routine analysis of active analytes in the bulk as well as pharmaceutical dosage form. It is going to be extended to review for its estimation in plasma and other biological fluids and may even be employed for quality control stability sample estimation and in cleaning method analysis during cleaning validation.

Keywords: Dolutegravir, Lamivudine, RP-HPLC, Acetonitrile, Methanol, Buffer

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Background

Lamivudine [1] (Fig. 1) (LAM) has activity against human immunodeficiency virus type I (HIV-I) as well as hepatitis B and may be a nucleoside reverse transcriptase inhibitor (NRTI). Dolutegravir [2] (Fig. 2) (DOL) may be a novel HIV-1 integrase inhibitor which acts by binding to site and blocking strand transfer step to retroviral integration of DNA. It is often an important phase of HIV replication cycle and can end in the viral activity inhibition. It is given in combination with novel drugs such as lamivudine, dolutegravir and lots of other drugs. As this drug is approved by FDA and made by GSK Healthcare, it is marketed as Dovato, a hard and fast dose combination product containing dolutegravir and lamivudine. Chemically, dolutegravir is (3S,7R)-N-[(2,4-difluorophenyl)methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclotetradeca-10,13-diene-13-carboxamide. Lamivudine is (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. These medications are available in the market and prescribed either individually or as a combination form.

Dolutegravir and lamivudine (Dovato), manufactured by GSK Healthcare, may be a combination antiretroviral agent approved by the FDA as an entire regimen for HIV-1 infection treatment in case of adults. It is a fixed-dose combination product consisting of dolutegravir 50 mg and lamivudine 300 mg. The recommended dose of the drug is once daily per oral with or without food in case of adults [3]. Dolutegravir, being an integrase strand transfer inhibitor, blocks replication of HIV by the amalgamation prevention of viral DNA into the host human immune T cells' genetic material [4]. In the pharmaceutical industry, an effective analytical method is needed to analyse a drug individually or simultaneously in combination with other drugs. Various methods of analysis were employed for quantitative determination of individual or multi-component combination assay of nucleoside polymerase inhibitor (NPI) in pharmaceutical dosage forms. Various HPLC [5–14], LC/MS/MS [15–19], HPTLC [20, 21], UV [22–24] and UPLC [25] assay methods were described within the literature regarding the estimation of lamivudine, abacavir, and a few other anti-retroviral drugs individually as well as in combination with other drugs. On the converse based on the review of literature, no official method for the stability-indicating simultaneous estimation of dolutegravir and lamivudine by RP-HPLC in the bulk and tablet dosage form was performed so far. Hence, there is an undertaken effort for research that has been made to develop and validate a novel and simple analytical method for the stability-indicating simultaneous estimation of dolutegravir and lamivudine in the bulk and pharmaceutical dosage form. The proposed novel method is able to separate all the active analytes present in the pharmaceutical dosage form and validated as per the guidelines of ICH (Q2 specification) [26].

