AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Simultaneous RP-HPLC Method Development and Validation for Lamivudine and Raltegravir in Bulk API Dosage Forms

Honeesha Nandimandalam*[1], D. Gowri Sankar[1]
1. Department of Pharmaceutical Analysis, Andhra University College of Pharmaceutical Analysis, Visakhapatnam-530003, Visakhapatnam (Dist.), Andhra Pradesh, India.

ABSTRACT

An accurate, precise and reproducible RP-HPLC method for the simultaneous determination of Lamivudine and Raltegravir in the bulk API dosage form has been developed and validated. Chromatographic separation was carried out on Agilent C18 (100×4.6mm, 3.5u particle size) column using mobile phase composed of phosphate buffer (P^H3.0): acetonitrile (ACN) in ratio 60:40 at a flow rate of 0.8ml/min. The analyte was monitored using DAD detector at 231nm. The retention times were found to be 1.12 and 4.08 for Lamivudine and Raltegravir respectively. The linearity for each were found in the range 10-60µg/ml and their regression values are 0.998 and 0.999 respectively. The developed method was validated as per ICH guidelines.

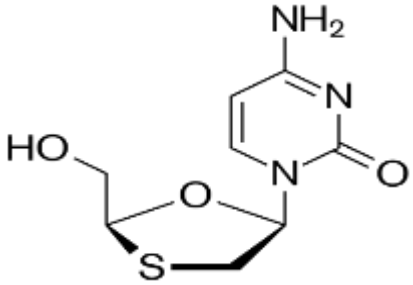
Keywords: Lamivudine, Raltegravir, RP-HPLC.

*Corresponding Author Email: honeesha.n@gmail.com
Received 13 August 2015, Accepted 03 September 2015

Please cite this article as: Honeesha N *et al*., Simultaneous RP-HPLC Method Development and Validation for Lamivudine and Raltegravir in Bulk API Dosage Forms. American Journal of PharmTech Research 2015.

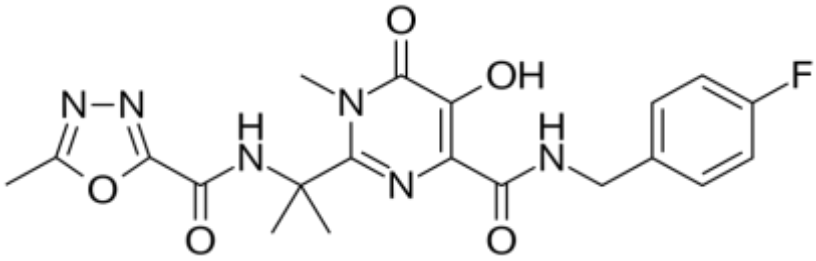
INTRODUCTION

Lamivudine[1]: The chemical name of Lamivudine is 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. 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.



Structure of lamivudine

Raltegravir[2]: the chemical name of Raltegravir is N-[(4-Fluorophenyl) methyl]-1, 6-dihydro-5-hydroxy-1-methyl-2[1-methyl-1-[[(5-methyl-1,3,4-oxadiazol-2-yl)carbonyl]amino]ethyl]-6-oxo-4-pyrimidinecarboxamide. 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 glucronidation.



Structure of raltegravir

Lamivudine (150) mg for effective HIV management. The combined formulation of Raltegravir (300mg) and Lamivudine (150mg) was recently approved by FDA to treat HIV. The combined action of Raltegravir (300mg) and Lamivudine (150mg) was comparable to that of individual doses of Raltegravir (400 mg) and Lamivudine 150 mg taken simultaneously. The Raltegravir content in the combined formulation was less compared to that of Raltegravir single formulation, but having similar action. The raltegravir intake can be reduced by using combined formulation. Several HPLC and UV methods are reported in literature for the estimation of raltegravir and lamivudine separately[3, 4]. As far as we are aware, no simultaneous RP-HPLC method for estimation of raltegravir and lamivudine has been reported[5, 6]. The objective of the present work

was to develop and validate a RP-HPLC method for simultaneous estimation of raltegravir and lamivudine.

MATERIALS AND METHOD

Chemicals and Reagents:

ACN (HPLC grade) and orthophosphoric acid from Fisher Scientific, and triple distilled water.

Instrumentation

Equipment	Apparatus
HPLC system	Agilent 1260 infinity series, with DAD detector, manual injector-20 µl.
Software	Open lab version A.02.01(1.3.3)

Preparation of Buffer

Potassium di-hydrogen orthophosphate buffer of P^H 3.0 was prepared according to the Indian Pharmacopoeia.

Preparation of Mobile Phase

Mobile phase was prepared by mixing buffer and ACN in the ratio 60:40v/v.

