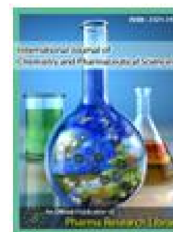
International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijcps



Research Article **Open Access**

Analytical Method Development and Validation for the Simultaneous Estimation of Raltegravir and Lamivudine by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

Parsa Sivarama Krishna*, G. Devika Subramaniyan, Ramesh Petchi Rajendran

Bomma Institute of Pharmacy, Allipuram, Khammam, Telangana

A B S T R A C T

A simple, accurate, precise method was developed for the simultaneous estimation of the Raltegravir and Lamivudine in Tablet dosage form. Chromatogram was run through Inertsil ODS 3V(250x4.6mm)5µm column by using a mixture of Phosphate buffer and acetonitrile in a ratio of 55:45 v/v was pumped through column at a flow rate of 1.0ml/min and detection wavelength was at 275nm. The %RSD of the Raltegravir and Lamivudine were and found to be 1.14 and 1.19 respectively. The % recovery was obtained as 100.68 and 102.33for Raltegravir and Lamivudine respectively. LOD, LOQ values are obtained from regression equations of Raltegravir and Lamivudine were 0.21, 2.4µg/ml and 0.6, 4.2µg/ml respectively. The developed method is applicable for routine quality control analysis of selected combined dosage forms.

Keywords: Raltegravir, Lamivudine, RP-HPLC

A R T I C L E I N F O

CONTENTS

1. Introduction 585
2. Materials and Method. 586
3. Results and Discussion. 588
4. Conclusion. 590
5. References 590

Article History: Received 15 September 2016, **Accepted** 21 October 2016, **Available Online** 27 November 2016

***Corresponding Author**
Parsa Sivarama Krishna
Bomma Institute of Pharmacy,
Khammam, Telangana, India.
Manuscript ID: IJCPS3227

PAPER-QR CODE

Citation: Parsa Sivarama Krishna. Analytical Method Development and Validation for the Simultaneous Estimation of Raltegravir and Lamivudine by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form. *Int. J. Chem, Pharm, Sci.*, 2016, 4(11): 585-591.

Copyright© 2016 Parsa Sivarama Krishna. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Analytical methods: The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Often a time lag exists from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias[1]. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors.

Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs[2]. Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the International Conference on Harmonization (ICH) guidelines (Q2A and Q2B). [3,4]

Method development is a continuous process that progresses in parallel with the evolution of the drug product. The goal and purpose of the method should reflect the phase of drug development. During early drug development, the methods may focus on API behaviour[5]. They should be suitable to support preclinical safety evaluations, pre-formulation studies, and prototype product stability studies. As drug development progresses, the analytical methods are refined and expanded, based on increased API and drug product knowledge.

The methods should be robust and uncomplicated, while still meeting the appropriate regulatory guidelines. Scouting experiments are frequently performed during method development to establish the performance limits of the method, prior to formal validation experiments[6-9]. These may include forced degradation studies, which are an integral part of development of a stability-indicating method.

API is typically subjected to degradation by acid, base, peroxide, heat, and light. This allows for a determination of the capability of the method to separate and quantify degradation products, while providing insight into the main mechanisms of degradation. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate potential degradation of the API in the presence of formulation excipients[10].

Raltegravir[11] is an antiretroviral drug produced by Merck & Co., used to treat HIV infection. It received approval by the U.S. Food and Drug Administration (FDA) on 12 October 2007, the first of a new class of HIV drugs, the integrase inhibitors, to receive such approval. This compound belongs to the class of organic compounds known as pyrimidine carboxylic acids and derivatives. These are compounds containing a pyrimidine ring which bears a carboxylic acid group.

Lamivudine[12] 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). This compound belongs to the class of organic compounds known as 3'-thia pyrimidine nucleosides. These are nucleoside analogues with a structure that consists of a pyrimidine base, which is N-substituted at the 1-position with a 3'-thia derivative (1,3-oxazolidine) of the ribose moiety that is characteristic of nucleosides.

