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Development and Validation of NP HPTLC Method for Estimation of Emtricitabine in Bulk and Pharmaceutical Dosage Form

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KEYWORDS
Emtricitabine, HPTL, Camag TLC scanner 3.

ABSTRACT:
Objective of the present work is to develop and validate a simple, precise, selective, cost effective, and sensitive, high-performance thin layer chromatography (HPTLC) method for the estimation of emtricitabine in bulk and pharmaceutical dosage form. The optimized mobile phase contained of toluene: methanol 3.5:1.5(v/v). The method used HPTLC precoated silica gel 60 F254 as a stationary phase. Densitometric estimation of emtricitabine was carried out in the absorbance mode at 240nm. The method was linear in the range of 300-1800 ng/band. The correlation coefficient is 0.998 and slope 3.611, intercept 1696. The limit of detection and limit of quantitation were 10.93 and 33.14 respectively. The developed method is precise accurate, sensitive for analysis of emtricitabine in bulk and pharmaceutical dosage form.

Keyword

1. INTRODUCTION

Emtricitabine (EMT) is nucleotide reverse transcriptase inhibitors (NRTI) with activity against human immunodeficiency virus (type I) (HIV-1). It is used for the prevention of perinatal HIV-reverse transcriptase. It is also active against hepatitis-B-virus. Chemically, it is 4-amino-5fluoro-1-[2-(hydroymethyl]-1, 3-oxathiolan-5-yl]-pyrimidin-2-one. It is white to off white crystalline powder with molecular formula C8H10FN3O3S and a molecular weight of 247.24g/mole. The structural formula is shown in fig.1.

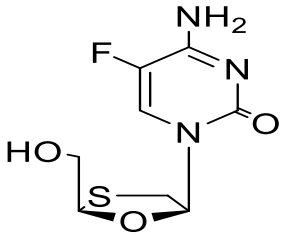

Fig.1. Structure of Emtricitabine

Literature survey indicated that few analytical method has been reported for the analysis of emtricitabine such as, spectrophotometric methods for the determination of emtricitabine in bulk and in its pharmaceutical formulations using aromatic aldehydes as chromogenic reagent, development and validation of HPTLC method for simultaneous estimation of emtricitabine,rilpivirine and tenofovir disoproxil fumarate in combined dosage form, development and validation of uv-spectrophotometric method for the simultaneous determination of emtricitabine and tenofovir in combined dosage form, validated HPTLC method for the determination of emtricitabine as bulk and in dosage form, stability indicating HPTLC method for the simultaneous estimation of rilpivirin, emtricitabine and tenofovir in bulk and combined pharmaceutical dosage form, A validated stability indicating rp-hplc method for simultaneous estimation of tenofovir disoproxil fumarate, cobicistat, emtricitabine and elvitegravir in bulk and pharmaceutical dosage form, Development and

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Validation of Spectrophotometric method for determination of Emtricitabine and Tenofovir Disoproxil Fumarate in Bulk and Tablet dosage form.[1-8].

2. EXPERIMENTAL

2.1. Chemical and reagent:

Pure emtricitabine were obtained as a gift sample from Cipla pharmaceuticals pvt.Ltd, Mumbai, India. Methanol purchased from merck Ltd, Worli,and Mumbai India.

2.2. Instrumentation and chromatographic condition

HPTLC aluminum plates pre-coated with silica gel $60F_{254}$ (10 cm X 10 cm) were obtained from Merck. Densitometry was carried out using Camag TLC Scanner 3 (Camag, Switzerland) fitted with win-CATS software version 1.3.0. Samples were applied as band on the HPTLC plates using the microliter syringe of CamagLinomat 5 under nitrogen gas flow and developed in a Camag 10 cm X 10 cm Muttenz twin trough chamber. Finally, the mobile phase consists of toluene and methanol in the ratio of 3.5:1.5 (v/v). Chromatographic condition is shown in table 1.

2.3. **Preparation of standard stock solution**:

An accurately weighed quantity of 10 mg Emtricitabine was transferred to a 10 mL volumetric flask and dissolved in methanol, and volume was made up to mark with the same solvent to obtain concentration of 1000 ng/µL. Aliquots of standard solutions 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 µL of emtricitabine were applied on TLC plates with the help of microliter syringe, using Linomat 5 sample applicator to obtain the concentration of 600, 900, 1200, 1500, 1800 ng per spot.

3. VALIDATION OF METHOD

The proposed method was validated as per ICH guidelines

3.1. LINEARITY:

Aliquots of standard stock solutions 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 µL of emtricitabine was applied on TLC plates with the help of microliter syringe, using Linomat 5 sample applicator to obtain the concentration of 300, 600, 900, 1200, 15000 and 1800 ng per spot. The data of peak area versus concentration were treated by linear least square regression.