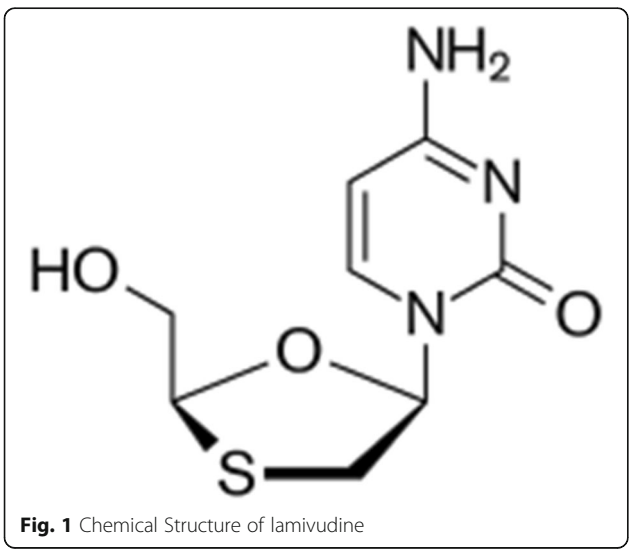

Fig. 1 Chemical Structure of lamivudine

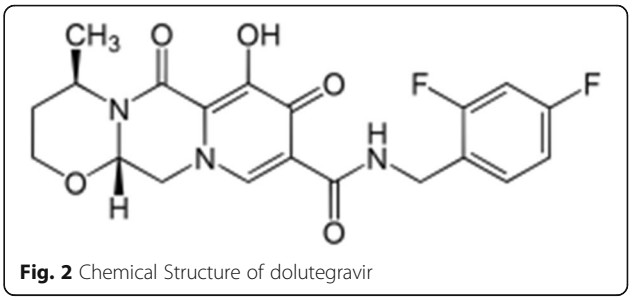

Fig. 2 Chemical Structure of dolutegravir

Methods

Materials

Dolutegravir and lamivudine were received as gift samples from Mylan Laboratories Limited, Hyderabad, India. The marketed pharmaceutical tablets of Dovato consisting of dolutegravir 50 mg and lamivudine 300 mg, respectively (manufactured by GSK Healthcare), were procured from the pharmacy. HPLC grade methanol, acetonitrile, Milli-Q water, orthophosphoric acid and analytical grade chemicals of sodium hydroxide, potassium dihydrogen phosphate, hydrogen peroxide and hydrochloric acid were purchased from Merck India Limited, Mumbai, India.

Instrumentation

HPLC-Waters alliance (Model-2695) was used to perform chromatographic separation which consists of a column oven, an in-built auto sampler and 2996 PDA detector. The information was obtained through the use of Empower-3-software. The column utilised was Inertsil ODS 250 × 4.6 mm, 5 μm. Meltronics sonicator was used to enhance the solubility of the drugs. For pH

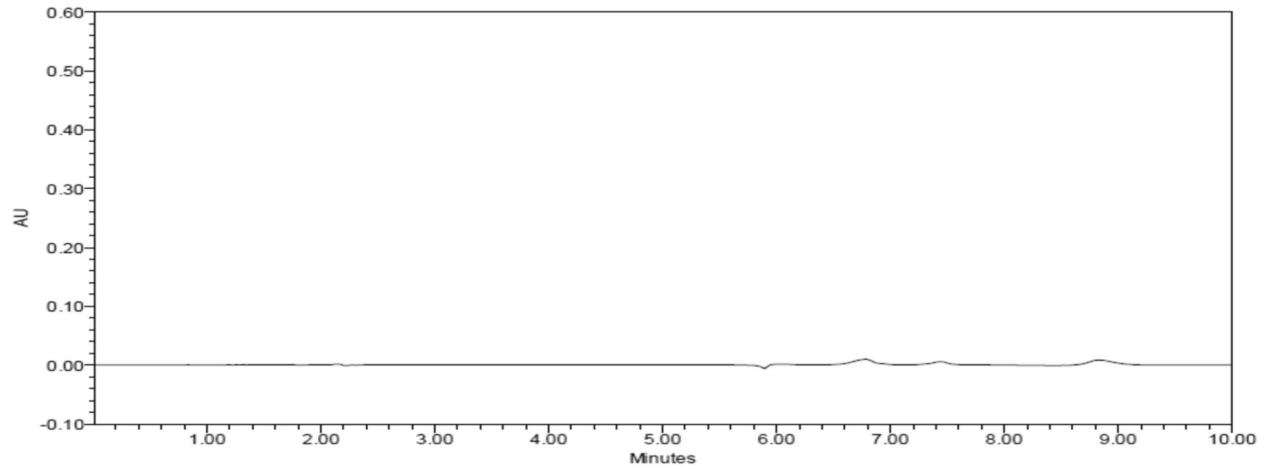

Fig. 3 Typical chromatogram of placebo

adjustment of the solution, Elico pH meter was employed. Sartorius balance was employed for weighing the samples.

Optimised chromatographic conditions

A chromatographic separation was achieved by using Inertsil ODS 3 V, 5 μm particle size, 250 × 4.6 mm column as a stationary phase and mobile phase composed of phosphate buffer, pH 3.0:acetonitrile:methanol (50:20:30% v/v/v) with a flow rate of 1.0 mL/min, and the eluents were detected at a wavelength of 257 nm utilising PDA detector. The HPLC system was operated at 30 °C and therefore the runtime was 10 min. Mode of separation was isocratic.

Phosphate buffer solution, pH 3.0 preparation

1.36 g of accurately weighed potassium dihydrogen orthophosphate was dissolved in 900 mL of HPLC grade water and the pH of the solution was adjusted to 3.0 with the addition of dilute phosphoric acid. Further, the volume was adjusted to 1000 mL with water and the prepared solution was filtered through 0.45 μm membrane filter and degassed to sonicate.

Preparation of mobile phase

Phosphate buffer solution pH 3.0, acetonitrile and methanol were taken within the ratio of 50:20:30% v/v/v.

Preparation of diluent

The prepared mobile phase was used as diluent.

