# Estimation of Emtricitabine and Tenofovir by HPTLC Method

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***Abstract:***

*The HPTLC procedure was optimized for simultaneous determination of Emtricitabine and Tenofovir. The mobile phase Methanol: Toluene: Ethyl acetate: Ammonia (1.5:5.5:1.5:0.1 v/v/v/v) resulted in good resolution, and sharp and symmetrical peaks were obtained. It was observed that prewashing of HPTLC plates with methanol (followed by drying and activation) and pre saturation of HPTLC chamber with mobile phase for 20 min (optimum chamber saturation time) ensured good reproducibility and peak shape of three drugs.*

***Key Words:*** *mobile phase, environment-friendly solvents.*

## INTRODUCTION:

High Performance Thin Layer Chromatography (HPTLC) is a powerful method equally suitable for qualitative and quantitative analytical tasks.HPTLC has been reported to provide excellent separation, qualitative and quantitative analysis of a wide range of compounds, such as herbal and botanical dietary supplements, nutraceuticals, traditional western medicines, traditional Chinese medicines and Ayurvedic (Indian) medicines and determination of radiolabeled substances in chemical, biochemical, biological, pharmaceutical, and medicinal samples.It includes the ability to analyze crude samples containing multi-components, application of large number of sample and a series of standards using the spray-on technique, choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step, processing of standards and samples identically on the same plate leading to better accuracy and precision of quantification, different and universal selective detection methods, and in situ spectra recording in sequence to obtain positive identification of fractions, storage of total sample on layer without time constrains[1-3].

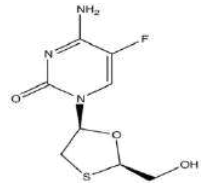
HPTLC is the most advanced form of modern TLC. It uses HPTLC plates featuring small particles with a narrow size distribution which results in homogenous layers with a smooth surface to be obtained. HPTLC uses smaller plates (10 × 10 or 10 × 20 cm). HPTLC plates provide improved resolution, higher detection sensitivity, and improved *in-situ* quantification and are used for industrial pharmaceutical densitometry quantitative analysis. Normal phase adsorption TLC on silica gel with a less polar mobile phase, such as chloroform– methanol, has been used for more than 90% of reported analysis of pharmaceuticals and drugs[4].Simple and precise HPTLC methods were developed for the simultaneous estimation of two anti-inflammatory drugs (curcuminand galangin). The method was tailored to analyze both drugs in their commercial dosage form (capsules) with no interference fromingredients. Chromatographic separation was performed over precoated TLC plates (60 F254, 20 cm× 10 cm, 250µm thickness,Merck, Darmstadt, Germany) via a linear ascending technique using n-hexane, ethyl acetate, acetic acid, and methanol as the mobilephase. Detection and quantification was achieved at 404 nm through spectrodensitometricanalysis[5].

The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyte. The mobile-phase systems are used based on their diverse selectivity properties are diethyl ether, methylene chloride, and chloroform combined individually or together with hexane as the strength adjusting solvent for normal-phase TLC and methanol, acetonitrile, and tetrahydrofuran mixed with water for strength adjustment in reversed-phase TLC. Separations byion pairing on C-18 layers are done with a mobile phase such as methanol–0.1 M acetate buffer (pH 3.5) containing 25 mM sodium pentanesulfonate (15.5:4.5).

A new high-performance thin-layer chromatographic (HPTLC) method has been established for determination of minocycline in human plasma. Chromatography was performed on aluminium plates coated with silica gel 60F254; the mobile phase was methanol: acetonitrile:isopropanol: water 5:4:0.5:0.5 (v/v)[6.]

## RESULT AND DISCUSSION-

Emtricitabine is 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2-(1*H*)-pyrimidon (Figure I)[7-8]. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Emtricitabine is structurally related with Lamivudine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. Tenofovir is [{(1R)-2-(6-amino-9H-purin-9-yl) -1-methylethoxy}methyl] phosphonic acid (Figure II)[9]. Tenofovirbelongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which block reversetranscriptase, an enzyme crucial to viral production in HIV-infected people. Literature review revealed that UV[10-14],HPLC[15-23]and HPTLC[19-22]methods have been reported for analysis of Emtricitabine and Tenofovir as a single form and in combination with other drugs. To date there have been no published reports on simultaneous quantitation of Emtricitabine and Tenofovir by HPTLC in bulk drug and in tablet dosage form. This present study reports for the first time the simultaneous quantitation of Emtricitabine and Tenofovir by HPTLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH Guidelines[23].