3.2. ACCURACY:

The accuracy of the method was checked by recovery study. The standard addition method was employed at 80, 100 and 120 % level.

Table 1: Finalized Chromatographic condition

Parameters	Specifications
Stationary phase	HPTLC aluminum plate pre-coated with silica gel 60F254 10 cm *10 cm, layer thickness 0.2mm E-Merck prewashed with methanol.
Mobile phase	Toluene:Methanol (3.5:1.5 v/v)
Chamber saturation	20 minutes
Migration distance	80mm
Activation of prewashed plate	10 min
Band width	6mm
Radiation source	Deuterium lamp
Scanning wavelength	240nm

3.3. PRECISION:

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was controlled by examining the three concentrations. (600, 900, 1200 ng/band), of emtricitabine for three times on the same day. Inter-day precision was controlled by examining the three concentrations (600,900,1200 ng/band) of emtricitabine for three times in different days.

3.4. REPEATABILITY:

Repeatability was measured by 1200 ng/band concentration of emtricitabine solution for six times.

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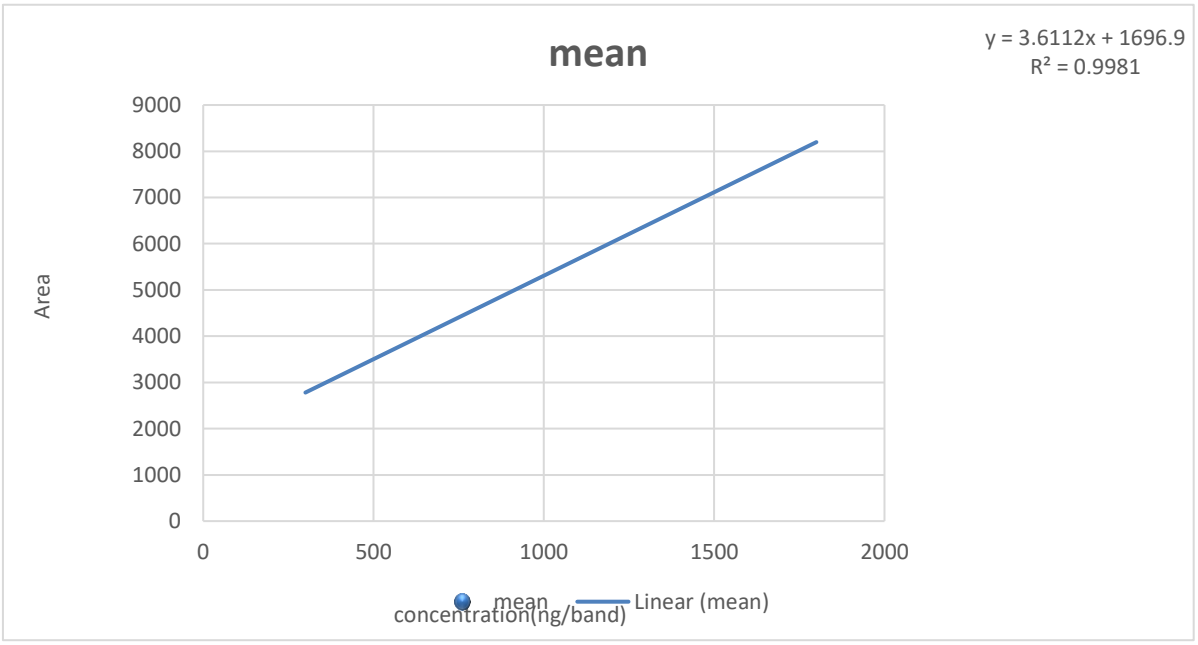

