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A NEW METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC OF LAMIVUDINE AND RALTEGRAVIR BULK AND ITS DOSAGE FORM

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ABSTRACT

The estimation of Lamivudine and Raltegravir was done by RP-HPLC. The assay of Lamivudine and Raltegravir was performed with tablets and the % assay was found to be 100.48 and 98.84 which shows that the method is useful for routine analysis. The linearity of Lamivudine and Raltegravir was found to be linear with a correlation coefficient of 0.998 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 1.31 and 0.96 for Lamivudine and Raltegravir which shows that the method is precise. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

Key Words: Lamivudine, Raltegravir, RP-HPLC

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INTRODUCTION

In the modern pharmaceutical industry, HPLC is a major analytical tool applied at all stages of drug discovery, development and production. Fast and effective development of rugged analytical HPLC methods is more efficiently undertaken with a thorough understanding of HPLC principles, theory and instrumentation. Liquid Chromatography (LC), Which is one of the forms of Chromatography, is an Analytical technique that is used to separate a mixture in solution into its individual components.

The separation relies on the use of two different "phases" or "immiscible layers," one of which is held stationary while the other moves over it. Liquid Chromatography is the generic name used to describe any chromatographic procedure in which the mobile phase is a liquid. The separation occurs because, under an optimum set of conditions, each component in a mixture will interact with the two phases differently relative to the other components in the mixture.

HPLC is the term used to describe Liquid Chromatography in which the liquid mobile phase is mechanically pumped through a column that contains the stationary phase. An HPLC instrument, therefore, consists of an injector, a pump, a column, and a detector.

Validation may be viewed as the establishment of an experimental data base that certifies an analytical method performs in the manner for which it was intended and is the responsibility of the method development laboratory. Method transfer, on the other hand, is the introduction of a validated method into a designated so that it can be used in the same capacity for which it was originally developed. (1-3).

Lamivudine (4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one) is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination.

Raltegravir (N-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-2-{2-[(5-methyl-1,3,4-oxadiazol-2-yl)formamido]propan-2-yl}-6-oxo-1,6-dihydropyrimidine-4-carboxamide) targets integrase, an HIV enzyme that integrates the viral genetic material into human chromosomes, a critical step in the pathogenesis of HIV. The drug is metabolized away via glucuronidation.

Literature reveals different methods for their analysis in their formulations. But our present plan is to develop a new, simple, precise& accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated (4-9).

MATERIALS AND METHOD

Wave length selection

UV spectrum of 10 µg / ml Lamivudine and Raltegravir in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 240. At this wavelength both the drugs show good absorbance.

Optimization of Column

Inersil ODS (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.9 ml/min flow.

Validation of the method was done according to ICH guidelines.

ASSAY

Standard Solution Preparation

Accurately weigh and transfer 15mg of Lamivudine & 30mg of Raltegravir working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1ml of Lamivudine & Raltegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of Lamivudine & Raltegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation

Accurately weigh and transfer equivalent to 15mg of Lamivudine & 30mg Raltegravir equivalent weight of the sample into a 10ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of Lamivudine & Raltegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of Lamivudine & Raltegravir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Lamivudine & Raltegravir peaks and calculate the %Assay by using the formulae (10).

RESULTS AND DISCUSSION

The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Lamivudine and Raltegravir by RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Lamivudine and Raltegravir by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Lamivudine and Raltegravir in pharmaceutical dosage form.

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Lamivudine and Raltegravir was obtained and the isobestic point of Lamivudine and Raltegravir showed absorbance's maxima at 240 nm.

The spectrums are shown in Fig-1.

