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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF LAMIVUDINE & RALTEGRAVIR IN PURE & PHARMACEUTICAL DOSAGE FORM.

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Abstract:

A simple, specific, precise, and efficient method for the Simultaneous estimation of Lamivudine and Raltegravir in pure and pharmaceutical dosage forms by a Reverse Phase-High Performance Liquid Chromatography method is developed and validated. Selected mobile phase were in a combination of ACN: Water (40:60% v/v). Optimized column is a Phenomenex Gemini C18 (4.6mm×250mm) 5µm particle size and at a flow rate of 1.0mL/min with detection wavelength at 255nm for Lamivudine and Raltegravir. In our study, the validation of analytical method for determination of Lamivudine and Raltegravir in pure and pharmaceutical dosage forms was performed in accordance the parameters including-system suitability, specificity, linearity of response, accuracy, precision (reproducibility & repeatability), robustness (change of wave length±2 nm). The method is validated according to ICH guidelines. The results obtained by RP-HPLC methods are rapid, accurate and precise. Therefore, proposed method can be used for routine analysis of Lamivudine and Raltegravir in the pure form as well as in combined pharmaceutical dosage form.

Keywords: *Lamivudine and Raltegravir, HPLC, Method Development, Validation.*

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INTRODUCTION:

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

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. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

Different methods of analysis:

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.

E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistance as a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [3]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma-Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

Introduction to hplc:

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates

Easy sample recovery, handling and maintenance

Analytical Method Development:

A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible and it should allow the use of sophisticated tools such as computer modeling. The important factors, which are to be taken into account to obtain reliable quantitative analysis, are

- Careful sampling and sample preparation.
- Appropriate choice of the column.
- Choice of the operating conditions to obtain the adequate resolution of the mixture.
- Reliable performance of the recording and data handling systems.
- Suitable integration/peak height measurement technique.
- The mode of calculation best suited for the purpose.
- Validation of the developed method.

Careful sampling and sample preparation [17]:

Before beginning method development it is need to review what is known about the sample in order to define the goals of separation. The sample related information that is important is summarized in following.

The sample related summarized relation

- Number of compounds present, chemical structures
- Molecular weights of compounds
- pKa values of compounds, UV spectra of compounds
- Concentration range of compounds in samples of interest
- Sample solubility

The chemical composition of the sample can provide valuable clues for the best choice of initial conditions for an HPLC separation.

Choice of the column:

The selection of the column in HPLC is somewhat similar to the selection of columns in G.C, in the sense that, in the adsorption and partition modes, the separation mechanism is based on inductive forces, dipole-dipole interactions and hydrogen bond formation. In case of ion-exchange chromatography, the separation is based on the differences in the charge, size of the ions generated by the sample molecules and the nature of ionisable group on the stationary phase. In the case of size-exclusion chromatography the selection of the column is based on the molecular weight and size of the sample components.

Choice of the operating conditions to obtain the adequate resolution of the mixture:

Most of the drugs come under the category of regular samples. Regular samples mean typical mixtures of small molecules that can be separated using more or less standardized starting conditions. Regular samples can be further classified as neutral or ionic. Samples classified as ionic include acids, bases, amphoteric compounds and organic salts. If the sample is neutral buffers or additives are generally not required in the mobile phase. Acids or bases usually require the

addition of a buffer to the mobile phase. For basic or cationic samples, less acidic reverse phase columns are recommended. Based on recommendations of the conditions, the first exploratory run is carried and then improved systematically. On the basis of the initial exploratory run isocratic or gradient elution can be selected as most suitable. If typical reverse-phase conditions provided inadequate sample retention it suggests the use of either ion-pair or normal phase HPLC. Alternatively the sample may be strongly retained with 100% Acetonitrile as mobile phase suggesting the use of non-aqueous reverse phase chromatography or normal phase HPLC.

Getting started on method development:
One approach is to use an isocratic mobile phase of some average organic solvent strength (50%). A better alternative is to use a very strong mobile phase first (80-100%) then reduce %B as necessary. The initial separation with 100% B results in rapid elution of the entire sample but few groups will separate. Decreasing the solvent strength shows the rapid separation of all components with a much longer run time, with a broadening of latter bands and reduced retention sensitivity. Goals that are to be achieved in method development are briefly summarized.