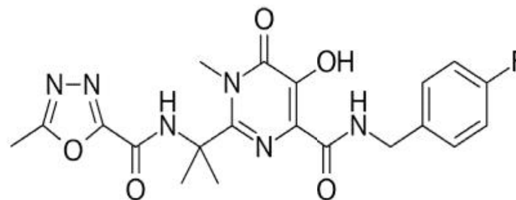


Figure 1: Raltegravir

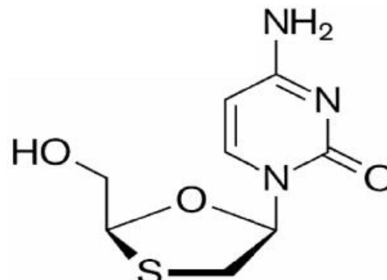


Figure 2: Lamivudine

2. Materials and Methods

Apparatus

The instrument used for the study was Shimadzu (LC20ATVP) HPLC, Separation module 2695, UV detector with Spin chrome software version 2.

Reagents and Materials

The solvents used were Methanol, Acetonitrile, Potassium dihydrogen ortho phosphate, Dipotassium hydrogen phosphate, ortho phosphoric acid and HPLC Water.

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 Raltegravir and Lamivudine were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 275 nm was selected as the detection wavelength for the present study.

Selection of mobile phase

Initially the mobile phase tried was Methanol and water, Methanol, Buffer and water in various proportions. Finally, the mobile phase was optimized to a mixture of 55 volumes of mixed phosphate buffer and 45 volumes of acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter

Chromatographic trials for Simultaneous Estimation of Raltegravir and Lamivudine by RP- HPLC.

Trial – 1 Chromatographic conditions

Mobile phase	: Methanol: CAN: Water
Column	: Analytical (Hyperchrom) ODS
pH	:5.0
Ratio	: 50:10:40
Column	: Inertsil ODS 3V (250×4.6× 5µ)
Wavelength	: 275 nm
Flow rate	: 1ml/min

Preparation of mixed standard solution

Weigh accurately 10 mg of Riltegravir and 10 mg of Lamivudine in 100 ml of volumetric flask and dissolve in

10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of Riltegravir and Lamivudine is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

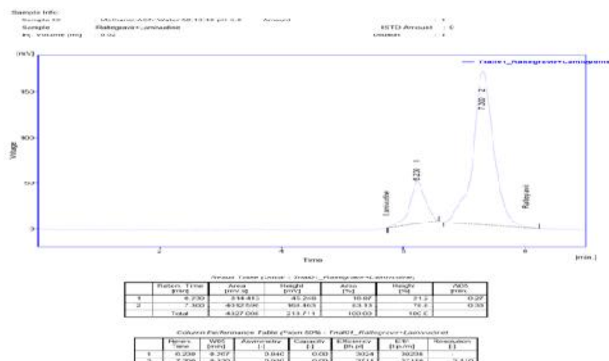


Figure 2: Trail 1 Chromatogram

Observation: Although the Efficiency was not satisfactory for Lamivudine and the peak response of Riltegravir was very less. Hence it was not taken for optimization.

Trial- 2Chromatographic conditions

Mobile phase : Methanol: ACN: Phosphate buffer
pH : 4.5
Ratio : 50:30:20
Column : Inertsil ODS 3V (250×4.6 ×5µ)
Wavelength : 275nm
Flow rate : 1ml/min

Preparation of mixed standard solution:

Weigh accurately 10 mg of Riltegravir and 10 mg of Lamivudine in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of Riltegravir and Lamivudine is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

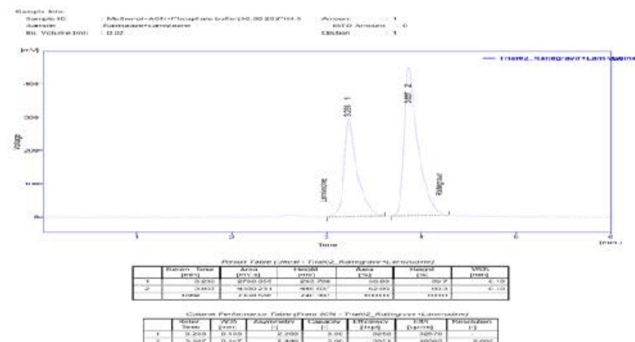


Figure 3: Trail 2Chromatogram

Observation:

Efficiency of both the drugs was good and the run time is very more.The peaks of Riltegravir and Lamivudine showed tailing. Hence it was not taken for optimization.

