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RP-HPLC METHOD FOR THE DETERMINATION OF LAMIVUDINE AND RALTEGRAVIR IN TABLET DOSAGE FORM

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ABSTRACT

Aim of 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.

Key Words: LC method, sildenafil citrate, Chromatographic separation

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INTRODUCTION

Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation.The technique employed in quantitative analysis is based upon the quantitative performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction, or ascertaining the amount of reaction product obtained. Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods there can be no "second quality" in drugs. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the Physico-chemical methods, the most important are optical (Refractometry, Polarimetry, Emission, Fluorescence methods of analysis, Photometry including Photocolorimetry and Spectrophotometry covering UV-Visible and IR regions and Nephelometry or Turbidimetry) and chromatographic (Column, Paper, TLC, GLC, HPLC) methods. Methods such as Nuclear Magnetic Resonance and Para Magnetic Resonance are becoming more and more popular. The combination of Mass Spectroscopy with Gas Chromatography and Liquid Chromatography are the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base,

precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis (1-4).

Literature reveals different methods for their analysis in their analysis in their formulation. 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.

MATERIALS AND METHODS

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 (5, 6).

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 20µL 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.

Accuracy

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

RESULTS AND DISCUSSION

System suitability

It was found from above data that all the system suitability parameters for developed method were within the limit (Fig-1 and Table-1).

Fig-1 Chromatogram for system suitability

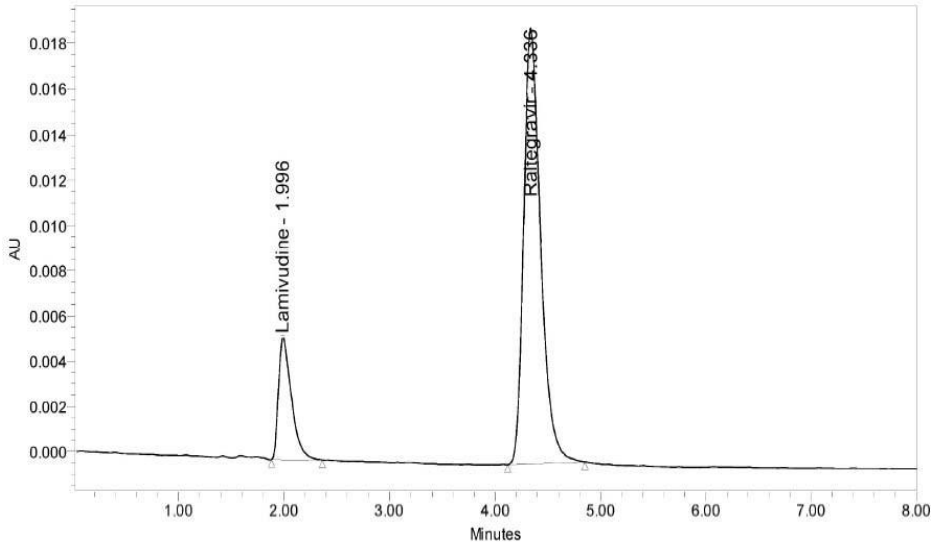

Table-1 Results of system suitability parameters

S. No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Lamivudine	1.996	42115	5573		1.65	2559.08
2	Raltegravir	4.336	215502	19212	9.46	1.35	3511.35

The linearity range was found to lie from 15µg/ml to 75µg/ml of Lamivudine, 30µg/ml to 150µg/ml 0f Raltegravir and results are shown in table-2 and fig-2 and 3.

Table-2 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

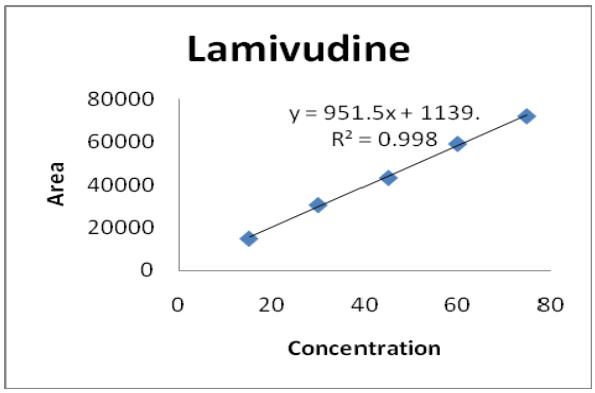

Fig-2 Calibration graph for Lamivudine

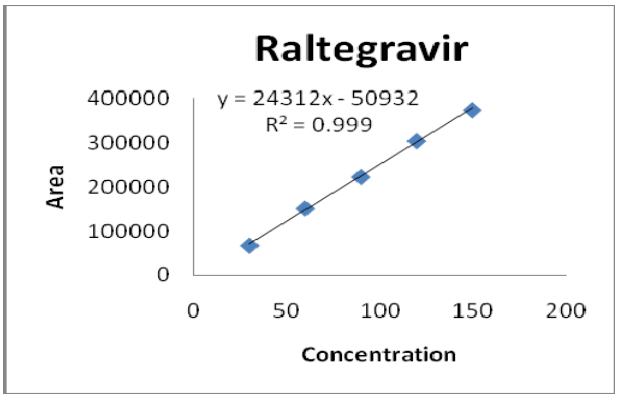

Fig-3 Calibration graph for Raltegravir

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated and results are given in table-3 and 4. The percentage recovery was found to be within the limit (97-103%). The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table-3 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%	43141	15	14.88	99.18	
150%	66636	22.5	22.98	100.60	100.12

***Average of three determinations**

Table-4 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	
100%	214518	30	29.90	99.67	
150%	326302	45	45.48	101.07	99.89

***Average of three determinations**

CONCLUSION

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 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 is 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.

REFERENCES

1. Becket and Stenlake, Practical pharmaceutical chemistry, part 24th edition CBS publications and distributors, 2005.
2. P.D. Sethi, HPLC quantitative analysis of pharmaceutical formulations CBS publications and distributors, 1st edition, 2001.
3. B.K Sharma, Instrumental method of chemical analysis, 23rd edition, goal publishers 2004.
4. Lloyd R.Snyder, Joseph J. Kirkland, Joseph L. Glajch Practical HPLC method development, second edition.
5. Validating chromatographic methods, David M.Bliesner.

6. International conference on harmonization: ICH Q 2 (R1) Validation of Analytical Procedures: Text and Methodology 1995.