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Stability Indicating Method Development And Validation Of Tenofovir And Lamivudine By Uv Spectrophotometer And Rp-Hplc

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Abstract

This study aimed to develop and validate stability-indicating methods for the analysis of Tenofovir and Lamivudine using UV spectrophotometry and RP-HPLC. Optimization of chromatographic parameters including selection of method, ionic strengths of mobile phase, mobile phase ratio, mobile phase pH, and flow rate was conducted. A binary combination of water and methanol was utilized for drug concentration determination. Wavelengths of 265 nm for Tenofovir and 271 nm for Lamivudine were chosen. Methanol and water at a ratio of 60:40% v/v were found suitable as the mobile phase. The retention times for Tenofovir and Lamivudine were 2.7 and 2.67 minutes, respectively. The developed method adhered to ICH standards and exhibited linear calibration curves for Tenofovir within the concentration range of 10 to 30 µg/mL. The LOD and LOQ were found to be 0.105 µg/mL and 0.32 µg/mL, respectively. The method demonstrated high precision with low RSDs for intraday and interday accuracy. Recovery experiments confirmed method reproducibility. Stability studies revealed degradation under oxidative conditions. The UV method proved suitable for routine analysis, while the RP-HPLC method offered simplicity, selectivity, precision, and accuracy, with better sensitivity. Overall, both methods are applicable for Tenofovir and Lamivudine analysis, with RPHPLC being more sensitive and accurate. The stability studies indicated significant degradation under oxidative conditions compared to other degradation types.it is concluded that the developed UV and RP-HPLC methods offer reliable means for the analysis of Tenofovir and Lamivudine in formulations, complying with ICH guidelines. The RP-HPLC method demonstrated superior sensitivity and accuracy, although both methods are applicable. Stability studies highlighted the susceptibility of the drugs to oxidative degradation.

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Keywords: Tenofovir, Lamivudine, UV spectrophotometry, RP-HPLC, stability-indicating method, optimization, degradation, ICH guidelines.

INTRODUCTION:

The threat of disease and the rising costs of medicine are some of the challenges that the people of the world face. For developing effective medicine, the costs must be brought down so that the people of the world can access it Making all analytical methods available will make it easy for other competitors entering into the market. Equally bio-available, safe, and quality products will be available for cheaper prices in the market and help researchers in formulating other conventional dosage forms[1].

Analytical method development has a great role throughout the whole "Life cycle" of New Drug Development. Simply put, a pharmaceutical analysis is the examination of a drug (s). In Webster's definition, a pharmaceutical is defined as a therapeutic agent. It is common knowledge that a pharmaceutical is a pharmacologically active chemical compound. Active Pharmaceutical ingredient (API) or active ingredient is a better phrase for a pharmaceutical. "Active" is a colloquial name for it, however, the usage of this phrase is discouraged. The recommended word is active pharmaceutical ingredient, even though the term active ingredient is more often used. API is also known as a drug material to differentiate it from the formed product or drug product. To create a therapeutic product that can be administered to patients, a drug component is combined with inert chemicals (excipients). When it comes to administering a patient with a therapeutic medicine, the drug product is more likely to be used than the drug ingredient. While this may not always be the case, it is worth noting that certain pharmacological substances may be delivered after simply dissolving in water. The availability and other safety factors must be taken into account even in the most difficult of circumstances. To conduct safety and effectiveness studies, drug substances and products must fulfill two key conditions[2].

- Established identity and purity.
- Established bioavailability/dissolution

As a result, it is fairly commonplace to alter the manufacturing process for both the drug material and the drug product. To keep up with these changes, the approach must be regularly enhanced and/or adjusted. Methods that are phase-appropriate should be created, in reality. After careful assessment of the required dose and place of administration, a drug substance may be transformed into a variety of drug products using input from a variety of departments.

ANALYTICAL TECHNIQUES[3,4]:

Analyzing a sample only in line with written instructions, without further inspection of the findings, is rare in the pharmaceutical industry. This is true even in the context of quality control, where it is typical to assume that testing is done per recipe. Many problems would go unnoticed if this were the case. Batch-to-batch variations may occur even in the most perfect production setting. Analytical research and development require a high degree of flexibility, attentiveness, and innovation. Consider all known and unknown pollutants when designing a strategy for selecting the optimal one.