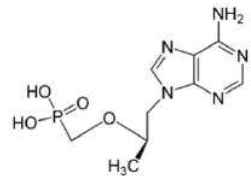




**Figure I:** Structure of Emtricitabine**Figure II:** Structure of Tenofovir

## EXPERIMENTAL SECTION-

### METHOD DEVELOPMENT:

The HPTLC procedure was optimized for simultaneous determination of Emtricitabine and Tenofovir. The mobile phase Methanol: Toluene: Ethyl acetate: Ammonia (1.5:5.5:1.5:0.1 v/v/v/v) resulted in good resolution, and sharp and symmetrical peaks were obtained at Rf 0.29 ± 0.02, 0.41 ± 0.02 for Emtricitabine and Tenofovir respectively. It was observed that prewashing of HPTLC plates with methanol (followed by drying and activation) and pre saturation of HPTLC chamber with mobile phase for 20 min (optimum chamber saturation time) ensured good reproducibility and peak shape of these drugs.

### VALIDATION OF THE METHOD:-

### LINEARITY-

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 320-1120 ng per spot Emtricitabine and 480-1680 ng per spot Tenofovir. Each concentration was applied in triplicate on the HPTLC plate (Table I).

| **Parameter** | **Emtricitabine** | **Tenofovir** |
|---|---|---|
| Linearity range | 320-1120 ng/spot | 480-1680 ng/spot |
| correlation coefficient ($r^2$) | 0.998 | 0.999 |
| Slope | 8.02 | 2.52 |
| Intercept | 178.8 | 50.14 |

*Table I: Linear regression data for drugs*

### LOD AND LOQ: -

The LOD & LOQ were determined from slope of the lowest part of the calibration plot.LOD and LOQ of respected drug shown in table (II).

| Parameter | Emtricitabine | Tenofovir |
|---|---|---|
| LOD | 30.24 | 51.90 |
| LOQ | 91.64 | 157.29 |

*Table II: LOD &LOQ for drugs*

### PRECISION:

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table (III )reveal the high precision of the method.

| Drug | Conc.(ng/band) | Intra day | | | Inter day | | |
|---|---|---|---|---|---|---|---|
| | | *%mean | *SD | *%RSD | *%mean | *SD | *%RSD |
| Emtricitabine | 800 | 99.90 | 0.73 | 0.73 | 99.96 | 0.90 | 0.90 |
| Tenofovir | 1200 | 99.98 | 0.72 | 0.72 | 99.73 | 0.82 | 0.82 |

*Table III: Statistical evaluation of precision of developed method (n=3)*

*Mean of three determinations, SD: Standard Deviation, R.S.D: Relative Standard Deviation

**RECOVERY STUDIES:**

When the method was used for extraction and subsequent analysis of these drugs from the pharmaceutical dosage forms and the extract was over applied with 100 and 120% of additional drug. As shown in the Table (IV) good recoveries of the Emtricitabine and Tenofovir in the range from 98.00 to 102.00 % were obtained at various added concentrations. The average recoveries of three levels (nine determinations) were 99.16±0.40 and 99.71±0.20 % for Emtricitabine and Tenofovir respectively.

| Drug | Level of % recovery | %mean | *S.D. | *%R.S.D. |
|---|---|---|---|---|
| Emtricitabine | 80% | 99.59 | 0.13 | 0.13 |
| | 100% | 99.23 | 0.32 | 0.32 |
| | 120% | 98.68 | 0.65 | 0.65 |
| Tenofovir | 80% | 98.59 | 0.60 | 0.60 |
| | 100% | 99.93 | 0.24 | 0.24 |
| | 120% | 100.63 | 1.64 | 1.64 |

*Table IV: Recovery study Data*

*Mean of three determinations, SD: Standard Deviation, R.S.D: Relative Standard Deviation

**ROBUSTNESS:**

The standard deviations of peak areas were calculated for the aforementioned four parameters (variation in composition of the mobile phase, amount of mobile phase, Time from spotting to chromatography, Time from chromatography to scanning) and coefficients of variation were found to be less than 2% in all cases as shown in Table (V).

| **Parameters** | **% RSD for Emtricitabine*** | **% RSD for Tenofovir*** |
|---|---|---|
| Mobile phase composition (± 0.1 ml) | 99.05 | 98.95 |
| Amount of mobile phase (± 1.0 %) | 99.08 | 98.55 |
| Time from spotting to chromatography (5 min) | 98.86 | 99.14 |
| Time from chromatography to scanning(10 min) | 98.94 | 98.90 |

*Table V: Results of Robustness*

*Mean of three determinations, R.S.D: Relative Standard Deviation

**FORCED DEGRADATION STUDIES:**

HPTLC studies of the samples obtained during the stress testing of Emtricitabine and Tenofovir under different conditions. Different degradations peak as shown in figures 2-10.The mass balance is a process of adding together the assay value and the levels of degradation products to see how closely these add up to 100% of initial value with due consideration of the margin of analytical error. The amount of drug recovered after degradation studies and the Rf of the degradation products are given in table (VI).