Fig.2: Calibration curve of emtricitabine

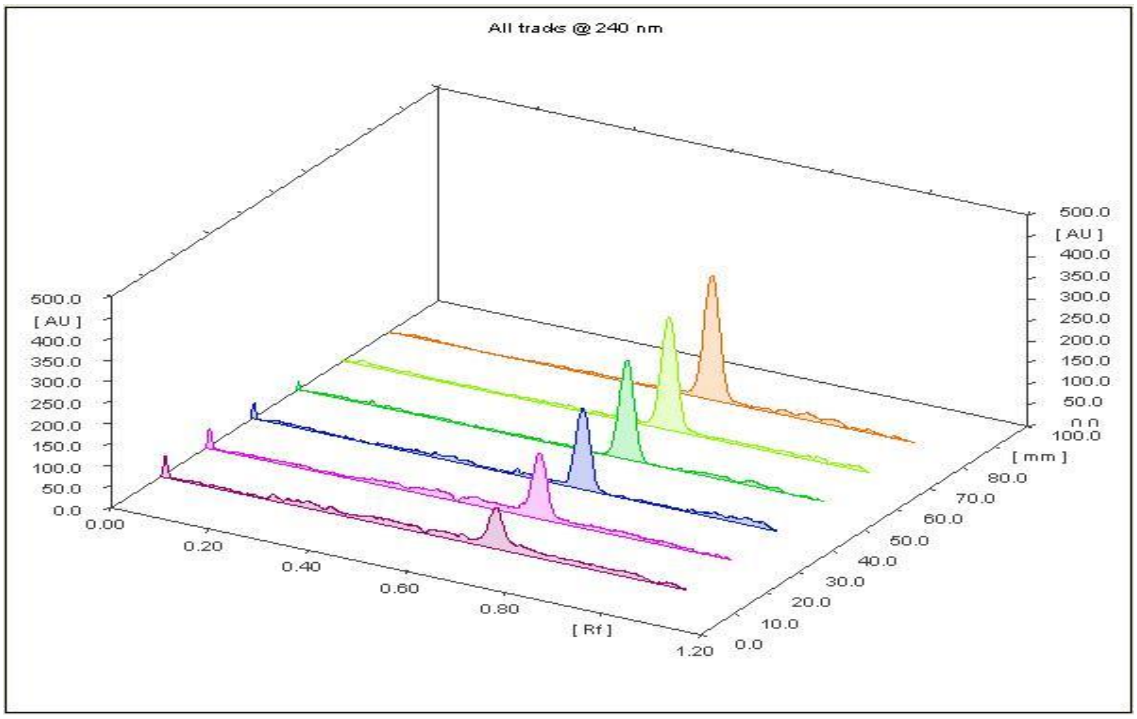

Fig-3: The 3D linearity spectra of emtricitabine standard drug solution

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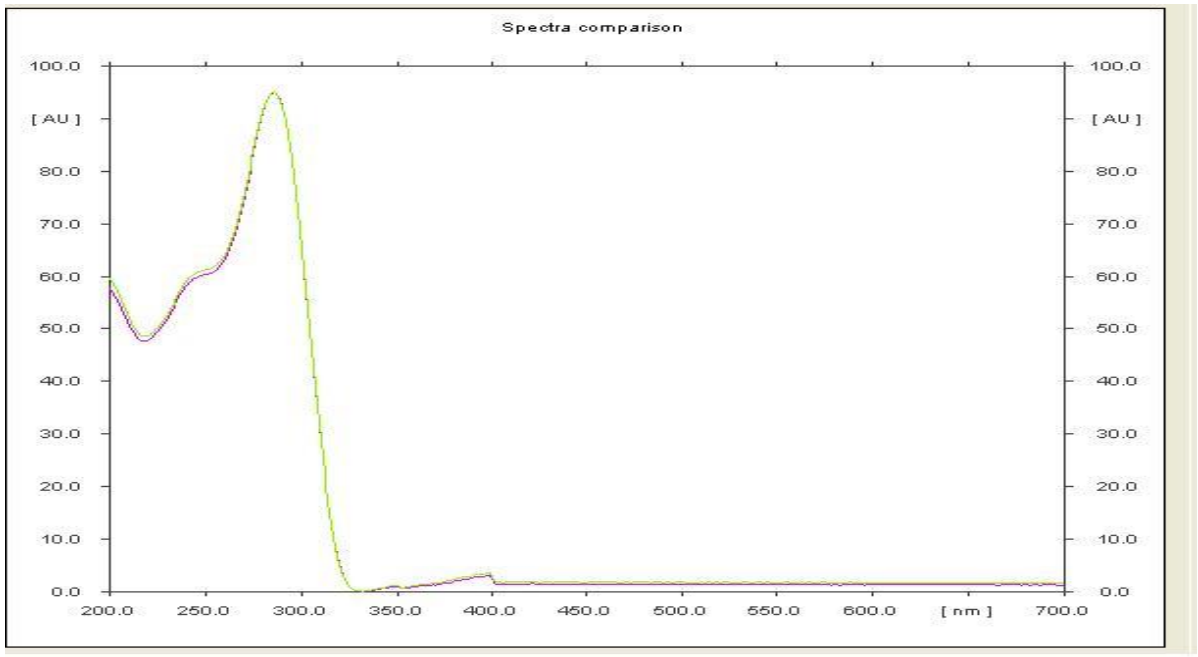

Fig-4: peak purity spectra of standard emtricitabine

3.5. RUGGEDNESS:

The ruggedness of the method was performed by spotting 1200 ng/band of emtricitabine by two different analysts, with same experimental and environmental conditions.

3.6. ROBUSTNESS:

For carrying out robustness, minor changes were made in the experimental conditions like change in proportion of mobile phase, activation of plates, chamber saturation times.