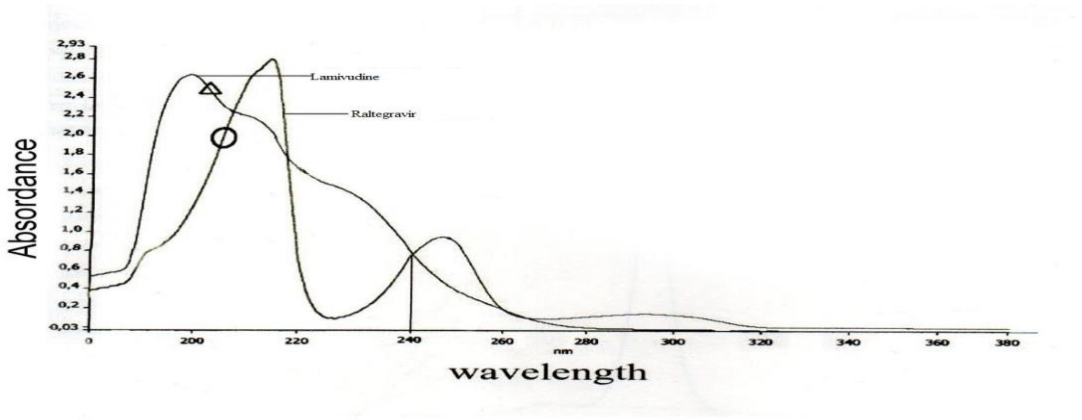

Figure-1 Wavelength selection Lamivudine and Raltegravir

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown in fig-2 and 3.