Separation or resolution is a primary requirement in quantitative HPLC. The resolution (R_s) value should be maximum (R_s>2) favors maximum precision. Resolution usually degrades during the life of the column and can vary from day to day with minor fluctuations in separation conditions. Therefore values of R_s=2 or greater should be the goal during method development for sample mixtures. Such resolution will favor both improved assay precision and greater method ruggedness. Some HPLC assays do not require base line separation of the compounds of interest (qualitative analysis). In such cases only enough separation of individual components is required to provide characteristic retention times for peak identification. The time required for a separation (runtime = retention time for base band) should be as short as possible and the total time spent on method development is reasonable (runtimes 5 to 10 minutes are desirable).

Repeatable separation:
As the experimental runs described above are being carried out, it is important to confirm that each chromatogram can be repeated. When we change conditions (mobile phase, column, and temperature) between method development experiments, enough time must elapse for the column to come into equilibrium with the new mobile phase and temperature.

Usually column equilibration is achieved after passage of 10 to 20 volumes of the new mobile phase through the column. However this should be confirmed by repeating the experiment under the same conditions. When constant retention times are observed in two such back-to-back repeat experiments (± 0.5% or better), it can be assumed that the column is equilibrated and the experiments are repeatable.

Optimization of HPLC method:
During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape, plate counts, asymmetry, capacity factor, elution time, detection limits, limit of quantitation and overall ability to quantify the specific analyte of interest.
The various parameters that include to be optimized during method development are

- Selection of mode of separation.
- Selection of stationary phase.
- Selection of mobile phase.
- Selection of detector.

Selection of mode of separation:
In reverse phase mode, the mobile phase is comparatively more polar than the stationary phase. For the separation of polar or moderately polar compounds the most preferred mode is reverse phase. The nature of the analyte is the primary factor in the selection of the mode of separation. A useful and practical measurement of peak shape is peak asymmetry factor and peak tailing factor. Peak asymmetry is measured at 10% of full peak height and peak tailing factor at 5%. Reproducibility of retention times and capacity factor is important for developing a rugged and repeatable method.

Buffers and buffer capacity:
Buffer and its strength play an important role in deciding the peak symmetries and separations. Some of the most commonly employed buffers are phosphate buffers.

MATERIALS AND METHODS:

Lamivudine (Pure)-Procured from Mylon, provided by Sura Pharma Labs, Raltegravir (Pure)-Procured from Mylon, provided by Sura Pharma Labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC -Merck.

Hplc method development:

Trails

Preparation of standard solution:
Accurately weigh and transfer 10 mg of Lamivudine and Raltegravir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.2ml of Lamivudine and 0.6ml of Raltegravir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to ACN: Water in proportion 40:60 v/v respectively.

Optimization of Column:
The method was performed with various C18 columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µm was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:
Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : 36ºC
Column : Phenomenex Gemini C18 (4.6×250mm) 5µm particle size
Mobile phase : ACN: Water (40:60% v/v)
Flow rate : 1ml/min
Wavelength : 255nm
Injection volume : 20µl
Run time : 6 minutes

Validation
Preparation of mobile phase:
Preparation of mobile phase:
Accurately measured 400ml of ACN (40%) of and 600ml of Water (60%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)
Mobile phase ratio : ACN: Water (40:60% v/v)
Column : Phenomenex Gemini C18 (4.6×250mm) 5µm particle size
Column temperature : 36ºC
Wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 6minutes

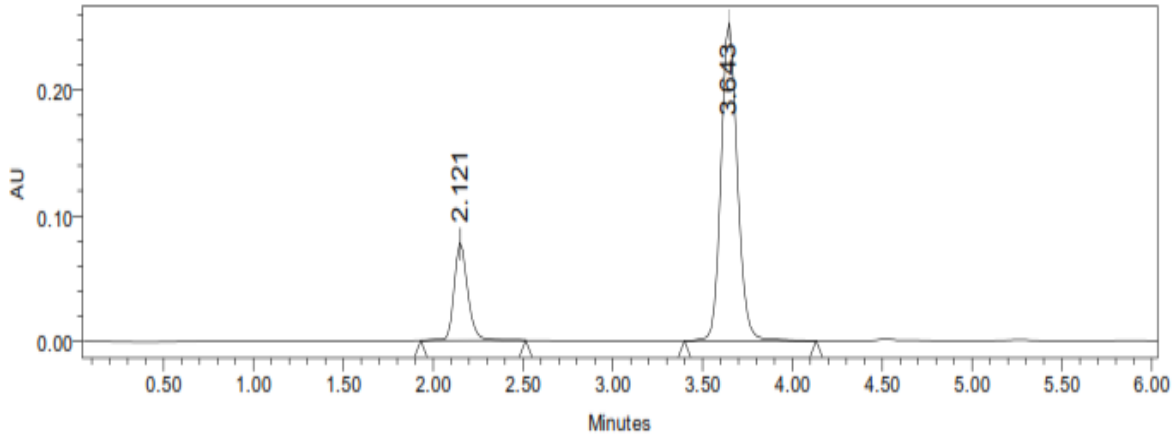

Figure-7.5: Optimized Chromatogram (Standard)

