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SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND RALTEGRAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Lamivudine and Raltegravir in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. Mobile phase ortho phosphoric acid buffer:Acetonitrile 40:60 were set (Buffer PH 2.45 adjusted with Triethylamine), Symmetry C 18 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 260 nm. As the methanol content was increased Lamivudine and Raltegravir got eluted with good peak symmetric properties. The retention times for Lamivudine and Raltegravir was found to be 2.335 min and 3.400 min respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to150 % levels, R2 value was found to be as 0.999.

Key Words: Lamivudine, Raltegravir, Chromatography

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INTRODUCTION

Lamivudine is a reverse transcriptase inhibitor and zalcitabine analog in which a sulfur atom replaces the 3' carbon of the pentose ring. It is used to treat Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV). [1]Lamivudine 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.[2-4]Raltegravir 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 Brand name is Dutrebis, Merck co & Ltd and is soluble in water. [5]

INSTRUMENTS AND EQUIPMENTS

System : **Waters 2690 separation module**
Pump : Analytical HPLC Binary Gradient pump
Detector : **photo diode array detector**
Software : Empower software
Column : Symmetry (250×4.6mm, 5µ) ODS C-18 RP-column
Injector : Auto injector with 130µl capacity
Sonicator : Analytical Technologies Limited- Ultrasonic cleaner

CHEMICALS AND SOLVENTS

Table 1: Particulars of Chemicals used

Chemical / Reagent	Grade	Manufacturers
Methanol	HPLC Grade	Merck
Ortho phosphoric acid	HPLC Grade	Merck
Potassium dihydrogen ortho phosphate	HPLC Grade	Merck
Methanol	HPLC Grade	Merck
Tri ethyl amine	HPLC Grade	Merck
Water	HPLC	Merck

Selection of chromatographic condition

Proper selection of the method depends upon the nature of the sample, its molecular weight and solubility. The drugs selected in the present study are polar in nature and hence reversed phase or ion-pair or ion exchange chromatography method may be used. The reversed phase HPLC was selected for the separation because of its simplicity and suitability.

Selection of detection wavelength:

The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected. Standard solutions of Lamivudine and Raltegravir were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 260 nm was selected as the detection wavelength for the present study.

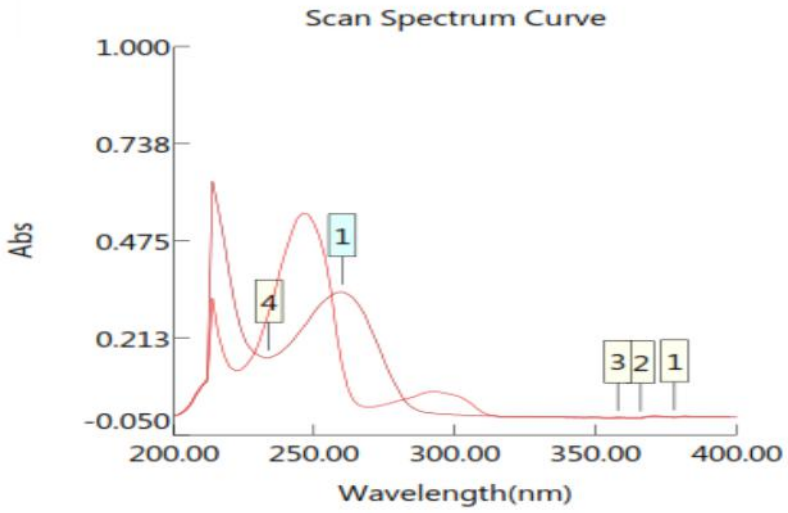

Fig. 1: Over line Spectrum of Lamivudine and Raltegravir

Preparation of Buffer: About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and pH 2.5 was adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

Preparation of mobile phase: Mobile phase consist of buffer: Acetonitrile of P^H 2.5 (40:60) was taken sonicated and degassed for 10min and filtered through 0.45 µm nylon membrane filter

Standard Preparation: Weigh accurately 10 mg Lamivudine Working Reference Standard and 10mg of Raltegravir Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase.

(Stock solution)

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Table 2: Chromatographic conditions of optimised Trial

Parameters	Description
Flow rate	1ml min^{-1}
Column	Symmetry C_{18} Column (250mm x 4.6mm)5µm.
Mobile Phase	Phosphate buffer: acetonitrile P^H 2.5 (40:60 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	260nm
Injection volume	20µl
Run time	10min

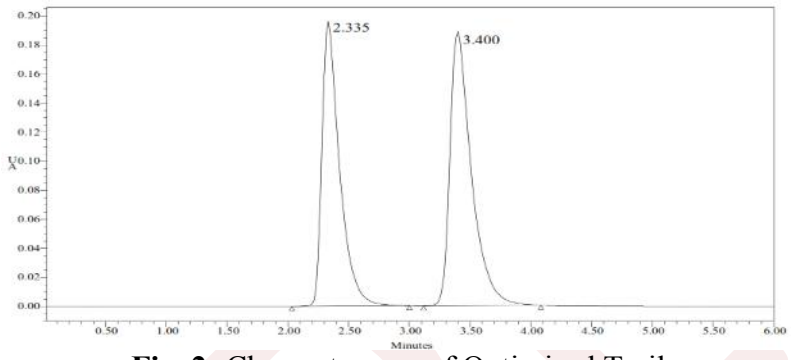

Fig .2: Chromatogram of Optimised Trail

Assay: The % assays of Lamivudine and Raltegravir were found to be 99.77% and 100.12% respectively. Thus, % Assay results were found to be within the limits i.e., 98-102% for both the drugs. Hence the developed method can be routinely used for the simultaneous estimation of Lamivudine and Raltegravir in the marketed formulations.