Preparation of Diluent

ACN and water in ratio 50:50v/v.

Preparation of Standard solutions

Accurately weighed and transferred 25mg of each drug into 25ml volumetric flask, volume was made up to 25ml with the diluent to get 1mg/ml solutions. From the above solutions 1ml each was pipetted out into 10 ml volumetric flask to get concentration 100µg/ml used as a working stock solution. From the working standard 2ml of Lamivudine and 4ml of Raltegravir were pipetted into 10ml volumetric flasks to get 20µg/ml and 40µg/ml used as final working standard solution.

Method Development and Optimization

The peak shapes and symmetry of each drug were good at following optimized conditions,

Optimized condition:

Flow rate	0.8ml/min
Mobile phase	60:40%v/v (buffer: acetonitrile)
Buffer P^H	Potassium di hydrogen orthophosphate buffer P^H 3.0 adjusted with OPA
Wavelength	231nm
Injection volume	20µl
Run time	6mins
Retention times	Lamivudine - 1.1min Raltegravir - 4.08min.

Method Validation

The optimized me RP-HPLC method was validated for system suitability, specificity, precision, linearity, accuracy, robustness according to ICH guidelines.

System Suitability

System suitability was carried out by injecting 20µl of standard solution in six replicates. The system suitability parameters were evaluated for tailing factor, theoretical plates, retention time and area. %RSD for peak areas was calculated (%RSD NMT 2) were within the limits. The obtained values are given in Table1.

Specificity

Specificity of method can be absence of any interference at retention times of samples. Specificity was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes. This is shown in Figure 1.

Linearity

The linearity of both drugs was studied by preparing six different concentrations of each solution in the range of 10µg/ml to 60µg/ml each concentration was injected in five replicates and mean value of peak area was taken for calibration. The calibration curve was found to be linear with correlation coefficient of 0.998 and 0.999 for lamivudine and raltegravir. Results obtained are shown in Figure 2, 3.

Accuracy

The accuracy of the assay method was calculated for lamivudine and raltegravir by recovery studies at three different concentrations i.e. 50%, 100% and 150% levels by using standard addition method and each concentration was injected three times. The accuracy of an analytical method should be established across its range. Results obtained are shown in Table 2 and 3.

Precision

The precision of the assay was studied with respect to both intra-day (repeatability) and inter-day (intermediate precision). Repeatability was calculated from six replicates injections, intermediate precision was carried out by injecting six replicates on the second day. %RSD values for retention time and peak area were found to be within limits. The obtained values are presented in Table 4.

Robustness

Robustness of the proposed method was determined by analyzing the standard solution by changing the physical parameters like flow rate, mobile phase composition, pH of the buffer. The results obtained are shown in the Table 5 and 6.

RESULTS AND DISCUSSION

System suitability studies

Table 1: System Suitability Parameters

Parameters	Lamivudine	Raltegravir
Retention time	1.09	4.1
Tailing factor	1.11	1.1
Theoretical plates	2477	6754
%RSD of peak areas	0.37	0.40