THIN LAYER CHROMATOGRAPHIC TECHNIQUE FOR IDENTIFICATION OF

DRUGS[5,6]: Its ease of use, cheap cost, great sensitivity, and quick separation time make TLC a very popular analytical instrument for a broad range of applications. A thin fixed phase and a liquid mobile phase are used to separate the components of a mixture. TLC may be used to identify the constituents of a combination, assess the purity of a chemical, and keep track of a reaction's development.

FT-IR AN IMPORTANT TOOL FOR IDENTIFICATION[7]:

FTIR is used for the identification of organic, inorganic, and polymeric materials utilizing infrared radiation for scanning the samples.

PARTICLE SIZE ANALYSIS - BY LASER DIFFRACTION-BASED TECHNIQUE:

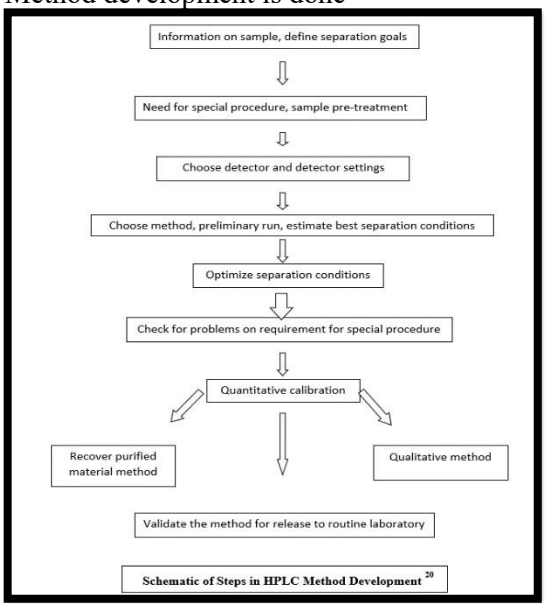
A particle can be defined as a minute portion, piece, fragment, or amount of matter. Particle size plays an important role in predicting dissolution rate and flow ability (of powders). Sieving and laser diffraction-based techniques are the methods available for measuring particle size distribution. The ideal concentration for a representative sample is usually a liquid or gas. The refractive index of the sample is determined by looking at its shape and transparency.

STABILITY INDICATING RP-HPLC ASSAY METHODS[8]:

Methods that can distinguish between the principal active (intact) pharmaceutical components and any degradation (decomposition) product created during the stability assessment period are called stability-indicating methods. In areas like formulation development, manufacturing, and packaging, where knowledge of chemical behavior may be applied to enhance a therapeutic product, forced degradation studies may aid pharmaceutical development. This criterion has been more evident since the ICH rules came into effect. HPLC is a key analytical method for determining the stability of a medicinal product6. Separate, identify, and quantify the numerous drug-related degradants that may occur during storage or production, as well as detect and quantify any drug-related impurities that may be introduced during synthesis. HPLC techniques.

ANALYTICAL METHOD DEVELOPMENT[9,10]

Method development is done



Schematic of Steps in HPLC Method Development [20]

- "For new products"
- "For existing products"

Strategy for Method Development[11]

ANALYTICAL METHOD VALIDATION:

Method validation ensures dependability in regular usage and is often referred to as "the act of giving recorded proof that the method achieves what it is meant to do." The primary goal of validation is to show that the analytical technique is appropriate for its intended purpose and is accurate, specific, and precise throughout the defined range of analytes to be evaluated.

Analytical Performance Characteristic	Identification	Testing for Impurities		Assay: Content/Potency
		Quantitation	Limit	
Accuracy	–	+	–	+
Precision • Repeatability • Intermediate precision	– –	+ +	– –	+ +
Specificity	+	+	+	+
Detection limit	–	–	+	–
Quantitation limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

- Indicates this characteristic need not be considered
+ Indicates this characteristic needs to be considered

Method Validation Parameters as per ICH guidelines

The duties of a pharmaceutical analyst include ensuring that all batches of a drug product are made to the specifications established by the regulatory authorities. The relevance of analytical techniques in establishing and ensuring pharmaceutical quality is obvious from the preceding discussion.

