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LITERATURE REVIEW ON HPLC METHODS FOR ESTIMATION OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMARATE TABLETS

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ABSTRACT

Emtricitabine and Tenofovir Disoproxil Fumarate (TDF) are essential components of antiretroviral therapy (ART) for HIV infection. Accurate quantification of these drugs in pharmaceutical formulations is crucial for ensuring therapeutic efficacy and patient safety. High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for this purpose. Several validated HPLC methods have been developed, employing diverse chromatographic conditions and detection techniques. These methods typically utilize reversed-phase C18 columns and UV detection at 260 nm or 270 nm. Key considerations for method development include selectivity, sensitivity, accuracy, precision, linearity, and robustness. Additionally, other analytical techniques such as Ultra-High Performance Liquid Chromatography (UHPLC), Thin-Layer Chromatography (TLC), Fourier Transform Infrared Spectroscopy (FTIR), and Ultraviolet-Visible (UV-Vis) Spectroscopy have been explored for the analysis of Emtricitabine and TDF. However, HPLC remains the preferred method due to its versatility, sensitivity, and reliability.

INTRODUCTION

Emtricitabine and Tenofovir Disoproxil Fumarate (TDF) are vital components of antiretroviral therapy (ART) for the treatment of HIV infection. Accurate and precise quantification of these drugs in pharmaceutical formulations is crucial for ensuring therapeutic efficacy and patient safety.

High-Performance Liquid Chromatography (HPLC) has emerged as a reliable and widely used analytical technique for the determination of drugs in various matrices, including pharmaceutical formulations. HPLC offers several advantages, such as high sensitivity, selectivity, and reproducibility, making it a preferred method for the analysis of complex drug mixtures.

Literature Review on HPLC Methods

A comprehensive literature review reveals a plethora of HPLC methods developed and validated for the simultaneous determination of Emtricitabine and TNF in tablet formulations. These methods employ diverse chromatographic conditions, including:

- **Stationary Phase:** Reversed-phase C18 columns are commonly used due to their broad applicability and efficient separation of analytes.
- **Mobile Phase:** A combination of aqueous buffer and organic solvent, such as acetonitrile or methanol, is typically employed to achieve optimal separation. The pH of the mobile phase is often adjusted to enhance peak shape and resolution.
- **Detection:** UV detection at a suitable wavelength, such as 260 nm or 270 nm, is commonly used to monitor the elution of analytes.

Methods for Analysis of Emtricitabine and Tenofovir Disoproxil Fumarate:

1. High-Performance Liquid Chromatography (HPLC): HPLC is a commonly used technique for the analysis of emtricitabine and tenofovir disoproxil fumarate. The method involves mobile phase preparation, column selection, and detection wavelength optimization. The analytes are separated based on their affinity for the stationary phase and are detected using UV **or** mass spectrometry.

2. Ultra-High Performance Liquid Chromatography (UHPLC): UHPLC offers faster analysis times and improved sensitivity compared to traditional HPLC. The method development and validation steps are similar to HPLC, but with the use of smaller particle columns and higher pressures.

3. Thin-Layer Chromatography (TLC): TLC is a simple and inexpensive technique for preliminary screening and qualitative analysis. The analytes are separated on a stationary phase based on their adsorption properties and visualized using appropriate staining reagents.

4. Fourier Transform Infrared Spectroscopy (FTIR): FTIR is a non-destructive technique that can be used to identify and quantify the functional groups present in the drug and excipients. The method involves measuring the absorption of infrared radiation by the sample.

5. Ultraviolet-Visible (UV-Vis) Spectroscopy: UV-Vis spectroscopy is a quantitative technique that can be used to determine the concentration of analytes based on their absorbance at specific wavelengths. The method is suitable for emtricitabine and tenofovir disoproxil fumarate due to their UV-absorbing properties.