Specificity chromatogram

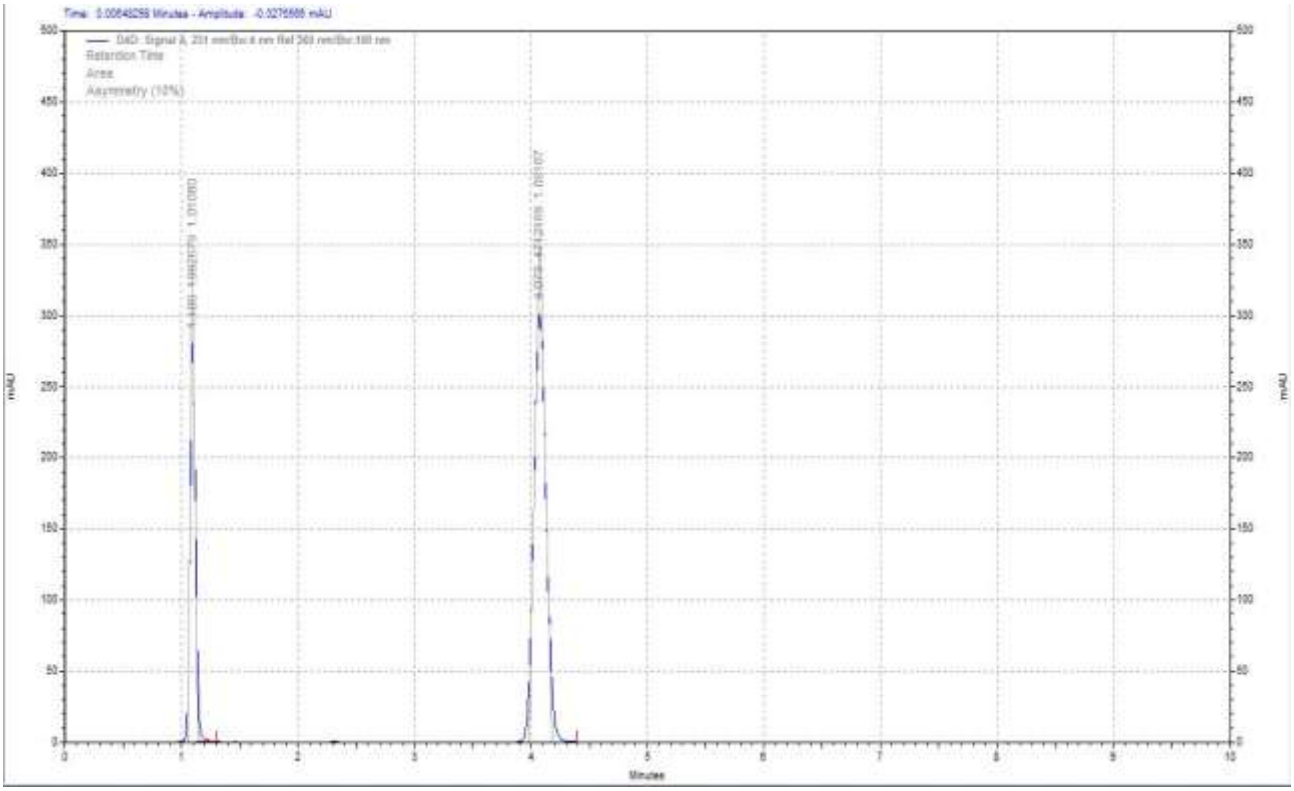


Figure: 1 Chromatogram of Standard

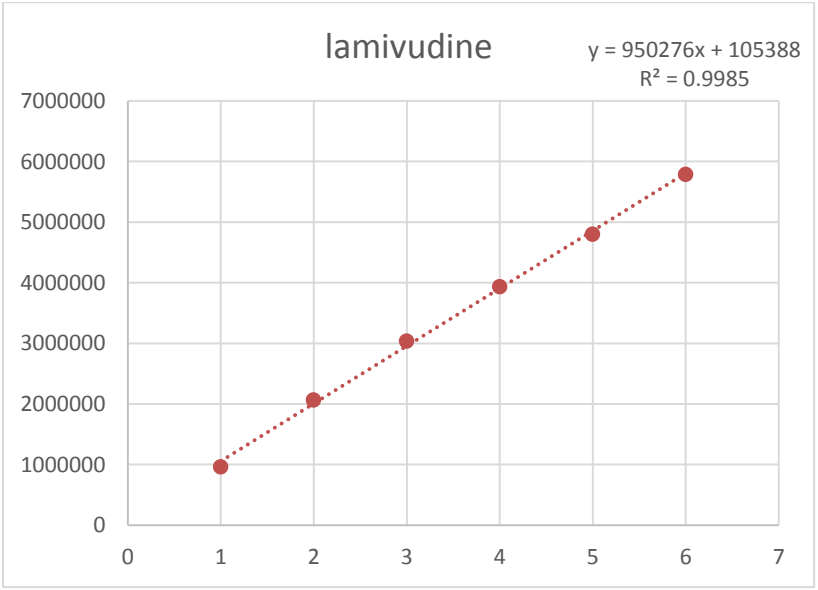


Figure 2: Linearity of Lamivudine

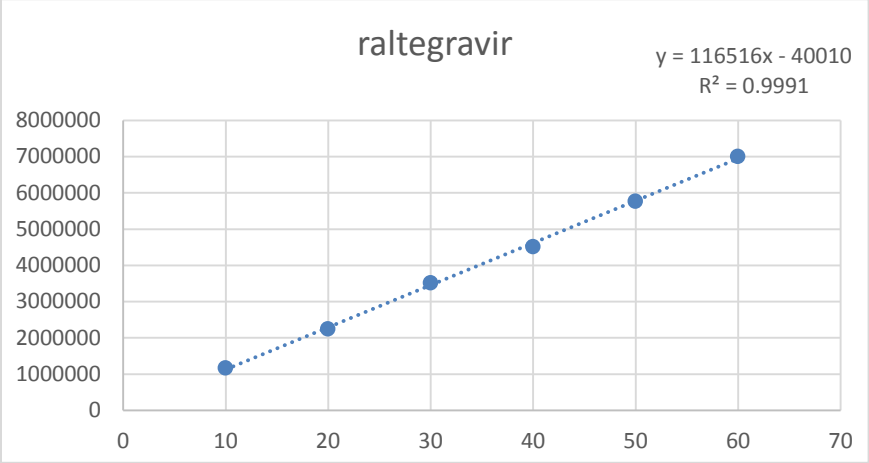


Figure 3: Linearity of Raltegravir

Table 2: Accuracy for Lamivudine

S.No	%Level	Amount Found	%Recovery	Mean Recovery
1	50%	9.97	99.76	Mean-99.41
2	50%	9.90	99.04	SD-0.3607
3	50%	9.94	99.44	%RSD-0.36
4	100%	19.85	99.25	Mean-99.1
5	100%	19.76	98.80	SD-0.2598
6	100%	19.85	99.25	%RSD-0.26
7	150%	30.23	100.7	Mean-100.17
8	150%	29.74	99.13	SD-0.90643
9	150%	30.22	100.7	%RSD-0.90

Table 3: Accuracy for Raltegravir

S.No	%Level	Amount Found	%Recovery	Mean Recovery
1	50%	19.76	98.32	Mean-98.93
2	50%	19.65	98.28	SD-0.716
3	50%	19.94	99.70	%RSD-0.72
4	100%	39.98	99.95	Mean-99.97
5	100%	39.99	99.99	SD-0.02
6	100%	39.99	99.97	%RSD-0.02
7	150%	59.50	99.17	Mean-99.06
8	150%	59.18	98.63	SD-0.395
9	150%	59.64	99.40	%RSD-0.40

Table 4: Precision Studies

Parameters		Lamivudine	Raltegravir
Intra-day precision	%RSD retention time:	0.10	0.11
	%RSD peak area:	1.15	0.95
Inter-day precision	%RSD retention time:	0.16	0.10
	%RSD peak area:	1.22	1.1

Robustness

The developed method was found to be robust for deliberate changes with variations of Flow rate, P^H of the Buffer, mobile phase organic composition.

Table 5: Results of Robustness study of Lamivudine

Parameter	Change Level	Lamivudine			
		RT (min)	Peak Area	Tailing factor	USP Plate Count
Flow Rate (±0.1ml/min)	0.7ml/min	1.20	2343451	1.03	2973
	0.9ml/min	0.99	1811386	1.01	2128
Mobile phase organic composition	59:41	1.10	2053144	1.04	2578
	61:39	1.10	2044146	1.09	2601
Buffer pH	P^H 3.1	1.09	2017761	1.03	2590