ANALYTICAL METHOD VALIDATION AS PER ICH GUIDELINES[12,13]

In order to ensure that the analytical approach used for a given test is appropriate for its intended application, method validation is required. Validation or revalidation of methods is necessary. Any time the circumstances under which a technique has been validated, such as a new
instrument with different properties, are introduced into normal usage
Every time a method is altered, and the change is beyond the method's initial scope.
A method's accuracy, specificity, reproducibility, and robustness throughout the stated range of an analyte are all checked out during method validation. Method validation, which is also referred to as "the act of supplying documented proof that the technique functions as intended," assures the dependability of a method in everyday usage. Compliance with FDA regulations necessitates the validation of laboratory methods.
The "Eight Steps of Method Validation" may be found in the USP Chapter on Method Validation and can be referred to as such. "Analytical performance parameters" or "analytical figures of merit" are sometimes used to describe this. The vast majority of these expressions are commonplace in the laboratory, where they are regularly used. Then then, there are certain words that have multiple meanings for different individuals. Method validation can only be furthered if all of the words and definitions involved have been fully grasped.
"Validation of Analytical Methods: Definitions and Terminology" was created by the International Conference on Harmonization in response to this situation. Figure 1 depicts how ICH broke down the "validation characteristics" into their several subcategories.

MATERIALS AND INSTRUMENTS[14,15] METHODOLOGY:

METHOD DEVELOPMENT AND VALIDATION OF TENOFOVIR BY HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY) METHOD

Instrument Specifications:

Instruments	Revers phase high-Performance Liquid Chromatography
Injector	Methanol (20µl loop)
Software	LC solutions
Detector	PDA

Initial chromatographic conditions[16,17]:

Selection of chromatographic method for separation

Reverse-phase chromatographic techniques were selected since the drug is polar.

Selection of wavelength:

Selectivity of HPLC method that uses PDA detector depends on proper selection of wavelength. A wavelength which gives distinct and specific response for the drugs is to be selected. From the UV spectra of Tenofovir drug, 265 nm was selected as the wavelength for the present study.

Selection of mobile phase:

Solvent selectivity (solvent type), solvent strength (percentage of organic solvent in the mobile phase), strength and pH of buffer, flow rate etc. were varied to determine the chromatographic conditions that gave the best separation.

Different mobile phases tried and their observations are given in the Table.

Selection of mobile phase trials[18,19]:

Optimized Chromatographic conditions:

MOBILE PHASE CONDITION	OBSERVATION
Methanol : Acetonitrile (75:25)	Tailing, Broad peaks
Methanol: Acetonitrile pH5 (70:30)	Fronting, Tailing, Splited Peaks
Methanol : Acetonitrile pH4.5 (70:30)	Tailing, Splited Peaks
Methanol : Acetonitrile pH4 1ml/min (70:30)	Tailing, Broad Peaks
Water: Methanol(60:40)	Good Symmetric Peaks

Method development and validation of Tenofovir[20,21]

System suitability studies

System suitability parameters like Retention time, number of theoretical plates (N), Tailing factor, resolution (Rs) etc., were studied, and results are given in Table. **System suitability studies of Tenofovir**

Drug	Theoretical plates (N)	Retention time (Rt)	Tailing factor
Tenofovir	3711	2.77	1.5

Concentration (µg/ml)	Mean peak area (A.U.)
5	**1320165**
10	**2510259**
15	**3678129**
20	**4928168**
25	**5972329**
Linear regression equation (y=mx+c)	**Y=234445x+165138.9**
Slope(m)	**23444.74**
Intercept(c)	**165138.9**
Correlation coefficient (R^2)	**0.999**

Linearity of Tenofovir:

Precision:

ANALYSIS OF FORMULATION[22,23]

For formulation analysis, fixed chromatographic conditions were used.

Preparation of standard solutions:

Tenofovir was dissolved in 10 ml of mobile phase and used to make stock solutions. Tenofovir standard stock solutions were aliquoted into 10-ml volumetric flasks and diluted to the desired concentrations within the Linearity limits.

