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Simultaneous HPTLC–Densitometric analysis of Tenofovir and Emtricitabine in Tablet dosage form

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Abstract: A new simple, precise, accurate, and selective high performance thin-layer chromatographic (HPTLC) method has been developed for simultaneous analysis of tenofovir and emtricitabine in a tablet dosage form. Chromatographic separation was achieved on aluminum foil plates precoated with silica gel $60F_{254}$, with toluene: methanol: ethyl acetate: acetic acid (4:2:5:0.1v/v/v/v) as mobile phase. Detection was performed densitometrically at 270 nm. The R_F of a tenofovir and emtricitabine were 0.52 ± 0.05 and 0.40 ± 0.02, respectively. The reliability of the method was assessed by evaluation of linearity (120– 600 ng spot^{-1} for tenofovir and 80-560 ng spot^{-1} for emtricitabine), accuracy (99.54 % for tenofovir and 99.87 % for emtricitabine), and specificity, in accordance with ICH guidelines. The method can be used for routine simultaneous analysis of tenofovir and emtricitabine in pharmaceutical formulations.
Key Words: Tenofovir and emtricitabine, HPTLC, simultaneous estimation.

INTRODUCTION

Tenofovir is chemically ({[(2*R*)-1-(6-amino-9*H*-purin-9-yl) propan-2-yl] oxy} methyl) phosphonic acid (Fig. 1a), belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors, which blocks reverse transcriptase, an enzyme crucial to viral production in HIV infected people [1]. A survey of literature revealed a HPLC- spectrofluorimetric detection [2], LC-MS method [3] and HPLC [4-5]. Emtricitabine, 4-amino-5-fluoro-1-[(2*R*, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2 dihydropyrimidin-2-one (Fig 1b), is a nucleoside reverse transcriptase inhibitor for the treatment of HIV infection in adults and children[6]. Literature survey reveals that there are a number of methods for the individual determination of emtricitabine like HPLC with fluorescence detector [7], RP-HPLC [8-10] and LC–MS/MS [11].

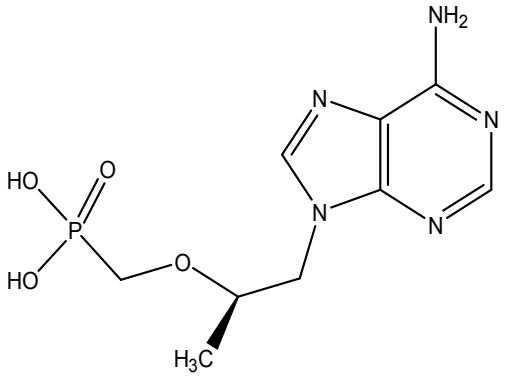

Figure 1 a Structure of Tenofovir

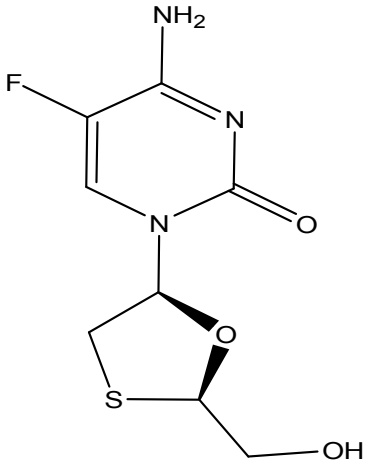

Figure 1 a Structure of Emtricitabine

Moreover the literature survey revealed that so far, no method has been reported for estimation of tenofovir and emtricitabine in combined dosage form. Therefore, it was thought worthwhile to develop simple, precise, accurate HPTLC method for the simultaneous estimation tenofovir and emtricitabine in tablets.

MATERIALS AND METHODS

Chemicals and reagents

Cipla Pvt.Ltd,Panvel, Maharashtra, India, generously gifted pure tenofovir and emtricitabine. Commercial tablets (Tavin-EM) containing tenofovir (300 mg) and emtricitabine (200 mg) were used for the study. All the other chemicals used were of analytical grade (E. Merck, India).