3.7. SENSITIVITY:

The sensitivity of the proposed method was measured in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). In order to determine detection and quantification limit, concentrations in the lower part of the linear range of the calibration curve were used. Emtricitabine solution of 300,400.500, 600,700, and 800 ng/band were prepared The LOQ and LOD were calculated using equation LOD=3.3x SD/B and LOQ=10x SD/B, whereSD is standard deviation of the peak areas of the drugs and B is the slope of the calibration curve.

4. RESULT AND DISCUSSION:

LINEARITY:

The linearity of proposed methods was evaluated by linear regression analysis, which was calculated by the least square method. The linear regression data for the calibration curves showed good linear relationship over the concentration range 0.3 - 1.8 ng/µL for emtricitabine (n = 6). The regression equations for the calibration curve were found to be y=3.611*+1696 (R^2=0.998) foremtricitabine. The calibration curve was plotted by area versusconcentration. (Fig-2).Results are shown in Table.2 and 3D-linearity spectra of emtricitabine show in Fig-3.

ACCURACY:

Sample solutions were prepared at three different concentration 80%, 100%, and 120% and known amount of sample was added to this solution and recovery of added sample was studied.The %RSD values that were determined and found to be less than 2 indicate that method is accurate. Results are shown in Table. 3

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Table: 2. Linearity study of Emtricitabine:

Sr.No	Concentration in(ng/band)	Peak area mean ±S. D	%RSD
1	300	2723.4±7.77	0.28
2	600	4001.67±10.12	0.25
3	900	4841.2±26.59	0.54
4	1200	6013.1±2.30	0.03
5	1500	7180.367±56.46	0.78
6	1800	8168.667±11.52	0.14

Table: 3. Results of recovery studies

Initial Amount (ng/ band)	Amount Added (%)	Amount recovered± SD.[ng/band] [n=3]	% recovered	% RSD
600	480	1078.28±3.60	99.64	0.75
600	600	1194.42±4.87	99.07	0.82
600	920	1322.01±5.53	100.28	0.76

Table: 4. Result of Precision studies.

Conc. (ng/band)	Intra-day %Amount found (ng)		Inter-day %Amountfound (ng)	
	Mean± SD (n=3)	%R. S.D	Mean± SD (n=3)	%R. S.D
600	100.20 ± 1.43	1.43	100.06 ± 0.46	0.46
900	99.21 ± 0.77	0.77	100.71 ± 1.07	1.07
1200	99.69 ±0.09	0.09	99.71 ± 1.86	1.86

REPEATABILITY:

It is a measure of how the HPTLC instrument performed under the given chromatographic conditions. The repeatability was determined by analyzing 1200ng/band concentration of emtricitabine solution for six times. With %RSD values less than 2. Results are shown in Table .5

Table: 5. Result of repeatability studies

Drug	Amount Taken	Amount Found	% Amount Found
	1200	1203.48	100.29
	1200	1215.75	101.31
Emtricitabine	1200	1119.33	99.94
	1200	1197.39	99.78
	1200	1198.64	99.88
	1200	1207.44	100.62
	Mean ± SD	1203.67 ± 6.98	100.30 ± 0.58
	%RSD	0.58	0.58

RUGGEDNESS:

Ruggedness of the proposed method was studies by two different analysts using the same experimental and environmental condition the 1200 ng/band emtricitabine were applied on TLC plate, developed and scanned. Results are shown in Table 6.

Table: 6. Result of ruggedness studies

Analyst	% Amount Found ± SD	%RSD
I	100.31 ± 0.61	0.60
II	100.28 ± 0.62	0.61

ROBUSTNESS:

The robustness of the method was established by introducing small changes in mobile phase composition and chromatograms were run. The amount of mobile phase, chamber saturation time, time from spotting to chromatography and from chromatography to scanning (±10min) result show in table 7.

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Table: 7.Results of Robustness Studies

Parameters	± SD of peak area	% RSD
Mobile phase composition		
toluene: methanol (4: 1.2 v/*v*)	63.30	1.09
toluene: methanol (3: 1.5 v/*v*)	113.58	2.07
Saturation Time		
15 Min	99.64	1.88
20Min	79.74	1.42
Activation of plates		
15Min	63.85	1.29
10Min	41.82	0.78

SENSITIVITY:

Sensitivity of the proposed method was measured in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOQ and LOD were calculated using equation LOD=3.3x SD/B and LOQ=10x SD/B, where,SD is standard deviation of the peak areas of the drugs and B is the slope of the calibration curve. The limit of detection and the limit of quantitation were 10.93 and 33.14 respectively.

CONCLUSION:

In the present work a simple, accurate, sensitive, selective and precise method was developed by using NP-HPTLC to determine emtricitabine (EMT) The method was validated according to ICH guidelines. Method validation was done by testing its linearity, precision, repeatability, ruggedness, accuracy, robustness, sensitivity.

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