Preparation of sample solutions:

The average weight of twenty Tenofovir pills was estimated, and each tablet was finely ground. Weighed and placed into a 10 ml volumetric flask, 100 mg of Tenofovir was used. To get rid of it, mobile phase is utilized. In order to achieve complete dissolution of the pharmaceuticals, the volumetric flask was sonicated for 20 minutes, then the solution was filled up with mobile phase and passed through a filter. Aliquots of the formulation solution were made and injected into the HPLC to attain a concentration within the linearity range.

Recording of chromatograms:

Standard drug solutions were administered and chromatograms obtained after a stable baseline was acquired using fixed chromatographic conditions. This drug has a half-life of 2.7 minutes. An injection was then made of a formulation-derived test sample. Peak areas of a standard medication vs the concentration of related standard solutions were used to draw calibration curves. The quantity of Tenofovir in the samples was determined by comparing the peak areas of the chromatograms.

Analysis of marketed formulation[24,25]

Drugs	Labeled amount(mg)	Amount found(mg)	% Label claim
Tenofovir	**60**	**59**	**98.33**

SL.NO	Degradation	% of degradation
1	**Acid degradation**	**10**
2	**Base Acid degradation**	**30**
3	**Oxidative degradation**	**50**

Summary of degradation:

RP-HPLC SYSTEM[26,27,28]:

Method development and validation of Lamivudine Instrument Specifications:

Instruments	Reverse phase high-performance liquid chromatography
Injector	Methanol (20µl loop)
Software	LC solutions

Initial chromatographic conditions[29,30]

Selection of the chromatographic method for separation

Reverse-phase chromatographic techniques were selected since the drug is polar. **Selection of wavelength**

The selectivity of the HPLC method that uses a PDA detector depends on the proper selection of wavelength. A wavelength that gives a distinct and specific response for the drugs is to be selected. From the UV spectra of the Lamivudine drug, 271 nm was selected as the wavelength for the present study.

Optimized Chromatographic conditions

Column	Trait C18 column (100 x 2 mm, 1.9 µm)
Flow rate	1 mL/ minute
Detector Wavelength	271 nm
Injection volume	20 µl
Column Temperature	600 °C
Auto sample Temperature	20 °C
Elution mode	Isocratic
Run time	5 minutes

System suitability studies

System suitability parameters like Retention time, number of theoretical plates (N), Tailing factor, resolution etc., were studied, and results are given in Table. **System suitability studies of Lamivudine**

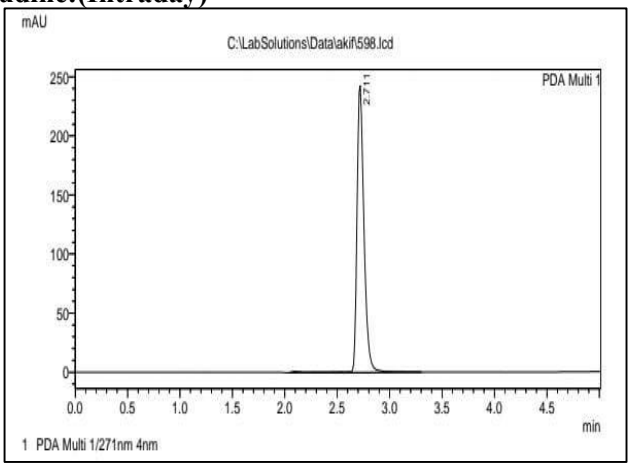
Drug	**Theoretical plates (N)**	**Retention time (Rt)**	**Tailing factor**
Lamivudine	3711	**2.67**	1.5

Calibration curve of Lamivudine Precision:

Intraday Precision of Lamivudine:

Drug	**Conc (µg/ml)**	**Time intervals of samples in the precision**					**Average**	**%RSD**
Lamivudine	5	2910583	2910054	2910129	2900248	2801352	2886473	1.48059