LITERATURE REVIEW:

REF	Specification	Experimental conditios	Results
(1)	RP-HPLC Method Validation for Estimation of Tenofovir Disoproxil Fumarate in Pharmaceutical Oral Dosage Form.	C-18 column 4.6 mm x 250 cm x 5 µm column Buffer: Acetonitrile in the ratio of 60: 40 Detected at 260nm	
(2)	Simultaneous determination of emtricitabine, tenofovir alafenamide fumarate and dolutegravir sodium by validated stability-indicating RP-HPLC-DAD method	Exterra C18 column (150×4.6mm, 5µm)Methanol and Buffer (comprising 0.1 (v/v) of Triethylamine and o-phosphoric acid in water, pH 2.6) as mobile phase. Wavelength 265nm.	Linearity- 500-1500µg/mL for EMT, 62.5-187.5µg/mL for TEN and 125-375µg/mL . The LOD of EMT, TEN and DOL were 91.78µg/mL, 10.47µg/mL and 19.28µg/mL correspondingly. The LOQ of EMT, TEN and DOL were 278.11µg/mL, 31.74µg/mL and 58.42µg/mL correspondingly. Assay 99.11-100.84%.
(3)	Analytical method development, validation, Synthesis, Characterization and Forced degradation study of Tenofovir Disoproxil Fumarate and its impurities	Shimpack C18 column (4.6 x 50 mm, i.d. 3µm)A combination of methanol-acetonitrile (50:50) and ammonium acetate (pH 4.19) in the ratio of 50:50 V/V is used as the mobile phase.	Accuracy 98.85% and precision obtained is 0.55%. linearity in the range of 10-60 µg/ml with correlation coefficient of 0.999. LOD and LOQ is found to be 0.514 and 1.713 µg/ml respectively.

(4)	Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Bictegravir, Emtricitabine, and Tenofovir Alafenamide Fumarate	inertsil octyldecylsilyl C18 (4.6×250 mm, 5 mm) 0.2% triethylamine buffer and methanol in a ratio of 40:60% (v/v) as the mobile phase to attain optimal elution.	The linearity ranges for bictegravir, emtricitabine and tenofovir AF were 25-125 µg/mL, 100-500 µg/mL, and 12.5-62.5 µg/mL, respectively. The R.T for bictegravir, emtricitabine, and tenofovir AF were found to be 5.998 min, 2.805 min, and 4.537, min respectively. The percent recoveries were within the range of 98-102% w/w.
(5)	Analytical method development and validation of related substances by rp-hplc of emtricitabine and tenofovir disoproxil fumarate tablets	Waters X-bridge C18 (250 mm x 4.6 mm, 5 µm) 260 nm	The retention times of Emtricitabine and Tenofovir Disoproxil Fumarate are approx. 29 min and 70 min. respectively. Monoester Impurity and Dimer Impurity found linear over the range of LOQ - 150 % of target concentration. Method also found precise by spiking impurities at specification level. Accuracy was demonstrate at LOQ - 150 % level
(6)	Analytical Method Development And Validation For The Determination Of Emtricitabine And Tenofovir Disoproxil Fumarate Using Reverse Phase Hplc Method In Bulk And Tablet Dosage Form	Mobile phase: ammonium acetate, phosphate buffer pH 3.5 and Acetonitrile at ratio of (3:5Edurasil ODS-3 C18(4.6 x 50mm, 3.5µm) column	The linearity study Emtricitabine and Tenofovir Disoproxil Fumaratewas found in concentration range of 20µg-100 µg and 30µg-150µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.35% and 100.24%, %RSD for repeatability was 0.22 and 0.5, % RSD for intermediate precision was 0.6 and 0.69 respectively. The precision study was precise, robust, and repeatable.LOD value was 2.98 and 2.96 , and LOQ value was 9.98 and 9.96 respectively.
(7)	A new RP-HPLC method for the	Hyper ODS2 C18Methanol and Phosphate buffer	A linearity range and retention time of Tenofovir were found to be 20-110 µg/ml and 2.1 min