Optimized Chromatographic Method

Mobile phase : Mixed phosphate buffer: ACN
pH : 3.0
Ratio : 55:45
Column : Inertsil ODS (250×4.6× 5µ)
Wavelength : 275 nm
Flow rate : 1ml/min

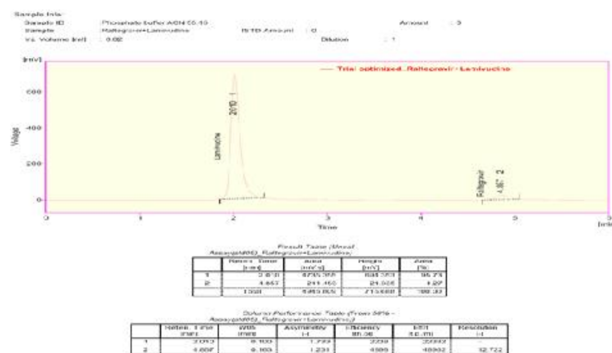


Figure 4: Chromatogram (Optimized Method)

Preparation of mixed standard solution

Weigh accurately 10 mg of Riltegravir and 10 mg of Lamivudine in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of Riltegravir and Lamivudine is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Observation:

All the system suitability requirements were met. The peak Asymmetry factor was less than 2 for both Lamivudine and Riltegravir. The efficiency was more than 2000 Lamivudine and Riltegravir and hence this method was selected as optimized method

Procedure

Mobile Phase:

A mixture of 45 volumes of acetonitrile and 55 volumes of mixed phosphate buffer were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Preparation of Mixed Phosphate Buffer:

1.625 gm of potassium Di hydrogen phosphate (KH2PO4) and 0.3 gm of Di potassium hydrogen phosphate was weighed and dissolved in 100ml of water and volume was made up to 550ml with water. Adjust the pH to 6.5 using orthophosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10 mg of Riltegravir and 10 mg of Lamivudine in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of Riltegravir and Lamivudine is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains Lamivudine 150mg and Raltegravir 300mg were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Lamivudine and Riltegravir were

prepared by dissolving weight equivalent to 150 mg of Lamivudine and 300 mg of Riltegravir and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 15μg/ml of Lamivudine and 30μg/ml of Riltegravir was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Lamivudine and Riltegravir present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of Lamivudine /Riltegravir in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

3. Results and Discussion

Method Validation Parameters

1. Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank and sample

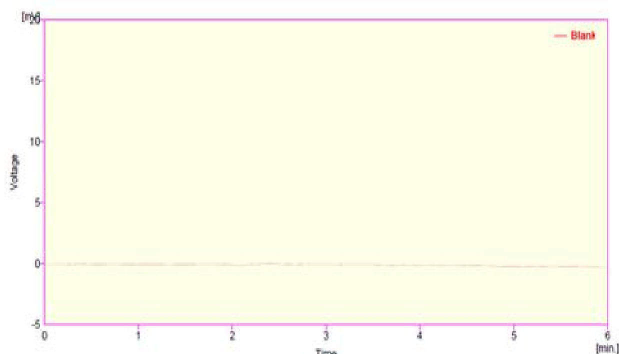


Figure 5: Chromatogram of Blank

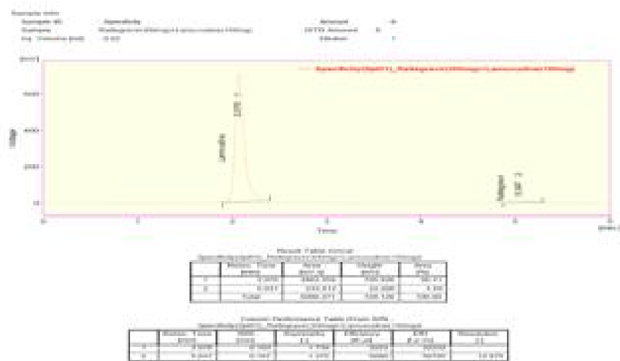


Figure 6: Chromatogram of Sample

2. 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.

Preparation of standard stock solution

Standard stock solutions of Raltegravir and Lamivudine (microgram/ml) were prepared by dissolving 10 mg of Raltegravir and 5 mg of Lamivudine dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. This solution contains 150-450 µg/ml of Raltegravir and 50-150 µg/ml of Lamivudine

Acceptance criteria: Correlation coefficient should be not less than 0.99.

3. Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 150-450 ppm of Raltegravir and 50-150 ppm of Lamivudine

4. Accuracy

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

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

Acceptance criteria:

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

Assay procedure

10µL of the standard and sample solutions of Raltegravir and Lamivudine were injected into the HPLC system and the chromatograms were recorded. Amount of drug present in the Tablets were calculated using the peak areas.