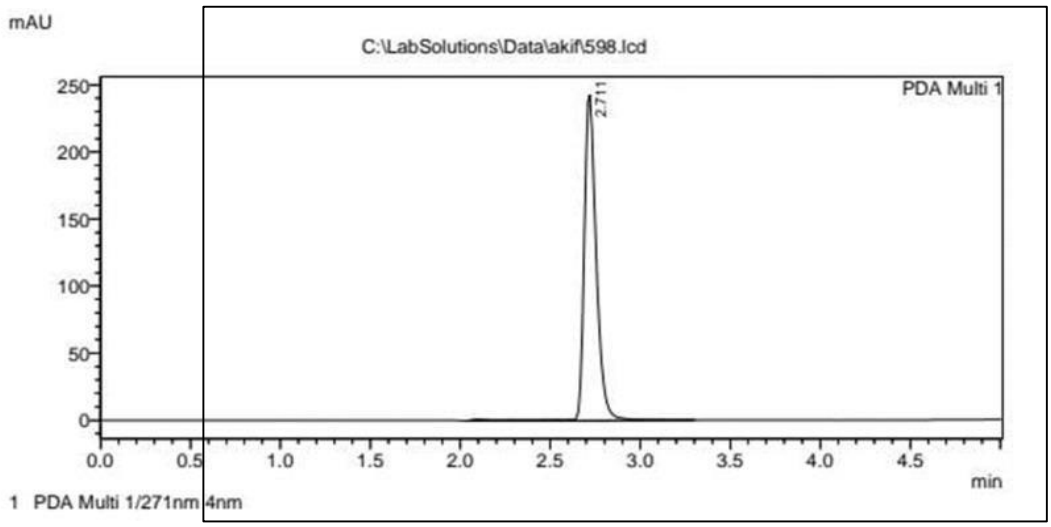
Precision of Lamivudine:(Intraday)



Inter-day Precision of Lamivudine:

Drug	**Conc (µg/ml)**	**Time intervals of samples in the precision**					**Average**	**%RSD**
Lamivudine	5	2891356	2900356	2912249	2923524	2930458	2911588	0.49402

Precision of Lamivudine: :(inter-day)



Limit of detection (LOD) and limit of quantification (LOQ):

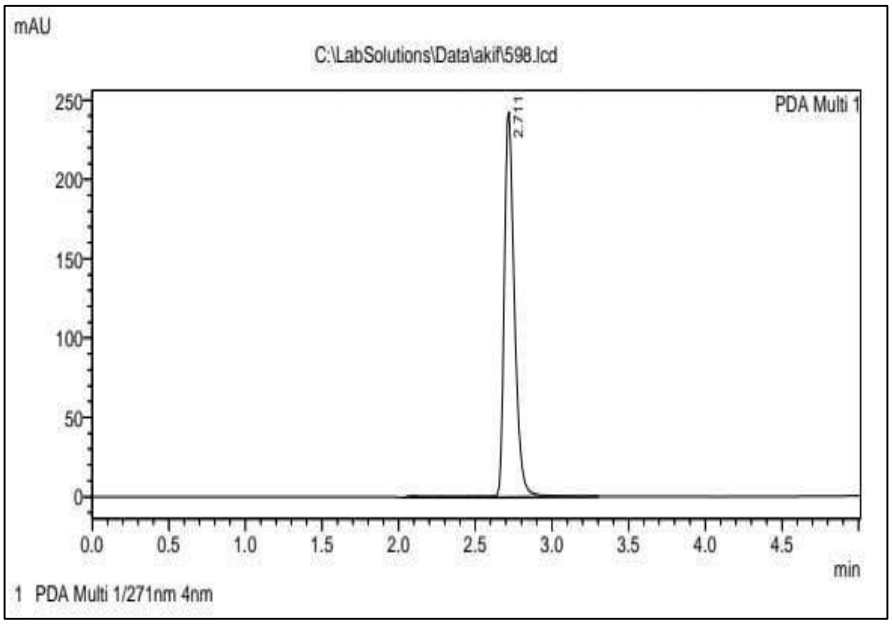
LOD and LOQ were calculated mathematically. The LOD and LOQ of Tenofovir were found to be 0.34 µg/ml and 0.112 µg/ml respectively.