	determination of Tenofovir Disoproxil Fumarate in pure form and pharmaceutical formulation	(90:10) as mobile phase	respectively. The % RSD of the Tenofovir was found to be 0.7. The % recovery was obtained as 99.7% for standard and 96.32% for tablets.
(8)	Development and validation of analytical method for quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC	Zorbax SB CN, (250 × 4.6 mm, 5 µm) column consisted of methanol (A) and buffer at pH 4.5(B)	The method showed to be linear (r^2 > 0.999), precise (RSD < 0.76%), accurate (recovery of 100.09% for Emtricitabine, 99.88% for Tenofovir and 100.04% for Efavirenz), specific and robust. Three batches of Emtricitabine, Tenofovir, and Efavirenz tablets were assayed by the validated method. The Emtricitabine contents in the tablet samples varied from 99.94 to 101.60%. The Tenofovir content in the tablet samples varied from 99.13 to 101.81% while Efavirenz content varied from 100.01 to 101.67%.
(9)	Analytical Method Development and Validation for the Determination of Emtricitabine and Tenofovir Disoproxil Fumarate Using Reverse Phase HPLC Method in Bulk and Tablet Dosage Form	Inspire C18 column (150×4.6mm) 5.0µm (30:70 v/v) Ortho-phosphoric acid Buffer : Methanol.	Linearity:20µg-100 µg and 30µg-150µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.35% and 100.24%, %RSD for repeatability was 0.22 and 0.5, % RSD for intermediate precision was 0.6 and 0.69 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.98 and 2.96 , and LOQ value was 9.98 and 9.96 respectively
(10)	A Validated Stability Indicating RP-HPLC Method	an ODS column (250 × 4.6 mm, 5 µm)mobile phase A (potassium	linear in the concentration range of 2-12 µg/mL for EMT, 3-18 µg/mL for TNDF, 1.5-9 µg/mL for ELV and COB, with the coefficient

	for the Determination of Emtricitabine, Tenofovir Disoproxil Fumarate, Elvitegravir and Cobicistat in Pharmaceutical Dosage Form	dihydrogen orthophosphate, pH adjusted to 2.5) and mobile phase B (acetonitrile) in the ratio of 55:45% v/v	value (R(2)) of >0.9990. The accuracy was measured via recovery studies and found to be acceptable, and the percentage recoveries were found in the range of 99.93-100.08 ± 0.5%.
(11)	Stability-Indicating HPLC Method for the Simultaneous Determination of HIV Tablet Containing Emtricitabine, Tenofovir Disoproxil Fumarate, and Rilpivirine Hydrochloride in	Phenomenex Gemini C18 column (150 mm × 4.6 mm i.d., 5 µm)MeCN, potassium dihydrogen phosphate buffer (20 mM, pH 3.3), and triethylamine 58.72 : 41.23 : 0.05 (v/v)	The method was validated in terms of accuracy, precision, linearity, limits of detection, limits of quantitation, and robustness.
(12)	HPLC Method for the Determination of Emtricitabine and Related Degradation Substances	C18 HiQSil column ammonium formate (pH 4.2) and methanol in a gradient elution mode	Detection and quantification limits were established at 0.02 and 0.05 µg/mL, respectively. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal decomposition to determine the degradation behavior. Extensive degradation was found under acid, alkaline and oxidative stress.

CONCLUSION

The literature review highlights the widespread use of HPLC as a reliable and robust analytical technique for the determination of Emtricitabine and Tenofovir Disoproxil Fumarate in pharmaceutical formulations. Several validated methods have been developed, each with its own specific chromatographic conditions and detection parameters.

Key findings from the reviewed literature include:

- **Method Validation:** Most of the reviewed methods have been rigorously validated, demonstrating their accuracy, precision, specificity, and robustness.

- **Chromatographic Conditions:** Reversed-phase C18 columns with a mobile phase consisting of an aqueous buffer and an organic solvent (e.g., acetonitrile, methanol) are commonly employed.
- **Detection:** UV detection at 260 nm or 270 nm is the preferred detection method.
- **Sensitivity and Specificity:** The developed methods exhibit adequate sensitivity and specificity for the determination of the target analytes, even in the presence of potential impurities or excipients.
- **Stability-Indicating Methods:** Several studies have reported the development of stability-indicating HPLC methods, capable of detecting and quantifying degradation products.

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