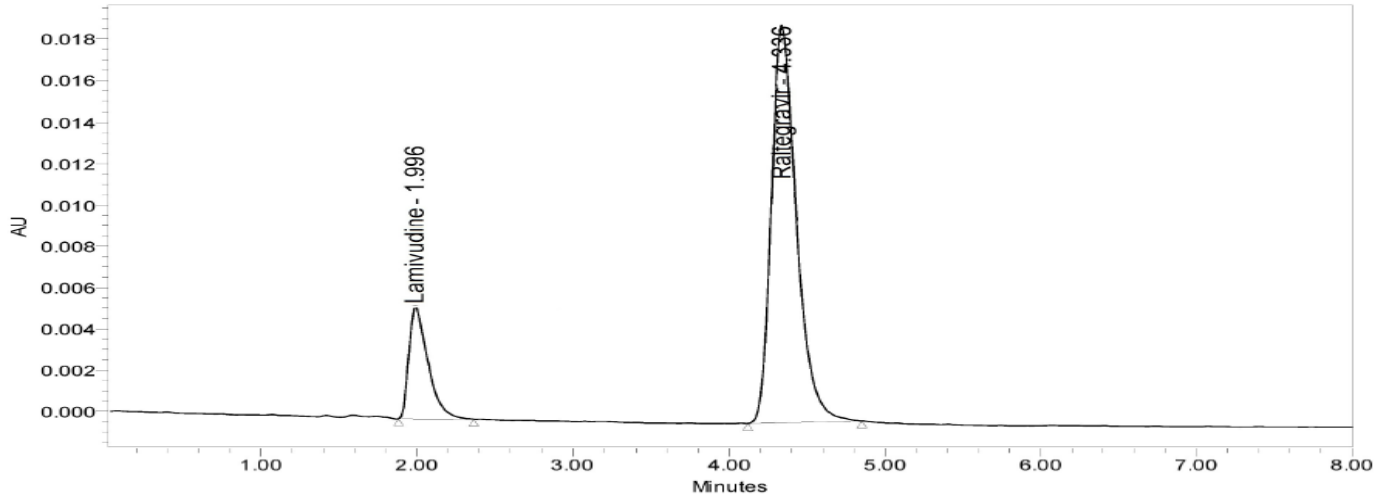

Figure-2 Chromatogram for Standard

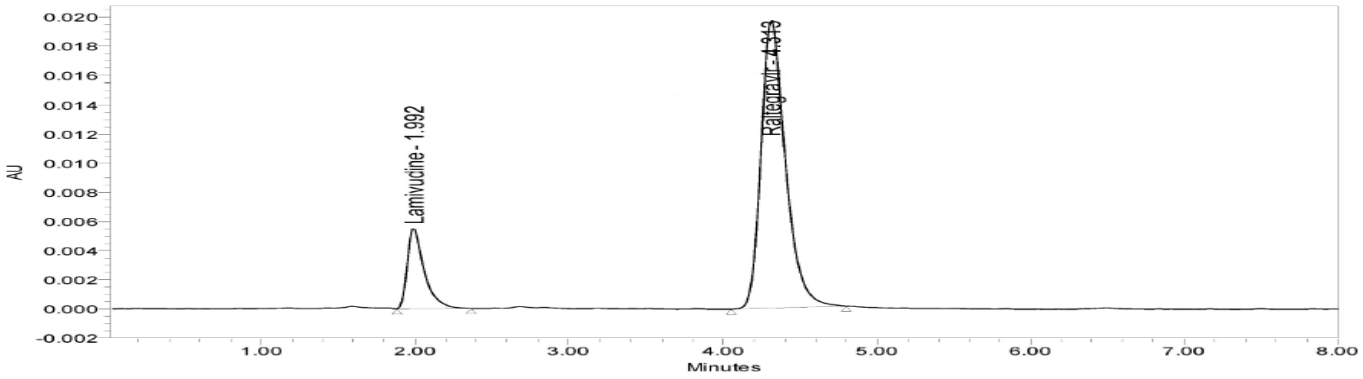

Figure-3 Chromatogram for Sample

The linearity range was found to lie from 15µg/ml to 75µg/ml of Lamivudine, 30µg/ml to 150µg/ml of Raltegravir and is shown in Table-1.

Table-1 Area of different concentration of Lamivudine and Raltegravir

S. No	Lamivudine		Raltegravir	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	15	14891	30	67496
2	30	30568	60	151923
3	45	43243	90	223324
4	60	59103	120	304753
5	75	71989	150	374626

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown in fig-4-8.