Table-7.5: Optimized Chromatogram (Standard)

S. no	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Lamivudine	2.121	513567	78659	1.2	4536	
2	Raltegravir	3.643	1625892	265321	1.1	7985	9.8

Observation: From the above chromatogram it was observed that the Lamivudine and Raltegravir peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

System Suitability:

Table-7.6: Results of system suitability for Lamivudine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Lamivudine	2.152	513652	78542	4698	1.2
2	Lamivudine	2.157	513524	78654	4785	1.2
3	Lamivudine	2.141	513425	78541	4682	1.2
4	Lamivudine	2.133	513647	78454	4854	1.2
5	Lamivudine	2.166	514824	78655	4872	1.2
Mean			513814.4			
Std. Dev.			572.2004			
% RSD			0.111363			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table-7.7: Results of system suitability for Raltegravir

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Raltegravir	3.674	1635285	265421	7985	1.1	10.1
2	Raltegravir	3.631	1635241	265484	7898	1.1	10.1
3	Raltegravir	3.625	1652547	253498	7954	1.1	10.1
4	Raltegravir	3.692	1658458	265241	7965	1.1	10.1
5	Raltegravir	3.629	1652894	265348	7985	1.1	10.1
Mean			1646885				
Std. Dev.			10865.58				
% RSD			0.659766				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Standard):

Table: Peak results for assay standard of Lamivudine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Lamivudine	2.152	513538	78074	1.2	4562	1
2	Lamivudine	2.198	513975	79001	1.2	4620	2
3	Lamivudine	2.179	513283	78048	1.2	4652	3

Table: Peak results for assay standard of Raltegravir

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Raltegravir	3.646	1625632	265325	1.1	7949	1
2	Raltegravir	3.604	1635458	265423	1.1	7919	2
3	Raltegravir	3.610	1635241	265874	1.1	7926	3

Assay (Sample):

Table: Peak results for Assay sample of Lamivudine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection	% of Assay
1	Lamivudine	3.651	513265	78548	1.2	4582	1	100.1
2	Lamivudine	2.150	513254	78547	1.2	4658	2	100.1
3	Lamivudine	2.187	513876	78498	1.2	4597	3	99.9

Table: Peak results for Assay sample of Raltegravir

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection	% of Assay
1	Raltegravir	3.646	1625284	78569	1.1	7985	1	100.0
2	Raltegravir	3.651	1624613	78547	1.1	7898	2	100.7
3	Raltegravir	3.601	1625874	78462	1.1	7854	3	100.6

Table: Showing Assay Results

S.No.	Name of Compound	Label Claim	Amount Taken (from Combination Tablet)	% Purity
1	Lamivudine	0.5mg	0.4	99.57%
2	Raltegravir	10 mg	9.8	99.57%

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Lamivudine and Raltegravir in pharmaceutical dosage form was found to be 99.57%

Linearity

Chromatographic data for linearity study:

Chromatographic data for linearity study of lamivudine:

Concentration µg/ml	Average Peak Area
10	245899
15	365687
20	481526
25	589854
30	705882