Standard solution preparation

Ten milligrammes of each dolutegravir and lamivudine working standards was weighed accurately and transferred into an individual 10-mL clean and dry volumetric flasks. Half volume of methanol was added and then the

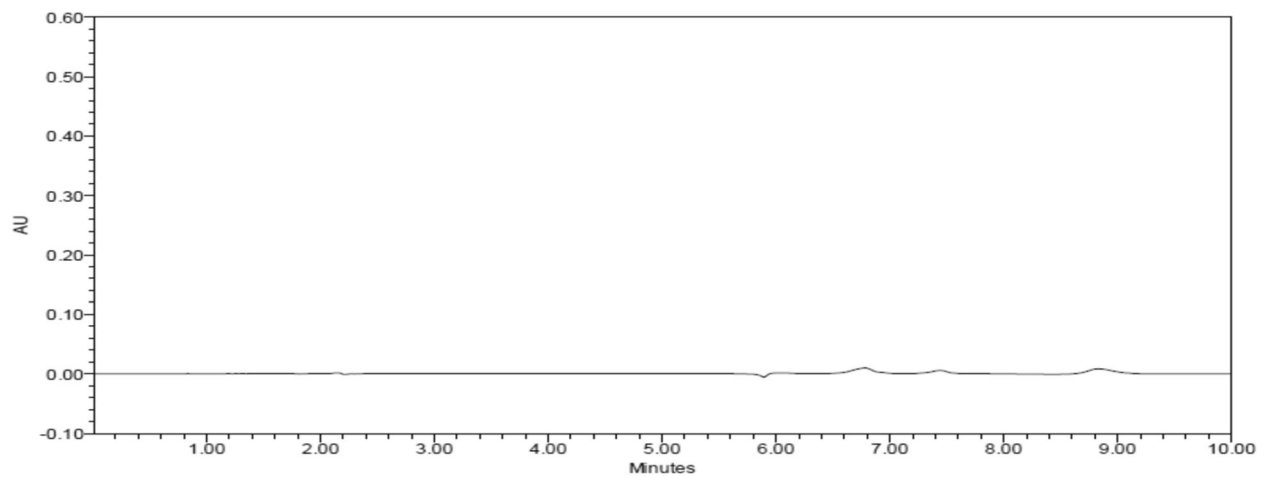

Fig. 4 Typical chromatogram of blank

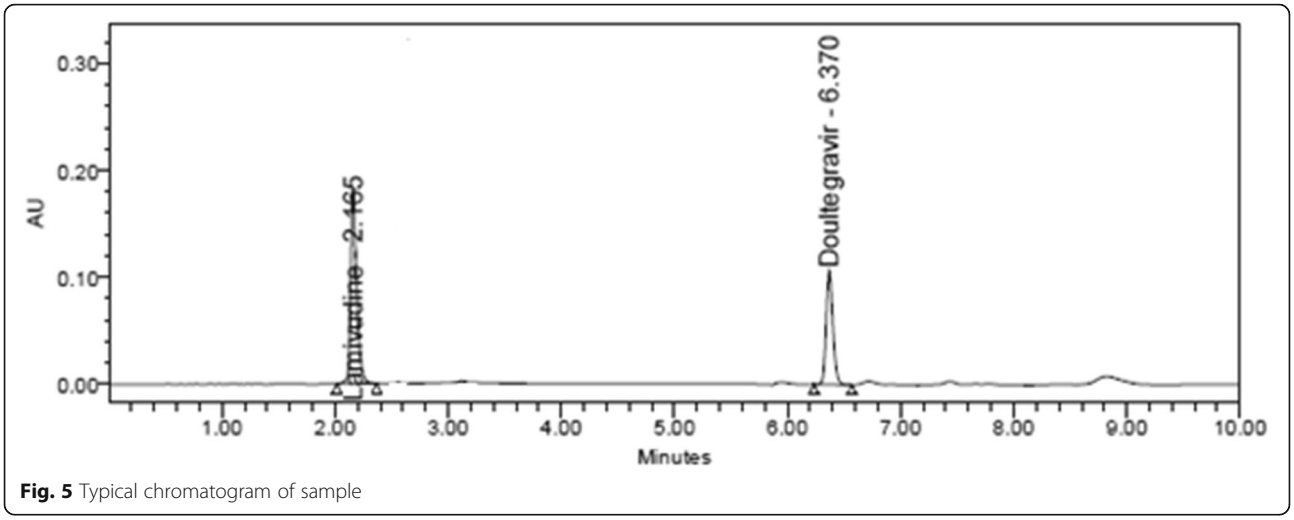

Fig. 5 Typical chromatogram of sample

volume was made up to the mark with diluent. 0.6 mL of lamivudine standard stock solution and 0.1 mL of dolutegravir standard stock solution were transferred into a 10-mL volumetric flask and the volume was made up to the mark with the diluent to get a standard solution containing 60 μg/mL of lamivudine and 10 μg/mL of dolutegravir.