Instrumentation

Camag HPTLC System (with TLC Scanner), WinCATS Softwar V 4.0 and Linomat 5 as application device used for the analysis. Precoated silica gel 60F254 on aluminium sheets (200µm thick) of E-Merck, Germany were used as stationary phase. Pre-washing of plate was done with methanol and then it was activated by keeping in an oven at 115^0C for 10 minutes. The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe. A constant application rate of 0.1 µL S^{-1} was used and the space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. Each chromatogram was developed over a distance of 8 cm. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

Preparation of Stock Solutions

Accurately weighed tenofovir (30 mg) and emtricitabine (20 mg) were transferred to a 25 mL volumetric flask and dissolved in and then diluted to the mark with methanol. The stock solution was further diluted with methanol to obtain a solution of tenofovir (120 µg mL^{-1}) and emtricitabine (80 µg mL^{-1}).

Validation of Method

The method was validated as per the ICH guidelines in terms of linearity, precision, limit of detection, limit of quantitation, robustness, specificity, accuracy and analysis of marketed formulation.

Linearity and Range

A stock solution containing 120 µg mL^{-1} for tenofovir and 80 µg mL^{-1} for emtricitabine were prepared in methanol. Different volumes of this solution were applied to the plate resulting in application of 120-600 ng $spot^{-1}$ for tenofovir and 80-560 ng $spot^{-1}$ for emtricitabine to the plate. The drugs were resolved using a mobile phase of toluene: methanol: ethyl acetate: acetic acid (4:2:5:0.1v/v/v/v) and UV detection was carried out at 270 nm. All measurements were repeated six times for each concentration and calibration curve was constructed by plotting peak areas against the corresponding drug concentration.

Precision

Intra-day and inter-day variation for determination of tenofovir and emtricitabine was measured at three different concentrations (120, 360 and 600 ng/spot for tenofovir and 80, 240 and 400 ng $spot^{-1}$ for emtricitabine) for three times on the same day and on three different days over a period of one week and each concentration was applied in triplicate and % RSD was calculated.

Limit of detection and limit of quantitaiton

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration plot.

Robustness:

Small changes in the chromatographic conditions were introduced and the effects on the results were examined.

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The peak

purity of both tenofovir and emtricitabine were determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

Recovery studies

Accuracy of the developed method was tested by standard addition method. Drug corresponding to 50, 100 and 150% was added separately to preanalysed sample. At each level, the amount recovered was calculated.

Analysis of the Marketed Formulation

Twenty tablets were weighed (each containing 300 and 200 mg each of tenofovir and emtricitabine and their average weight was calculated. The tablets were finely powdered and powder equivalent to 300 mg of tenofovir and 200 mg of emtricitabine was accurately weighed and dissolved in 70 mL of methanol. The solution was filtered through Whatman filter paper no. 41 and the residue was washed with methanol and volume was adjusted to 100 ml with the same solvent. Both the solutions were further diluted with methanol to obtain the final concentration 120 ng μl^{-1} of tenofovir and 80 ng μl^{-1} emtricitabine, respectively. The amount of tenofovir and emtricitabine present per tablet was calculated by comparing peak area of sample with that of standard.

Table 1 Precision Study

Concentration (ng $spot^{-1}$)	Repeatability (n=3)			Intermediate precision (n=3)		
	Measured Conc. ± SD	(%) RSD	Recovery (%)	Measured Conc. ±SD	(%) RSD	Recovery (%)
Tenofovir						
120	120.30 ± 2.03	1.68	100.25	119.78 ± 1.23	1.03	99.82
360	361.54 ± 4.81	1.33	100.40	360.70 ± 1.56	0.43	100.2
600	600.73 ± 3.12	0.52	100.12	597.88 ± 10.42	1.74	99.65
Emtricitabine						
80	80.15 ± 0.80	1.00	100.2	79.91 ± 1.39	1.74	99.88
240	239.39 ± 0.75	0.31	99.75	239.20 ± 4.87	2.00	99.66
400	401.66 ± 1.09	0.27	100.42	400.98 ± 2.19	0.54	100.25

Table 2 Robustness testing (n=3)

Parameter	Tenofovir ± SD	% RSD	Emtricitabine ± SD	% RSD
Mobile phase composition (± 0.1 ml)	5.21	1.29	3.45	1.52
Amount of mobile phase (± 0.5 %)	3.65	1.21	2.17	1.23
Time from spotting to chromatography (± 20 min)	1.32	1.56	4.24	1.14
Time from chromatography to scanning (± 20 min)	1.53	1.42	2.36	1.34

Table 3 Recovery studies

Drug	Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	Recovery (%)
Tenofovir	300	50	450	449.800	99.95
		100	600	598.180	99.69
		150	750	749.910	99.98
Emtricitabine	200	50	300	298.240	99.41
		100	400	399.624	99.906
		150	500	496.545	99.30