	P^H 2.9	1.14	2039955	1.06	2629

Table 6: Results of Robustness study of Raltegravir

Parameter	Change Level	Raltegravir			
		RT	Peak Area	Tailing factor	USP Plate Count
Flow Rate (±0.1ml/min)	0.7ml/min	4.83	5407582	1.04	7156
	0.9ml/min	3.82	4224638	1.05	6974
Mobile phase organic composition	59:41	3.91	4732661	1.10	6910
	61:39	4.61	4768778	1.05	6924
Buffer pH	P^H 3.1	3.92	4715111	1.02	6833
	P^H 2.9	4.11	4781123	1.08	6898

CONCLUSION

An accurate, precise and reproducible RP-HPLC method with DAD detection was developed and validated for lamivudine and raltegravir in bulk API dosage form. The method is rapid as run time is 6 minutes. The method can also be adapted for routine quality analysis.

ACKNOWLEDGEMENT

The author is indebted to Andhra University College of Pharmacy, Visakhapatnam dist., Andhra Pradesh, India for providing research facilities.

REFERENCES

1. Drugbank.ca. DrugBank: Lamivudine (DB00709) 2015. Available at http://www.drugbank.ca/drugs/DB00709
2. Drugbank.ca. DrugBank: Raltegravir (DB06817). 2015. Available at: http://www.drugbank.ca/drugs/DB06817.
3. Lakshmana Rao, Validated Reverse Phase HPLC Method for Determination of Raltegravir in Pharmaceutical Preparations. Int J Res Pharma Chem 2012, 2(1).

4. Rambabu Kuchi, New RP - HPLC Method Development and validation for Analysis of Antiviral drug Raltegravir. Int J Res Pharma Biological Sci 2011 ; 2 (1).
5. Mary C. Long A sensitive HPLC–MS–MS method for the determination of raltegravir in human plasma. J Chromatography B 2008; 867(2): 165–171**.**
6. NV. Krishnareddy. New RP - HPLC Method Development for Analysis and Assay of Lamivudine in Formulation. Int J Res Pharma Biological Sci 2011; 2 (1).