Preparation of sample solution

Twenty tablets were weighed and the average weight of each tablet was calculated. Then a quantity of powder equivalent to one tablet was weighed and transferred into a volumetric flask (100 mL), followed by addition of 3/4th volume of diluent and sonicated for half-hour. Further, the volume was made up with the diluent and filtered through 0.45 μm filter. 0.5 mL of the above solution was transferred into a 25-mL volumetric flask, and the volume was made up to the mark with the diluent and mixed up well to get a sample solution containing 10 μg/mL of dolutegravir and 60 μg/mL of lamivudine.

Method validation

The developed method was validated according to the ICH guidelines with reference to accuracy, precision, system suitability, specificity, linearity, limit of quantification, limit of detection and forced degradation studies [26].

Table 1 Results of system suitability

Parameters for system suitability	Result		Acceptance criteria
	LAM	DOL	
Retention time	2.169	6.367	For information
%RSD for area count of 6 standard replicate injections	0.7	0.8	NMT 2.0
Tailing factor	1.34	1.12	NMT 2.0
Theoretical plates	7643	55924	NLT 2000
Resolution	N/A	22.51	NLT 2.0

System suitability

The system suitability was checked by the use of 6 replicate injections of freshly made standard solution. The perceived RSD values were within suitable limits (≤ 2.0%). Theoretical plates, resolution and tailing factor of dolutegravir and lamivudine were determined and originated to be well within the suitable limits.

Linearity

The linearity was performed for the prepared standard solutions of dolutegravir and lamivudine at various concentration levels, i.e. within a range of 14.98 to 91.25 μg/mL for lamivudine and 2.54 to 15.35 μg/mL for dolutegravir. Each measurement was administered in triplicate. The linearity was proven by multivariate analysis by least square method.

Precision

The precision of the method was demonstrated by intra-day and inter-day analysis. The concentration used for the precision studies was assumed as 100% (10 μg/mL for dolutegravir and 60 μg/mL for lamivudine). To review the intra-day precision, the analysis of drugs was repeated sixfold on the similar day, and for inter-day precision, the analysis of drugs was made for six

Table 2 Results of linearity study

S.No.	Analyte name	Linearity range (μg/mL)	Calibration curve equation	Correlation coefficient
1	Lamivudine	14.98–91.25	$y = 11{,}326.640x + 5694.835$	0.999
2	Dolutegravir	2.54–15.35	$y = 49{,}345.031x - 1016.567$	0.999

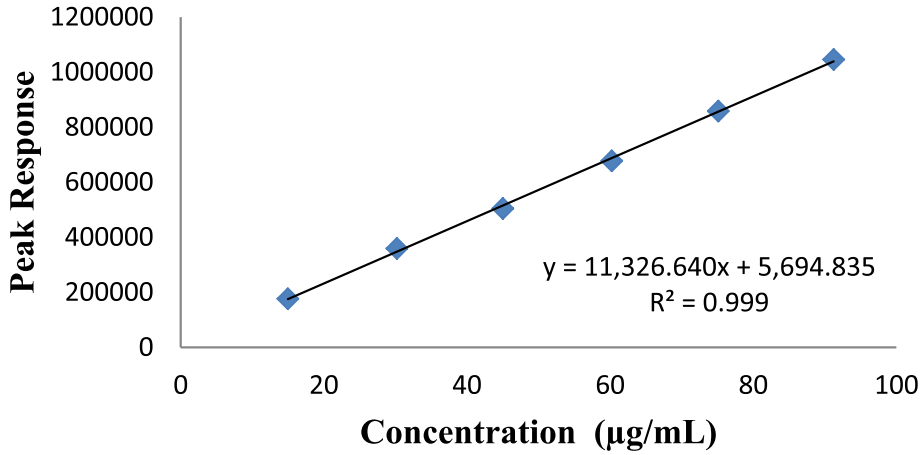

Fig. 6 Linearity plot of lamivudine

consecutive days for a concentration of 10 µg/mL for dolutegravir and 60 µg/mL for lamivudine.