Drug	Limit of Detection		Limit of Quantification
Lamivudine	0.112		`0.34

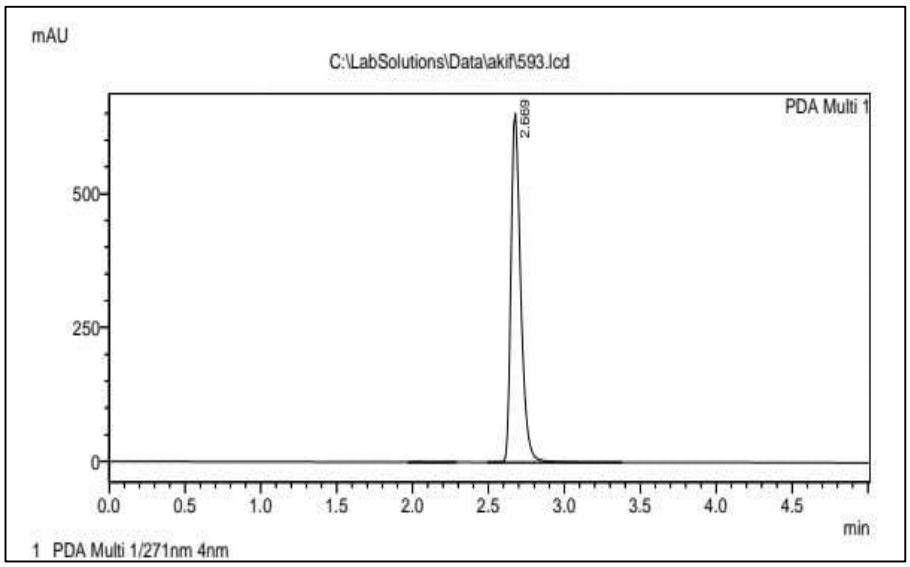
Accuracy studies of Lamivudine:

Drug	Levels (%)	Amount taken(µg/mL)	Amount added(µg/mL)	Amount recovered (µg/mL)	% recovered
Lamivudine	80	50	30	80.25	100.31%
	100	50	50	100.52	100.52%
	120	50	70	119.21	99.34%

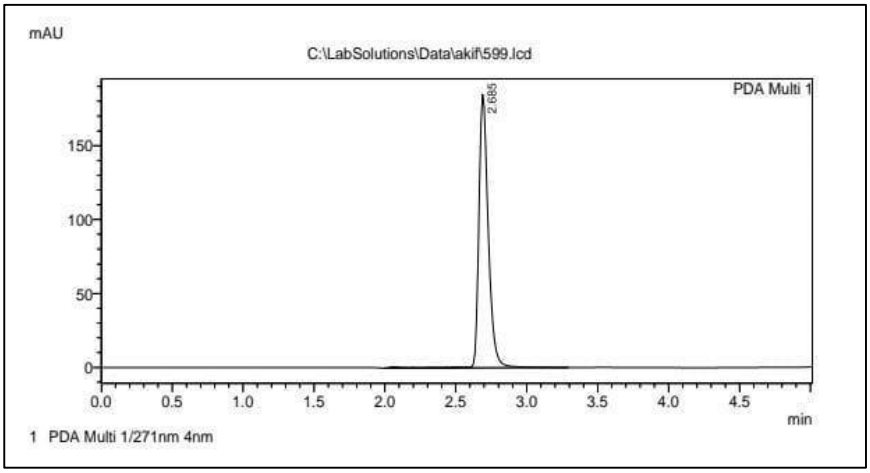
Accuracy of Lamivudine :80%



Accuracy of Lamivudine:100%



Accuracy of Lamivudine:120%



ANALYSIS OF FORMULATION:

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Preparation of standard solutions

Lamivudine was dissolved in 10 ml of mobile phase and used to make stock solutions. Lamivudine standard stock solutions were aliquoted into 10-ml volumetric flasks and diluted to the desired concentrations within the Linearity limits.

Preparation of sample solutions:

The average weight of twenty Lamivudine pills was estimated, and each tablet was finely ground. Weighed and placed into a 10 ml volumetric flask, 100 mg of Lamivudine was used. To get rid of it, mobile phase is utilized. In order to achieve complete dissolution of the pharmaceuticals, the volumetric flask was sonicated for 20 minutes, then the solution was filled up with mobile phase and passed through a filter. Aliquots of the formulation solution were made and injected into the HPLC to attain a concentration within the linearity range.

Preparation of sample solutions:

The average weight of twenty Lamivudine pills was estimated, and each tablet was finely ground. Weighed and placed into a 10 ml volumetric flask, 100 mg of Lamivudine was used.