**a) ACID INDUCED DEGRADATION:** The drugs were degraded in the acidic condition and shows different degradation products at Rf 0.15, 0.24 for Emtricitabine and 0.14, 0.29, 0.79 for Tenofovir as shows in the fig. III-IV.

**b) BASE INDUCED DEGRADATION:**The drugs were degraded in the alkaline condition and shows different degradation products at Rf 0.25 for Emtricitabine and 0.02 for Tenofovir as shows in the fig. V-VI.

**c) HYDROGEN PEROXIDE INDUCED DEGRADATION:** The drugs were degraded in hydrogen peroxide (3%) at room temperature shows different degradation products at Rf 0.57, 0.37 for Emtricitabine and 0.58 for Tenofovir as shows in the fig.VII-VIII.

| Stress condition | Drug | Mass balance (%assay of recovered + %impurities + % degradents) | Rf values of degradation Products |
|---|---|---|---|
| Acid hydrolysis (0.1N HCl) | Emtricitabine | 99.99 | 0.15,0.24 |
| | Tenofovir | 99.12 | 0.14,0.29,0.79 |
| Alkali hydrolysis (0.1N NaOH) | Emtricitabine | 100.10 | 0.25 |
| | Tenofovir | 98.96 | 0.02 |
| Oxidation ($3\%H_2O_2$) | Emtricitabine | 99.96 | 0.57 |
| | Tenofovir | 100.02 | 0.37,0.58 |

*Table VI: Results of Forced Degradation studies*

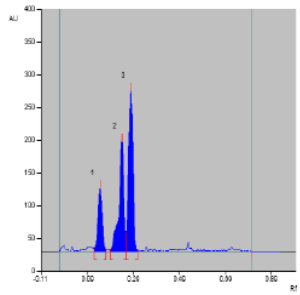


Fig. III: Densitogram of acid hydrolysis Of Emtricitabine

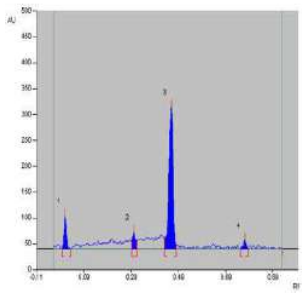


Fig. IV: Densitogram of acid hydrolysis of Tenofovir

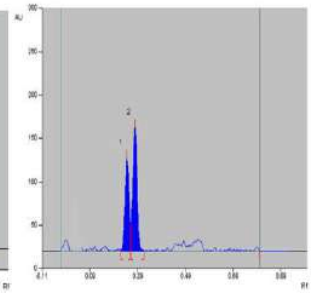


Fig. V: Densitogram of alkali hydrolysis ofEmtricitabine

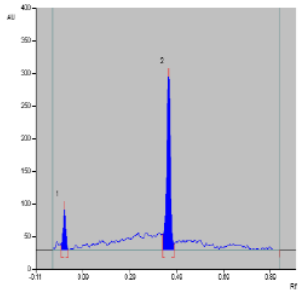


Fig. VI: Densitogram of alkali HydrolysisofTenofovir

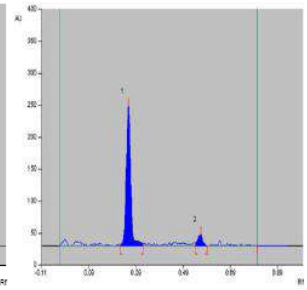


Fig. VII: Densitogram of oxidative degradation of Emtricitabine

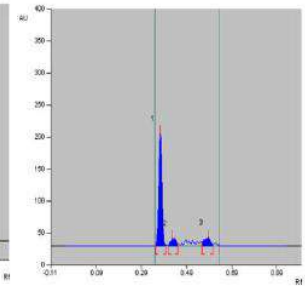


Fig. VIII: Densitogram of oxidative degradation of Tenofovir

**CONCLUSION:-**

The proposed method based on the HPTLC was developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy for the proposed method. Hence, it can be concluded that the developed chromatographic method is accurate, precise and selective and can be employed successfully for the estimation of Emtricitabine and Tenofovir in bulk and formulation.

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