Table 4: Results of Assay

Parameters	Lamivudine	Raltegravir
Standard peak area	810802	681469
Test peak area (mean)	828933	687178
Average Weight	694.2mg	694.2mg
% Purity of Standard	99.50	99.58
Amt obtained	399.88 mg	150.10 mg
% Assay	99.77%	100.12%

Linearity: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Serial dilutions of Lamivudine and Raltegravir (10-50µg/ml and 20-100 µg/ml) were injected into the column and detected at a wavelength set at 260 nm. The calibration curve was obtained by plotting the concentration vs. peak area.

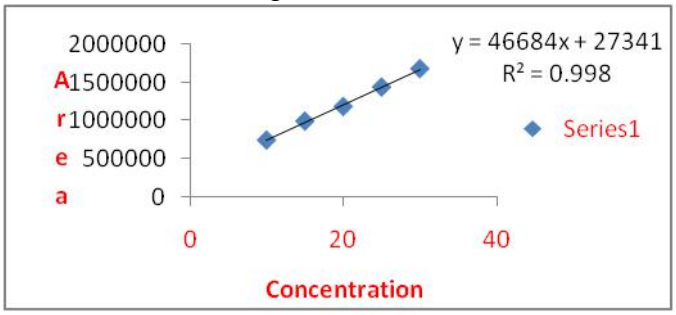

Fig.3: Calibration curve for lamivudine

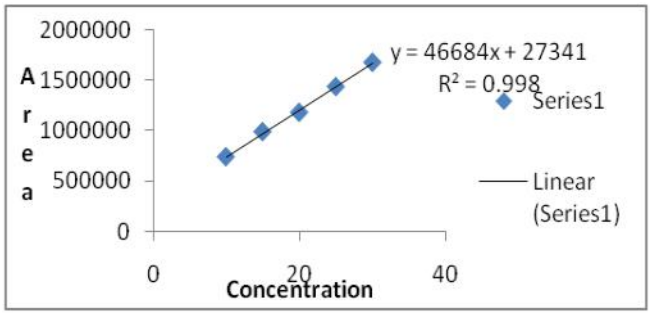

Fig .4: Calibration curve for Reltrigavir

PRECISION: The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported.

The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported.

Table 5: Precision results for Lamivudine **Table 6:** Precision results for Raltegravir

Name: Lamivudine

	Name	RT	Area
1	Lamivudine	2.335	1963566
2	Lamivudine	2.332	1964716
3	Lamivudine	2.333	1965030
4	Lamivudine	2.330	1960856
5	Lamivudine	2.331	1966445
Mean			1964123
Std. Dev.			2094.9
% RSD			0.11

Name: Raltegravir

	Name	RT	Area
1	Raltegravir	3.408	2304558
2	Raltegravir	3.406	2299453
3	Raltegravir	3.409	2296908
4	Raltegravir	3.404	2295001
5	Raltegravir	3.407	2299613
Mean			2299107
Std. Dev.			3597.7
% RSD			0.16

Accuracy: Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

a) Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

b).Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

c). Acceptance criteria: The mean % recovery of the Lamivudine and Raltegravir at each level should be not less than 95.0% and not more than 105.0%.

Table 7: Accuracy Study of Lamivudine

Sample Id	Conc found (µg/ml)	Concn Obtained (µg/ml)	%Recovery	Mean recovery	Statistical Analysis
50%	5	5.01	100.2	99.73	**%RSD= 0.505**
50%	5	4.96	99.2		
50%	5	4.99	99.8		
100%	10	9.95	99.5	98.8	**%RSD=0.66**
100%	10	9.87	98.7		
100%	10	9.82	98.2		
150%	15	14.64	97.6	98.8	**%RSD=1.45**
150%	15	14.76	98.4		
150%	15	15.06	100.4		

Table 8: Accuracy Study of Raltegravir

Conc (µg/ml)	Conc Obtained (µg/ml)	%Recovery of drug	Mean accuracy	%RSD
5	4.92	98.0		
5	4.96	99.2		
5	5.02	100.4	**99.2**	**1.2**
10	9.95	99.5		
10	9.94	99.4		
10	9.98	99.8	**99.5**	**0.2**
15	14.78	98.6		**0.530**

15	14.94	99.6	**99.0**	
15	14.83	98.8		

Table 9: LOD and LOQ Data of Lamivudine and Raltegravir

Lamivudine			**Raltegravir**		
Conc.(x) (µg/ml)	**Peak Areas (y)**	**Statistical Analysis**	**Conc.(x) (µg/ml)**	**Peak Areas (y)**	**Statistical Analysis**
40	2004682	S = 39092 c = 618048 LOD: 0.001µg/ml LOQ: 0.004µg/ml	20	1184227	S = 39092 c =369381 LOD:0.005 µg/ml LOQ:0.015µg/ml
40	2004587		20	1186425	

CONCLUSION

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate title ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The mobile phase containing mixture of orthophosphoric acid buffer solution: Acetonitrile (40:60v/v, pH 2.45) with a flow rate of 1.0 ml/min is quite robust. The optimum wavelength for detection was 260 nm at which better detector response for both the drugs was obtained. The retention times for Lamivudine and Raltegravir was found to be 2.335 ± 0.004 min and 3.400 ± 0.005 min, respectively. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 10 to 50 µg/ ml and 20 to 100 µg/ml, with regression 0.9979 and 0.9999, Lamivudine and Raltegravir respectively. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found above 99.3 % for both the drugs.Robustness of the proposed method was determined by varying various parameters, the %RSD reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

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