5. Precision

Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions. The % RSD of peak areas of six samples was calculated. The method precision was performed on Raltegravir and Lamivudine formulation.

Acceptance criteria

The % RSD for the area of sample injections results should not be more than 2.

Selection of solvent

Solutions of Raltegravir and Lamivudine were prepared by dissolving in mobile phase and UV spectrum of each was recorded by scanning between 200-400 nm.

Validation of the method

Linearity

Raltegravir and Lamivudine: Serial dilutions of 150-450 µg/ml of Raltegravir and 50-150 µg/ml of Lamivudine were injected into the column and detected at a wavelength at 275 nm. The calibration curve was obtained by plotting the concentration vs. peak area and the correlation coefficient was found to be 0.9998 and 0.9989 respectively.

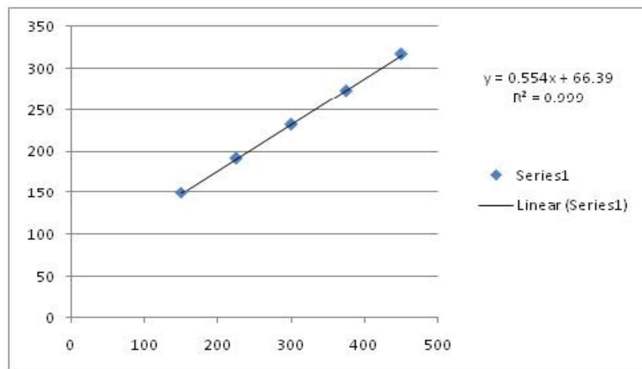


Figure 6: Linearity Graph of Raltegravir

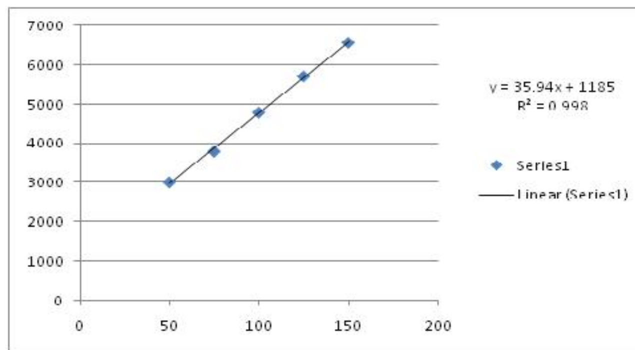


Figure 6: Linearity graph of Lamivudine

Table 1: Linearity of Raltegravir

S.No.	Conc.(µg/ml)	Area
1	150	149.905
2	225	191.759
3	300	232.136
4	375	273.288
5	450	317.219

Table 2: Linearity of Lamivudine

S.No.	Conc.(µg/ml)	Area
1	50	3023.467
2	75	3805.027
3	100	4792.760
4	125	5711.185
5	150	6563.077

Table 3: Recovery results for Raltegravir

Recovery level	Accuracy Raltegravir					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount Recovered	%Recovery	
50%	150	246.172	271.3697	150.89	101.629	100.68
	150	274.421				
	150	293.516				
100%	300	215.416	205.8547	298.65	96.36669	
	300	201.063				
	300	201.085				
150%	450	325.034	326.8517	453.76	104.0461	
	450	315.004				
	450	340.517				

Table 4: Recovery results for Lamivudine

Recovery level	Accuracy Lamivudine					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
50%	75	5649.981	5636.751	75.98	101.5941	102.33
	75	5595.240				
	75	5665.033				
100%	100	4784.610	4721.589	100.01	100.1172	
	100	4638.914				
	100	4741.244				
150%	125	6582.420	6533.965	126.98	105.2958	
	125	6489.801				
	125	6529.673				

Table 5: Result of Robustness study

Parameter	Raltegravir		Lamivudine	
	Retention time (min)	Tailing factor	Retention time (min)	Tailing factor
Flow Rate **0.8 ml/min** **1.2 ml/min**	5.983 4.047	1.357 1.353	2.427 1.667	1.852 1.750
Wavelength **323nm** **334nm**	5.047 4.853	1.375 1.385	2.070 2.003	1.739 1.739