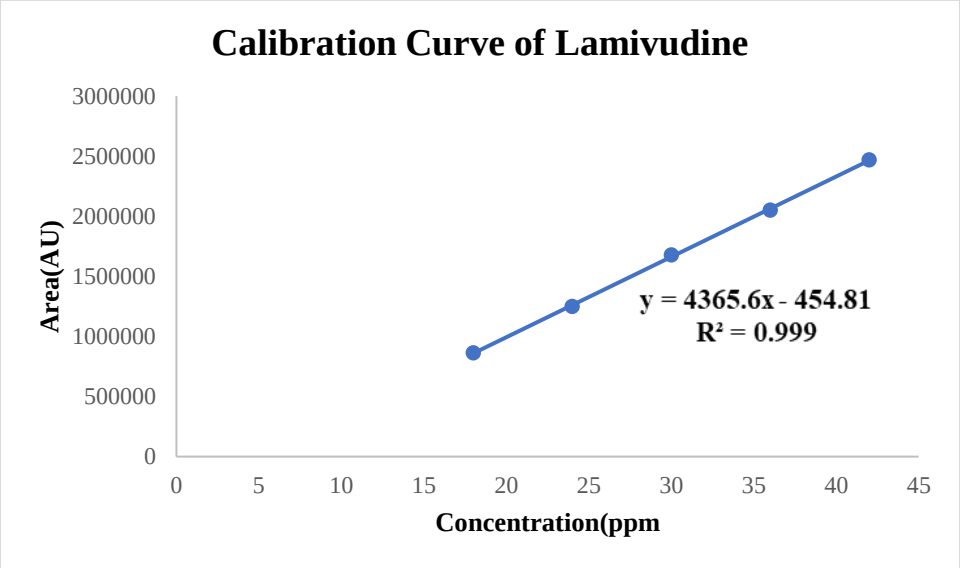

Fig: Calibration Graph of Lamivudine

Chromatographic data for linearity study of raltegravir:

Concentration µg/ml	Average Peak Area
30	863094
45	1249397
60	1678592
75	2050412
90	2468444

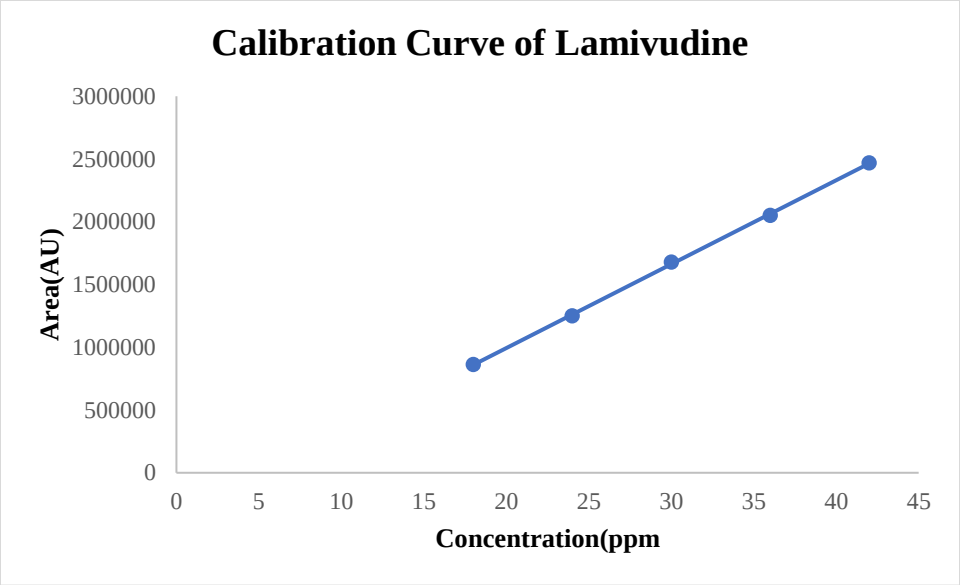

Fig: Calibration Curve of Raltegravir

Repeatability:

Table: Results of repeatability for Lamivudine:

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Lamivudine	2.157	513568	78546	1.2	4528
2	Lamivudine	2.159	513685	78541	1.2	4572
3	Lamivudine	2.186	513659	79852	1.2	4598
4	Lamivudine	2.160	513254	78498	1.3	4529
5	Lamivudine	2.170	513647	77898	1.2	4572
Mean			513562.6			
Std.dev			177.9475			
%RSD			0.03465			

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table: Results of repeatability for Raltegravir:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Raltegravir	3.603	1635625	265325	1.1	7985
2	Raltegravir	3.608	1658744	264588	1.1	7859
3	Raltegravir	3.600	1652985	265985	1.2	7845
4	Raltegravir	3.696	1645898	264898	1.1	7969
5	Raltegravir	3.629	1652364	268489	1.1	7846
Mean			1649123			
Std.dev			8811.631			
%RSD			0.534322			

Intermediate precision:

Table: Results of Intermediate precision for Lamivudine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Lamivudine	2.198	514658	78698	4658	1.2
2	Lamivudine	2.196	514354	78599	4598	1.2
3	Lamivudine	2.160	513985	79854	4652	1.2
4	Lamivudine	2.160	514875	79879	4561	1.2
5	Lamivudine	2.160	514658	79865	4659	1.2
6	Lamivudine	2.186	516452	79854	4589	1.2
Mean			514830.3			
Std. Dev.			852.3705			
% RSD			0.165563			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table: Results of Intermediate precision for Raltegravir