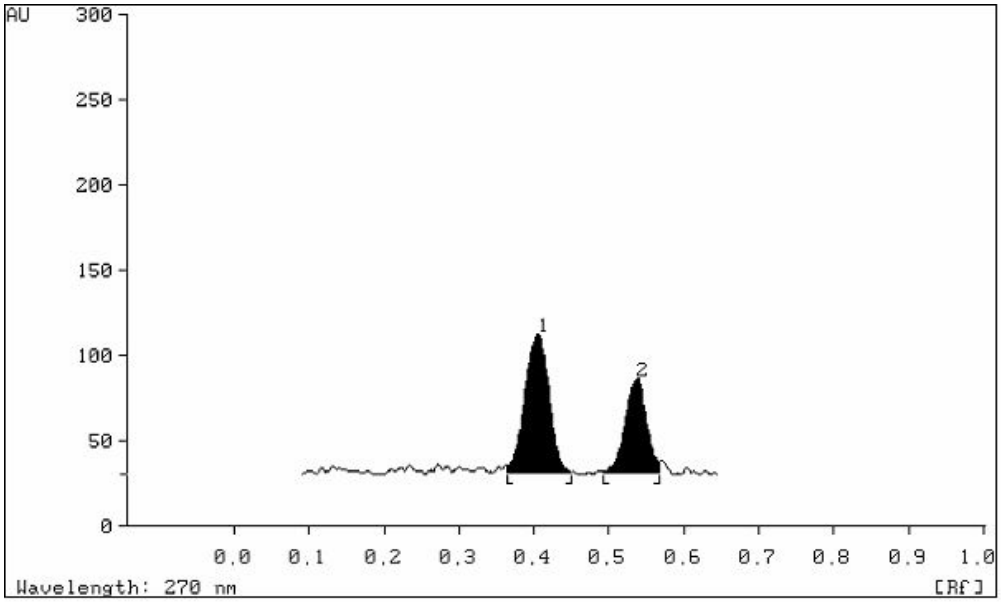

Figure 1 Chromatogram of Tenofovir and Emtricitabine. Peak 1 and 2 are emtricitabine and tenofovir, respectively.

RESULTS AND DISCUSSION

Method Development

The TLC procedure was optimized for simultaneous determination of tenofovir and emtricitabine. The mobile phase toluene: methanol: ethyl acetate: acetic acid (4:2:5:0.1v/v/v/v) resulted in good resolution and sharp and symmetrical peaks of R_F 0.52 ± 0.05 for tenofovir and 0.40 ± 0.02 for emtricitabine. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min (optimum chamber saturation time) ensured good reproducibility and peak shape of both drugs (Fig. 1).

Validation

Linearity

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 120-600 ng $spot^{-1}$ for tenofovir and 80-560 ng $spot^{-1}$ for emtricitabine. The linear regression equations were Y= 4.702x +101.5 (r^2 = 0.9996) for tenofovir and Y=11.04x +222.5 (r^2 = 0.998) for emtricitabine.

Precision

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table 1 reveal the high precision of the method.

Limits of Detection and Quantitation

The limits of detection and quantitation, calculated as described above, were 40 and 100 ng $spot^{-1}$ for tenofovir and 30 and 60 ng $spot^{-1}$ for emtricitabine. This indicates the method is sufficiently sensitive.

Robustness

The relative standard deviation of peak areas was less than 2%. The RSD shown in Table 2 indicate the robustness of the method.

Specificity

The peak purity of tenofovir and emtricitabine was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9996 and r (M, E) =0.999 for tenofovir and r (S, M) = 0.998 and r (M, E) = 0.9992 for emtricitabine. Good match was obtained between standard and sample spectra of tenofovir and emtricitabine respectively.

Accuracy

When the method was used for extraction and subsequent analysis of both drugs from the pharmaceutical dosage forms, and the extract was overapplied with 80, 100, and 120% of additional drug, the recovery was 99–100 %, as listed in Table 3.

Analysis of marketed formulation

When the Tavin-EM tablets were analysed, sharp and well defined peaks for tenofovir and emtricitabine were obtained at R_F 0.52 and 0.40, respectively, when scanned at 270 nm. The amount of the label claim measured was 100.06 % for tenofovir and 98.02 % for emtricitabine.

CONCLUSION

The developed HPTLC technique is simple, precise and accurate and can be used for simultaneous analysis of tenofovir and emtricitabine in tablets. The method was validated in accordance with ICH guidelines.

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