Accuracy

For the determination of accuracy in the sample preparation method of the standard pattern, additions were done for the measurement of the recovery of drugs. A quantity of sample was taken and the standard drug was added at the levels of 50, 100 and 150%. The results obtained were analysed and observed to be within bounds.

Ruggedness

The ruggedness of the method was confirmed by analysing the sample by various analysts on various instruments on various days.

Robustness

The robustness was determined by varying three parameters from the optimised conditions of chromatography like mobile phase composition (± 5%), making small changes in the flow rate (± 0.1 mL/min) and column temperature (± 5 °C).

Limit of detection and limit of quantification (LOD and LOQ)

LOD and LOQ were computed with the use of waters Empower software for signal-to-noise ratio method.

Specificity

The specificity of the method was carried out by the injection of a blank solution (i.e. without any sample), followed by injection of 10 µL drug solution into the column under the optimised conditions of chromatography, in order to demonstrate the separation of two drugs

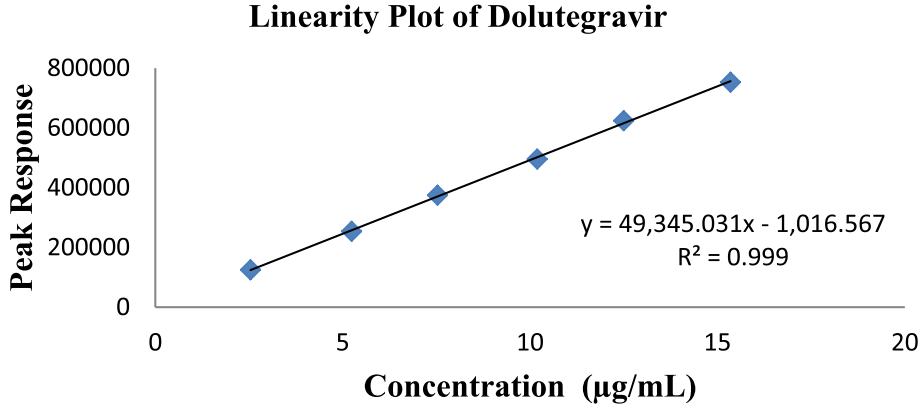

Fig. 7 Linearity plot of dolutegravir

Table 3 Limit of detection and limit of quantification

S.No.	Analyte name	Obtained concentration values (μg/mL)	
		LOD	LOQ
1	Lamivudine	0.40	0.88
2	Dolutegravir	0.04	0.11

dolutegravir and lamivudine from impurities present, if any.

Forced degradation studies

Such studies were carried out in order to demonstrate the optimised stability-indicating method and to find out if the method is able to quantify active pharmaceutical ingredient (API) accurately in the presence of degradation products that might be formed during various kinds of degradations pertained to drug sample. ICH guidelines (Q1A, Q1B, Q2B) highlight particular degradation environments such as base, acid, oxidation, photo and thermal stability.

Results

Development of RP-HPLC method

Suitable method was selected based on the character of the sample (neutral or ionic molecule), its relative molecular mass and solubility. Various conditions of chromatography were applied for the analysis of dolutegravir and lamivudine in both the bulk and pharmaceutical dosage form. The result of the analysis was within the suitable limit (not less than 2.0). Figs 3, 4 and 5 represent the chromatograms of placebo, blank and sample, respectively. This is often a sign of the specificity of developed RP-HPLC method.

System suitability

It was decided by making six replicate injections from the freshly made standard solutions. The RSD values were observed to be within the generally acceptable limit (≤ 2.0%). Theoretical plates, resolution and tailing factor of dolutegravir and lamivudine were determined and located to be within the suitable limits as shown in Table 1.

Linearity, limit of detection and limit of quantification

The test for linearity was performed by making standard solutions of dolutegravir and lamivudine at various concentration levels, i.e. in a range of 14.98 to 91.25 μg/mL for lamivudine and 2.54 to 15.35 μg/mL for dolutegravir. Each measurement was administered in triplicate. The linearity was proven by multivariate analysis by least square method. The results show that a linear relationship exists between peak area and concentration of drugs within the given range as shown in Table 2 and Figs. 6 and 7.

LOD and LOQ were observed to be 0.40 and 0.88 μg/mL, respectively, for lamivudine and 0.04 and 0.11 μg/mL, respectively, for dolutegravir. Signal-to-noise ratio values for LOD and LOQ were found to be 3.3 and 10.2 for lamivudine and 3.1 and 9.7 for dolutegravir and observed all the obtained results were found to be within acceptance criteria according to the guidelines of ICH and the results obtained were as shown in Table 3.