Table 6: Results for Method precision of Raltegravir and Lamivudine

Raltegravir			Lamivudine		
S.No.	Rt	Area	S.No.	Rt	Area
1	5.130	224.536	1	2.103	4937.655
2	5.100	219.059	2	2.093	4884.214
3	5.073	227.238	3	2.083	4921.208
4	5.047	219.466	4	2.070	4846.820
5	4.993	223.489	5	2.037	4814.949
6	5.047	211.508	6	2.070	4841.024
Avg	5.065	220.8827	avg	2.076	4874.312
Stdev	0.047636	5.545217	stdev	0.023065	48.36835
%RSD	0.940496	2.510481	**%RSD**	1.111037	0.992311

Table 7: Results for Ruggedness

Raltegravir	% Assay	Lamivudine	% Assay
Analyst 01	100.12	**Analyst 01**	100.23
Anaylst 02	99.98	**Anaylst 02**	100.01

Table 8: Results for LOD & LOQ

Drug name	LOD (μg)	LOQ (μg)
Raltegravir	0.21	0.6
Lamivudine	2.4	4.2

4. Conclusion

A new method was established for simultaneous estimation of Raltegravir and Lamivudine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Raltegravir and Lamivudine by using C18 column (4.6×250mm)5μ, flow rate was 1ml/min, mobile phase ratio was (55:45 v/v) mixed phosphate buffer: Methanol, detection wavelength was 275nm. Precision and recovery studies were also found to be with the range.The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Raltegravir and Lamivudine in pharmaceutical 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. Hence the suggested RP-HPLC method can be used for routine analysis of Raltegravir and Lamivudine in API and Pharmaceutical dosage form

5. References

[1] International Conference on Harmonization, "Q2A: Text on Validation of Analytical Procedures," *Federal Register.* 1995, *60*, 11260–11262.

[2] International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," *Federal Register*. 1997, *62*, 27463–27467.

[3] Michael Swartz, E.; Ira Krull, S, Analytical Method development. In Analytical Method Development and Validation, 1st ed.; Marcel Dekker, Inc: New York, 2009; 17-80.

[4] Particle Sciences Drug Development Services. Analytic Method Development and Validation. *Technical Brief*. 2009, *5*, 1-2.

[5] Brian, L. H.; Thomas, E. B. The Influence of Column Temperature on HPLC Chiral Separation on Macrocyclic Glycopeptide CSPs. Advanced Separation Technologies Inc. (Astec). New Jersey, USA.

[6] Ghulam, A. S. PLC Method Development and Validation for Pharmaceutical Analysis. *Pharmaceutical Technology Europe*. 2004, 7, 55 -63.

[7] Radhika, R.; Alfred, D. G. Guidance for Industry-Analytical Procedures and Methods Validation. *Federal Register*, 2000, *2396*, 1-32.

[8] Rajesh, K. P. Overview of Pharmaceutical Validation and Process Controls in Drug Development. *Der Pharmacia Sinica*. 2010, *1*, 11 - 19.

[9] Jay, B.; Kevin, J.; Pierre, B. Understanding and Implementing Efficient Analytical Methods Development and Validation. *Pharmaceutical Technology Analytical Chemistry & Testing*. 2003, *5*, 6 - 13.

[10] Ludwig, H. Validation of Analytical Methods. Agilent technologies. 2007, 1- 65.

[11] Venkatesh M, Sumanth ALM and Venkateswa Rao P. Analytical method development and validation of simultaneous estimation of Lamivudine and Raltegravir in bulk and Pharmaceutical dosage forms by using RP-HPLC. Asian J Pharma Anal Med Chem, 1(2): (2013), 60 - 69.

[12] Medenica M, Jancic B, Ivanovic D and Malenovic A. Experimental design inreversed-phase high-performance liquid chromatographic analysis of Lamivudine and Raltegravir and its impurity J Chromatogr A, 1031: (2004), 243–248.

[13] Ana B. Baranda, Nestor Etxebarria, Rosa M. Jiménez and Rosa M. Alonso.Improvement of the chromatographic separation of several Lamivudine and Raltegravir drugs by Experimental Design. J Chromatogr Sci, 43: (2005), 505-512.

[14] Sivakumar T, Manavalan R, Muralidharan C and Valliappan K. Multi-criteria decision making approach and experimental design as chemometric tools tooptimize HPLC separation of Lamivudine and Raltegravir .J Pharma Bio Med.

[15] Suresh R, Manavalan and Valliappan K. Developing and optimizing a validated RP-HPLC method for the analysis of Lamivudine and Raltegravir in pharmaceutical dosage forms applying response surface methodology. Int J Pharm Pharm. Sci, 4(3): (2012), 550-558.