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Raltegravir	3.623	1645875	266589	7985	1.1	10.1
2	Raltegravir	3.611	1658554	265898	8001	1.1	10.1
3	Raltegravir	3.696	1649854	265415	7985	1.1	10.1
4	Raltegravir	3.696	1659842	265154	7956	1.1	10.1
5	Raltegravir	3.696	1645985	266598	7985	1.1	10.1
6	Raltegravir	3.642	1659852	265341	8002	1.1	10.1
Mean			1653327				
Std. Dev.			6838.733				
% RSD			0.413635				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table: Results of Intermediate precision Day 2 for Lamivudine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	US Plate count	USP Tailing
1	Lamivudine	2.198	514658	78572	4672	1.2
2	Lamivudine	2.196	514895	78516	4639	1.2
3	Lamivudine	2.178	514658	78572	4783	1.2
4	Lamivudine	2.142	514784	78372	4623	1.2
5	Lamivudine	2.177	515268	78592	4639	1.2
6	Lamivudine	2.177	514598	78526	4737	1.2
Mean			514810.2			
Std. Dev.			248.5224			
% RSD			0.048275			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table: Results of Intermediate precision Day 2 for Raltegravir

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Raltegravir	3.611	1638732	264384	7985	1.1	10.1
2	Raltegravir	3.623	1637438	265827	7946	1.1	10.1
3	Raltegravir	3.684	1638474	266382	7943	1.1	10.1
4	Raltegravir	3.697	1634273	269183	7964	1.1	10.1
5	Raltegravir	3.684	1636372	261931	7968	1.1	10.1
6	Raltegravir	3.684	1639283	264356	7982	1.1	10.1
Mean			1637429				
Std. Dev.			1860.366				
% RSD			0.113615				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Accuracy:

Table: The accuracy results for Lamivudine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	245954	10	10.179	101.79%	101.36%
100%	483747	20	20.316	101.58%	
150%	715961	30	30.	100.72%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

Table: The accuracy results for Raltegravir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	842287	30	30.114	100.38%	100.26%
100%	1659744	60	60.068	100.113%	
150%	2483885	90	90.268	100.297%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Lamivudine:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	513567	2.179	4536	1.2
Less Flow rate of 0.9 mL/min	523652	2.210	4462.3	0.9
More Flow rate of 1.1 mL/min	502146	2.184	4325.1	1.0
Less organic phase	521574	2.200	4632.4	0.9
More Organic phase	502416	2.172	4190.8	0.8

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Raltegravir:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1625892	3.610	4536	1.1
Less Flow rate of 0.9 mL/min	1758455	4.498	4426.4	0.9
More Flow rate of 1.1 mL/min	1742514	3.505	4421.5	0.8
Less organic phase	1726451	4.504	4355.1	0.9
More organic phase	1725466	3.512	4426.6	0.9

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

A Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method has been developed and validated for the simultaneous estimation of Lamivudine and Raltegravir in both pure and pharmaceutical dosage forms. The method employs a mobile phase consisting of ACN (Acetonitrile) and Water (40:60% v/v) and utilizes a Phenomenex Gemini C18 column (4.6mm×250mm, 5µm particle size) operated at a flow rate of 1.0 mL/min. Detection of Lamivudine and Raltegravir is performed at a wavelength of 255nm.

The validation of the analytical method was conducted according to established parameters including system suitability, specificity, linearity of response, accuracy, precision (reproducibility & repeatability), and robustness (evaluation of wavelength ±2 nm variation). The validation was carried out in compliance with ICH (International Council for Harmonisation) guidelines.

The RP-HPLC method demonstrated rapid, accurate, and precise results for the determination of Lamivudine and Raltegravir. Therefore, the proposed method is deemed suitable for routine analysis of these compounds in their pure form as well as in combined pharmaceutical dosage forms.

In conclusion, the developed RP-HPLC method offers a reliable and efficient means for the simultaneous quantification of Lamivudine and Raltegravir. The method's use of a simple mobile phase composition and a specific chromatographic column ensures robust performance in terms of sensitivity and resolution. The validation results confirm the method's suitability for its intended application, meeting all necessary analytical criteria outlined in ICH guidelines.

This method holds promise for routine quality control analysis of Lamivudine and Raltegravir in pharmaceutical formulations, providing pharmaceutical scientists and regulatory authorities with a valuable tool for ensuring the accurate and precise determination of these important drugs. Future studies may explore its application in analysing these compounds in complex matrices or in monitoring their stability under various conditions.

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