Recovery, precision and accuracy

Within-day precision and accuracy of the method were analyzed from replicate analysis (n = 6). Precision of the method was finalised by intra-day and inter-day analysis. The percentage relative variance (%RSD) was computed and was within the suitable criterion of < 2.0. Accuracy was represented as the percentage of the respective active analyte recovery and also plotted linearity graph (Figs. 8 and 9) between the typical amounts added and the average amount recovered for all the active analytes and located to be linear for all the concentration levels (Tables 4, 5 and 6).

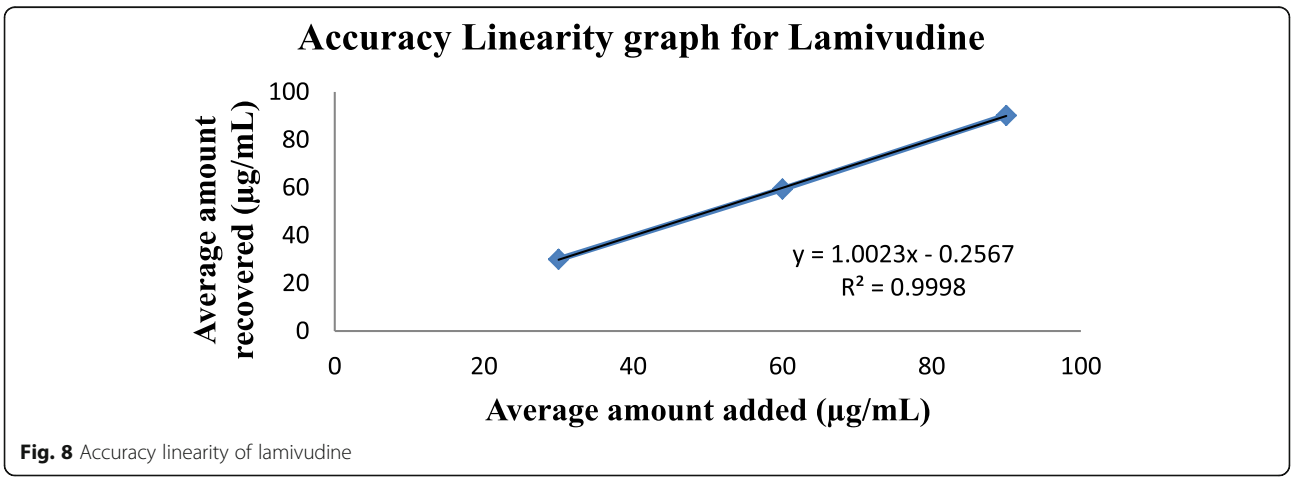

Fig. 8 Accuracy linearity of lamivudine

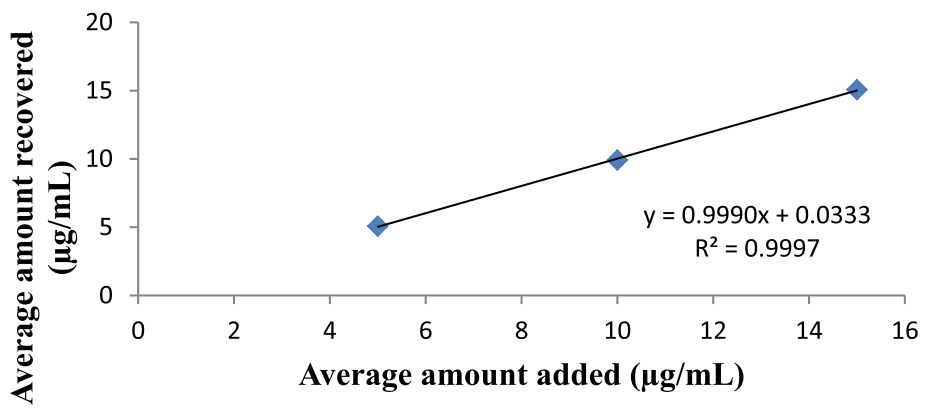

Fig. 9 Accuracy linearity of dolutegravir

Ruggedness

The ruggedness of the method was confirmed by the analysis of sample by various analysts on various instruments on various days. The results in Table 7 show that there were no significant changes within the chromatograms, which means that the developed RP-HPLC method is rugged in nature.