To get rid of it, mobile phase is utilized. In order to achieve complete dissolution of the pharmaceuticals, the volumetric flask was sonicated for 20 minutes, then the solution was filled up with mobile phase and passed

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Recording of chromatograms

Standard drug solutions were administered and chromatograms obtained after a stable baseline was acquired using fixed chromatographic conditions. This drug has a half-life of 2.67 minutes. An injection was then made of a formulation-derived test sample. Peak areas of a standard medication vs the concentration of related standard solutions were used to draw calibration curves. The quantity of Lamivudine in the samples was determined by comparing the peak areas of the chromatograms.

Analysis of marketed formulation:

Drug	Labeled amount(mg)	Amount found(mg)	% Label claim
Lamivudine	60	59	98.33

DISCUSSION

In this method, optimizations of different chromatographic parameters like selection of

- Chromatographic method
- ionic strengths of mobile phase
- Mobile phase ratio ➢ Mobile phase pH
- Flow rate etc., were done.

Drug concentrations were determined by using a binary combination of water and methanol. For this investigation, 265nm for Tenofovir and 271 nm for Lamivudine was chosen as the wavelength. Initially, several combination of concentrations were tested. Out of these results, Methanol and water were discovered to be excellent for the task. Then, by adjusting the ratio of Methanol to water, the mobile phase ratio was established. An alcohol/water solution (60:40 percent) was used to determine the drug's potency. Tenofovir was shown to have a retention time of 2.7 and Lamivudine was shown to have a the retention time 2.67 minutes. The new procedure was put to the test in accordance with ICH standards and found to be safe. The standard peak regions vs. concentration of standard solutions were used to produce calibration graphs. Tenofovir was shown to be linear in the concentration range of 10 to 30g/ml, with slope, intercept, and correlation coefficients of 234444.74, 165138.9, and 0.999, respectively. Tenofovir's LOD and LOQ were determined to be 0.105 g/ml and 0.32 g/ml, respectively, in this study. Intraday and interday accuracy of the devised approach were analysed. Indicators of high precision include low relative standard deviations (RSDs). Recovery experiments were conducted at two levels, i.e. 80 percent, 100 percent, and 120 percent, in order to establish the method's reproducibility by adding known amounts of standard pharmaceuticals and conducting analysis as per the formulation technique. The approach was shown to be accurate based on the recovery values. The procedure that was devised was proven to be dependable.Retention time (Rt), Tailing factor and Resolution were all examined to determine the system's appropriateness. For the purpose of determining the stability of Tenofovir and Lamivudine researchers used the previously established liquid chromatographic technique. Studies on the stability of Tenofovir and Lamivudine were carried out using a variety of different types of forced degradation, including acid, alkali, neutral, oxidative, photolytic, and thermal. Degradation was observed to be more pronounced under Oxidative conditions.

CONCLUSION:

The direct UV method development for the analysis of Tenofovir and Lamivudine can be applied for the routine analysis of formulation and the result was found within the limits according to ICH guidelines. The developed gradient Reverse Phase-HPLC method offers simplicity, selectivity, precision and accuracy. In this proposed method symmetrical peaks with good resolution were obtained. Out of all the methods developed, the RP-HPLC method was more sensitive and accurate. However, all these methods can be used for the analysis of Tenofovir and Lamivudine. By performing the stability studies the drug was degraded nearly 50 percent in Oxidative degradation when compared to Acid, Alkaline and thermal degradations.

REFERENCES:

1. Sharma B, Instrumental methods of chemical analysis, 19th edition, Goel Publishing House, 2003.
2. Willard H, Instrumental method of analysis, 7th edition, CBS Publishers and Distributors, New Delhi, 1986.
3. Remington, The Science and practice of pharmacy, 21st edition, (1), 2006.
4. Satoshkar R, Bhandarkar S and Ainapure S, Pharmacology and pharmacotherapeutics, 15th edition, Popular Prakashan, Mumbai, 1996.
5. Wolff ME. Berger's, A Medicinal Chemistry, 4th edition, Wiley Inter science, New York, 1981.
6. Doserge RF. Wilson and Gisvold's, Text book of organic medicinal and pharmaceutical chemistry, 8thedition, Lippincott Company, 1982.
7. AshutoshKar, Pharmaceutical analysis, 1st edition, Vol I, CBS Publishers and Distributers, New Delhi, 2007.
8. Beckett AH and Stenlake JB, Practical pharmaceutical chemistry, 4th edition, CBS Publishers and Distributers, New Delhi, 2007.
9. Indian Pharmacopoeia, Indian Pharmacopoeia commission, Ghaziabad, 2007.
10. British Pharmacopoeia, Medicines and health care product regulatory agency, London, 2010.
11. Reynolds JEF, Martindale- The extra Pharmacopoeia. 28th edition, The Pharmaceutical press, London, 1982.
12. Willard H, Merritt L, John A. Dean and Frank A. Settle, Instrumental methods of analysis, 7th edition, CBS Publishers and Distributers, New Delhi, 1986.
13. Fulton GK, Barbara S. Larsen and Charles N. McEwen, Gas chromatography and mass spectroscopy-A practical guide, Academic Press, London, 1996.
14. Basett J, Denney RC, Jerry GH and Mendham J, Vogel's text book of quantitative inorganic analysis, 4th edition, London, 1986.
15. Howard GA and Martin AJP, the separation of the C_{12}- C_{18} fatty acids by reversed-phase partition chromatography, Biochemical Journal, 1950; 46(5): 532-538.
16. Krusulovic AM and Brown RP, Reverse Phase High performance liquid chromatography, Theory practice and biomedical applications, 1982.
17. Gurdeep Chatwal and Sham Anand, Instrumental methods of chemical analysis, 1st Edition, Himalaya publishing House, Delhi, 1999.
18. Joachim Ermer and John H, Method validation in pharmaceutical analysis- A guide to best practice, Wiley –VCH Verlag Gmbh and Weinheim, 2005.
19. Thomas H, Stout, John G and Dorsey, High-Performance Liquid Chromatography, Eurand America, Inc. Vandali, Ohio, 2002.
20. Synder LR and Kirkland JJ, Introduction to modern liquid chromatography, Wiley interscience, New York, 1979.
21. Dr. Chowdary KPR, Devala Rao G and Himabindu G, Validation of analytical methods, The Eastern pharmacist, 1999; 42(497): 39-41.
22. International Conference on Harmonization, Validation of analytical procedures: methodology. Federal Register, 1996.
23. ICH harmonized tripartite guideline, Text on validation of analytical procedures, recommended for adoption at step 4 of the ICH process by the ICH steering committee.
24. Priti. H. Vansiya ,Kunjan B. Bodiwala , Sandesh R et al., Development and Validation of Spectrophotometric Method for the Estimation of EdoxabanTosylate Monohydrate in its Synthetic Mixture, Ajptr,2016, 8(2),297-306.
25. Panchumarthy Ravisankar*, D. Srikanth, C. Venkateswara Reddy et al., Development and validation of UV spectrophotometric method for the determination of EdoxabanTosylate Monohydrate in pharmaceutical dosage form,ijrpb,2018,(2),73-78.
26. P. Ravi Sankar*, M.M. Eswarudu, P. Siva Krishna, D. Srikanth,et al., Novel Validated RP- HPLC Method For Determination Of EdoxabanTosylate Monohydrate In Bulk And Its Pharmaceutical Dosage Form,jpsr,2021,(5),232-237.
27. Mohammed Hamad 2 , Ramadan Al-Shdefat 3 , Kenza Mansoor et al., Development And Validation Of A Stability Indicating RP-HPLC Method Of Apixaban In Commercial Dosage Form,ijpsr,2021,12(1),241-251.

28. Swarup Suresh Prabhune *, Rajendra Shankar Jaguste, Prakash Laxman Kondalkar,et al., Stability-Indicating High-Perform ance Liquid Chromatographic Determination of Apixaban in the Presence of Degradation Products,2014,(82),777-785.
29. Shashikant B. Landge1, Sanjay A. Jadhav1, Sunil B. Dahale et al., Development and Validation of Stability Indicating RP-HPLC Method on Core Shell Column for Determination of Degradation and Process Related Impurities of Apixaban—An Anticoagulant Drug,2015,6,539-550.
30. Thomas H, Stout, John G and Dorsey, High-Performance Liquid Chromatography, Eurand America, Inc. Vandali, Ohio, 2002.