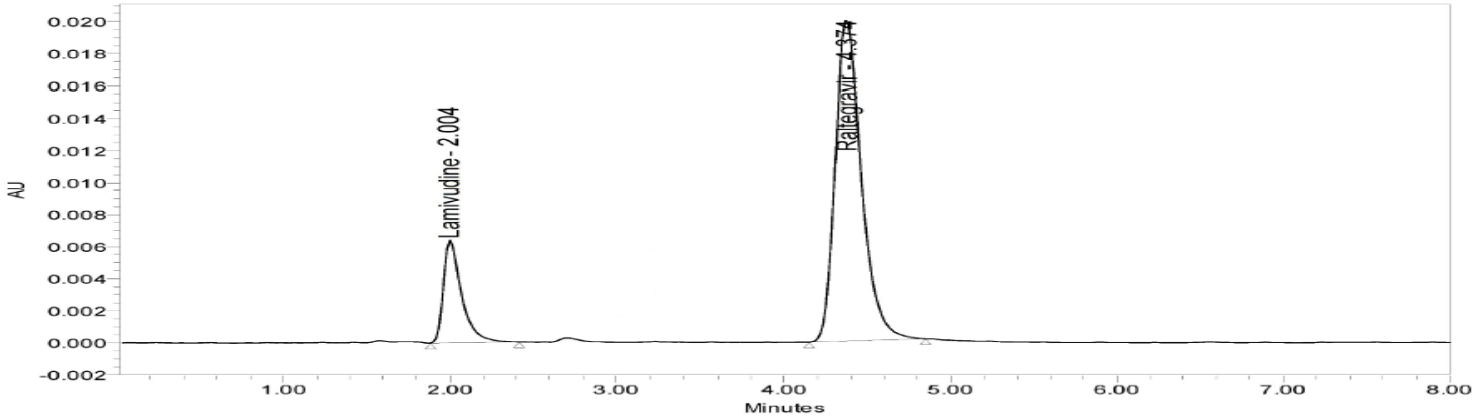

Figure-4 Chromatogram for Precision -1

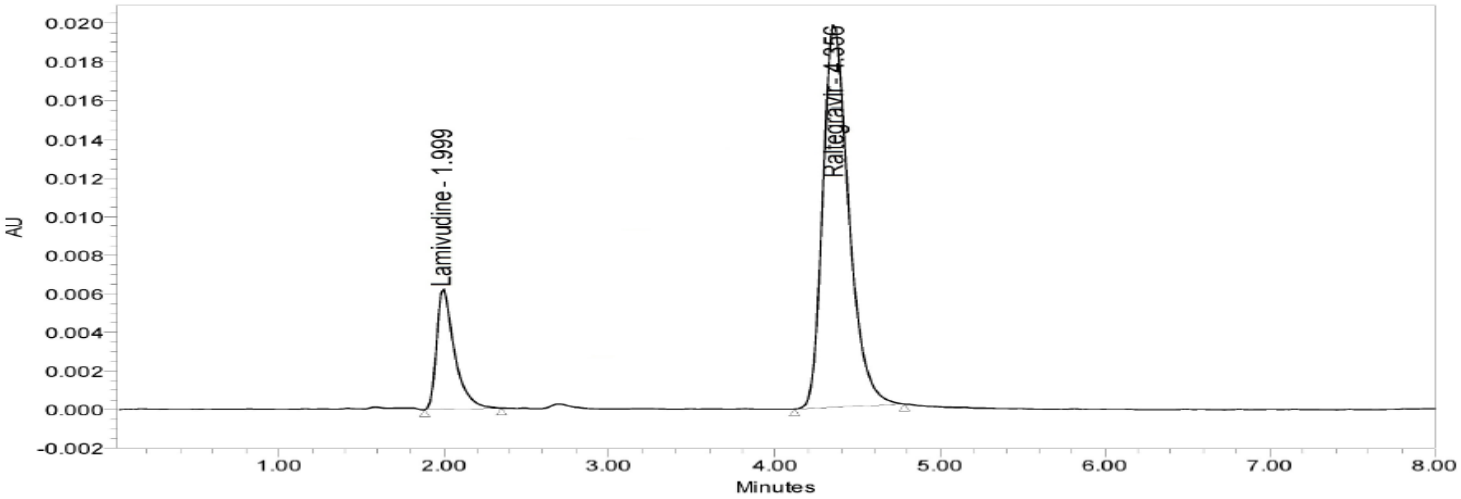

Figure-5 Chromatogram for Precision -2

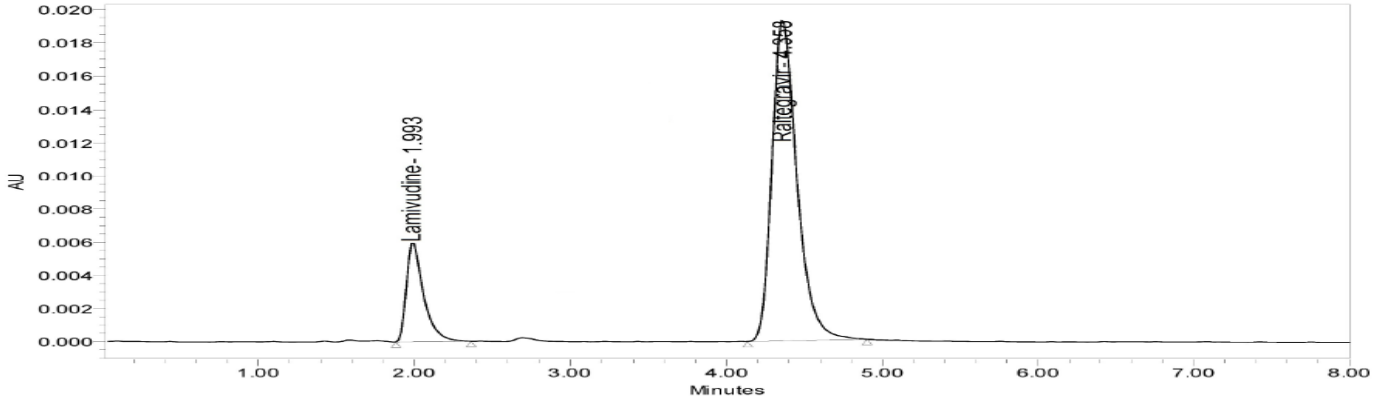

Figure-6 Chromatogram for Precision -3

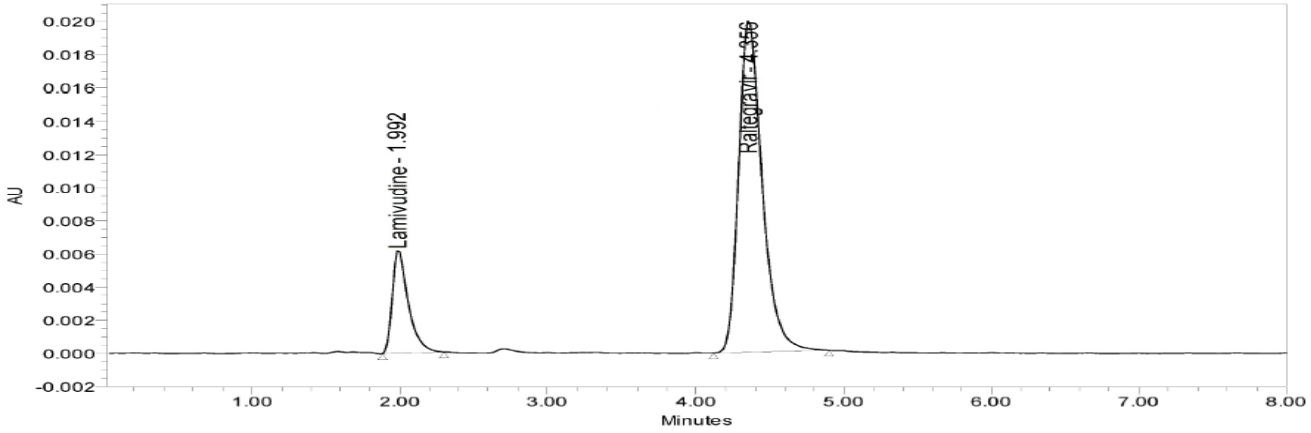

Figure-7 Chromatogram for Precision -4

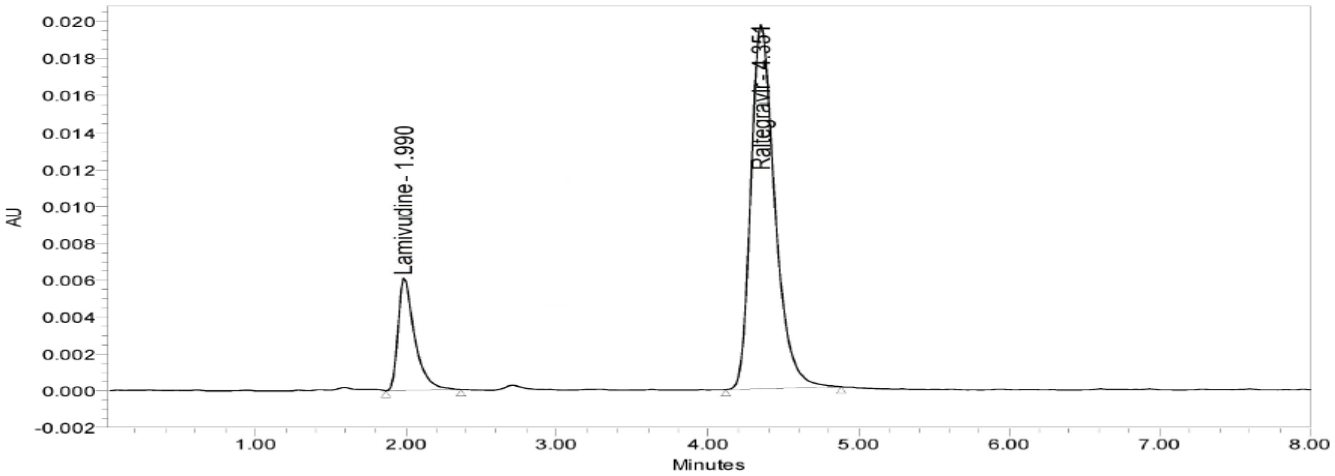

Figure-8 Chromatogram for Precision -5

The %RSD for the standard solution is below 1, which is within the limits hence method is precise.
There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.
Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated and is given in Table-2 and 3.

Table-2 Accuracy (recovery) data for Lamivudine

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	22057	7.5	7.61	100.58	100.12
100%	43141	15	14.88	99.18	
150%	66636	22.5	22.98	100.60	

*Average of three determinations

Table-3 Accuracy (recovery) data for Raltegravir

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	106481	15	14.84	98.94	99.89
100%	214518	30	29.90	99.67	
150%	326302	45	45.48	101.07	

*Average of three determinations

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.
The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. The result obtained is within the limit.

The standard and samples of Lamivudine and Raltegravir were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

CONCLUSION

Development of new RP-HPLC method for the estimation of Lamivudine and Raltigravir in the tablet dosage form and validation of the developed method was carried out. The assay of Lamivudine and Raltegravir was performed with tablets and the % assay was found to be 100.48 and 98.84 which shows that the method is useful for routine analysis. The linearity of Lamivudine and Raltegravir was found to be linear with a correlation coefficient of 0.998 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 1.31 and 0.96 for Lamivudine and Raltegravir which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 1.48 and 1.35 for Lamivudine and Raltegravir which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.12% and 99.89% for Lamivudine and Raltegravir. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ are 3 and 10.The LOD and LOQ for Lamivudine was found to be 2.96 and 9.96 and LOD and LOQ for Raltegravir was found to be 2.95 and 9.98. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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