Robustness

The robustness was determined by varying three parameters from the optimised conditions of chromatography like mobile phase composition (± 5%), making small changes in flow rate (± 0.1 mL/min) and column temperature (± 5 °C). It had been observed that small changes in such operating parameters did not cause drastic changes of the retention time of the peak of interest, tailing factor (NMT 2.0), plate count (NLT 2000), resolution (NLT 2.0) and %RSD for six replicate injections (NMT 2.0) which were found to be within the acceptance criteria. The results show that the developed method is robust as shown in Tables 8 and 9.

Forced degradation studies

In the case of forced degradation studies, aliquots of stock solutions were treated separately with 1 mL of 2N sodium hydroxide (alkaline stability), 1 mL of 2N hydrochloric acid (acid stability), 1 mL of 20% hydrogen peroxide (oxidative degradation), photo stability degradation (exposure of drug at 200 watt h/m^2), exposure of sample drug solution at 105 °C for 6 h (dry heat degradation) and neutral degradation (refluxing with water at 60 °C for 6 h). The stability of such samples was cross-checked with a fresh sample on the particular day of the study. No interference was

Table 4 System precision, intra-day and inter-day results for the developed method

Injections	Peak response for system precision		Peak response for intra-day		Peak response for inter-day	
	LAM	DOL	LAM	DOL	LAM	DOL
1	562,424	409,726	554,251	414,526	532,548	409,726
2	565,574	412,864	556,214	412,754	529,874	408,725
3	575,969	414,115	564,875	413,548	535,412	406,548
4	561,345	426,527	541,482	422,547	532,571	410,254
5	578,738	413,765	567,422	419,875	536,948	405,874
6	586,696	411,675	559,642	412,548	529,824	413,487
AVG	571,791	414,779	557,314	415,966	532,862	409,102
S.D	10,231.19	5971.93	9230.43	4207.37	2882.31	2757.89
%RSD	1.78	1.44	1.66	1.01	0.54	0.67

Table 5 Method precision

Sample preparations	% assay	
	Lamivudine	Dolutegravir
Sample-1	99.7	99.6
Sample-2	100.1	100.1
Sample-3	99.6	99.7
Sample-4	100.8	99.2
Sample-5	99.5	100.4
Sample-6	100.3	100.0
Average	100.0	99.8
S.D	0.50	0.42
%RSD	0.5	0.4

Table 7 Intermediate precision results

Sample preparations	% assay	
	Lamivudine	Dolutegravir
Sample-1	98.9	99.2
Sample-2	100.1	100.0
Sample-3	99.4	99.6
Sample-4	100.2	98.9
Sample-5	99.7	100.1
Sample-6	100.4	99.9
AVG	99.8	99.6
S.D	0.56	0.48
%RSD	0.6	0.5

exhibited in the HPLC chromatograms of the degraded products at the respective analyte peaks. Hence, the individual analyte peak purity values found to be within the suitable limits; hence, the method was specific and stability-indicating (Tables 10 and 11).

Discussion

The major aim for the development of the chromatographic method was to urge a reliable technique for the quantification of dolutegravir and lamivudine from the bulk and pharmaceutical dosage form that can be employed also for degradation products. Various conditions of chromatography were applied for the analysis of dolutegravir and lamivudine in both the bulk and pharmaceutical dosage form. The results of analysis obtained was within the suitable limit (not but 2.0). The calibration curves were found to be linear over the concentration range of 14.98 to 91.25 μg/mL for lamivudine and 2.54 to 15.35 μg/mL for dolutegravir. The samples were analysed at 257 nm, the injection volume was 10 μL and the separation was done by utilising Inertsil ODS-3 V, (5 μm, 250 × 4.6 mm) column. The tailing factor and retention times were computed. The retention time of dolutegravir and lamivudine were found to be 2.169 and 6.367 min, respectively. The proposed column was selected which gave a pointy and symmetrical peak with 1.34 tailing factor and theoretical plates of 7643 for lamivudine and 1.12 tailing factor and theoretical plates of 55924 for dolutegravir. The resolution between dolutegravir and lamivudine is 22.51. The linearity of the method was confirmed statistically. The RSD values obtained for the accuracy and precision studies were but 2.0% which proved that the developed analytical method was accurate and precise.

It was concluded from the forced degradation studies that all the degradant peaks exhibited during the degradation were well resolved from major drugs, i.e. dolutegravir and lamivudine, and therefore the peak purity was passed, i.e. purity angle was less than purity threshold as per waters Empower-3 software. Therefore, the method is established to be stability-indicating.

Conclusion

The developed chromatographic method is simple, accurate and selective as well as proved to be stability-indicating for the simultaneous estimation of dolutegravir and lamivudine in the bulk and pharmaceutical dosage form. Preparation of sample is straightforward, the analysis time is short and therefore the elution is by isocratic method. To our present knowledge, no

Table 6 Results of accuracy study

% of target concentration	Lamivudine (% recovery)	Dolutegravir (% recovery)
50	100.16	101.48
100	99.03	99.21
150	100.21	100.48
Mean % recovery (n = 3)	99.8	100.4
%RSD	0.67	1.13

At each % level mean % recovery in the acceptable limit of 98.0 to 102.0%

Table 8 Robustness Results for Lamivudine

S. No.	Parameter	As such method	Used	%RSD for peak area (n = 6)	Average retention time	Plate count	Tailing factor
1	Flow rate (± 0.1 mL/min)	1.0 mL/min	0.9 mL/min	0.2	2.374	9457	1.35
			1.0 mL/min	0.7	2.169	7651	1.32
			1.1 mL/min	1.0	2.152	9145	1.30
2	Column temperature (± 5 °C)	30 °C	25 °C	0.3	2.134	8640	1.25
			30 °C	0.7	2.169	7657	1.31
			35 °C	1.5	2.150	9128	1.33
3	Mobile phase composition	Buffer: ACN: Methanol, 55:20:30% v/v	55:25:25	0.5	2.136	8648	1.30
			55:20:30	0.7	2.169	7653	1.31
			55:15:35	0.9	2.164	8475	1.33

Table 9 Robustness results for dolutegravir

S. No.	Parameter	As such method	Used	%RSD for peak area (***n*** = 6)	Average retention time	Plate count	Tailing factor	Resolution
1	Flow rate (± 0.1 mL/min)	1.0 mL/min	0.9 mL/min	0.8	6.687	50185	1.12	23.47
			1.0 mL/min	0.9	6.367	55945	1.14	22.51
			1.1 mL/min	1.2	6.354	53434	1.06	21.34
2	Column temperature (± 5 °C)	30 °C	25 °C	0.5	6.321	52290	1.11	22.49
			30 °C	0.9	6.367	55945	1.16	22.51
			35 °C	1.5	6.353	53457	1.07	22.37
3	Mobile phase composition	Buffer:ACN:Methanol, 55:20:30% v/v	55:25:25	0.7	6.322	52235	1.08	22.48
			55:20:30	0.9	6.367	55985	1.13	22.51
			55:15:35	1.2	6.364	51769	1.04	22.58

Table 10 Forced degradation study results for lamivudine

S.No	Sample name	% assay	% degradation	Purity angle	Purity threshold	Purity flag
1	Controlled sample	100.1	--	0.310	1.375	No
2	Acid degradation	95.1	5.0	0.325	0.463	No
3	Alkaline degradation	96.9	3.2	0.575	1.081	No
4	Peroxide degradation	94.8	5.3	1.612	2.175	No
5	Thermal degradation	98.3	1.8	0.268	0.294	No
6	UV degradation	99.7	0.4	0.318	0.335	No
7	Water degradation	98.4	1.7	0.264	0.298	No

Table 11 Forced degradation study results for dolutegravir

S. No	Sample name	% assay	% degradation	Purity angle	Purity threshold	Purity flag
1	Controlled sample	99.9	–	0.122	0.378	No
2	Acid degradation	95.1	4.8	0.397	0.475	No
3	Alkaline degradation	96.3	3.6	0.761	1.199	No
4	Peroxide degradation	91.4	8.5	1.618	2.154	No
5	Thermal degradation	97.6	2.3	0.397	0.498	No
6	UV degradation	98.1	1.8	0.454	0.492	No
7	Water degradation	98.6	1.3	0.292	0.458	No

attempts have yet been made to estimate this multidrug mixture by the stability-indicating analytical procedure. All the active ingredients were profitably resolved with good resolution and quantified. Hence, the suggested validated stability-indicating method was successfully employed to work out dolutegravir and lamivudine in the bulk and pharmaceutical dosage form. It is going to be extended to review for its estimation in plasma and other biological fluids and may even be employed for quality control stability sample estimation and in cleaning method analysis during cleaning validation.

Abbreviations

DOL: Dolutegravir; HPLC: High-performance liquid chromatography; ICH: International Conference on Harmonisation; LAM: Lamivudine; LOD: Limit of detection; LOQ: Limit of quantification; NMT: Not more than; NLT: Not less than; RSD: Relative standard deviation

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Authors' contributions

NK conducted the literature study and designed, developed and validated the new RP-HPLC method. NK and SN compiled, analyzed and interpreted the data. NK and SN wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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