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Research Article

Validated UV Spectrophotometric and HPTLC Method for Determination of Fosamprenavir Calcium in Pharmaceutical Formulation

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Abstract

A simple, rapid and precise, UV spectrophotometric and High Performance Thin Layer Chromatographic (HPTLC) methods were developed and validated for quantitative determination of Fosamprenavir calcium in bulk and pharmaceutical formulations. In UV spectrophotometric method, estimation of Fosamprenavir calcium was carried out using 0.5 N Hydrochloric acids as solvent at 262.8 nm. The drug obeyed Beer–Lambert's law in the concentration range of 2–90 µg/mL with coefficient of correlation (R^2) of 0.9997. In the HPTLC method, the chromatographic development was carried out on HPTLC plates precoated with silica gel 60 F254 using Methanol as mobile phase. Detection was carried out at 270 nm. The R*f* value of drug was 0.69 ± 0.01. The calibration curve was linear over a range of 200–800 ng/spot with a regression coefficient of 0.996. Both the methods were validated as per ICH guideline with respect to linearity, accuracy, precision, robustness etc. The methods can be adopted in routine analysis of Fosamprenavir Calcium in tablet dosage form.

Keywords: Fosamprenavir Calcium; HPTLC method; UV method; Validation

Abbreviations

SD: Standard Deviation; RSD: Relative Standard Deviation; ICH: International Conference on Harmonization.

Introduction

Fosamprenavir Calcium, chemically (3S)-Tetrahydro-3-furyl {(αS)-α-[(1R)-1-hydroxy-2-(N1-isobutylsulfanilamido) ethyl] phenethyl} carbamate calcium phosphate (Figure 1), is a novel antiretroviral drug. Fosamprenavir calcium is one of the most recently approved HIV-1 protease inhibitor and is rapidly and extensively converted to amprenavir after oral administration. It is administrated orally with a dose of 700 mg two times a day [1-5].

The literature review revealed a dissolution method [6], and an electrochemical method [7] for evaluation of Fosamprenavir Calcium in tablet formulation. However, some HPLC and HPLC-MS methods had been reported for estimation of Fosamprenavir Calcium along with other antiretroviral drugs in human blood plasma and body fluids [8-10]. One HPLC method is available for quantitative determination of Fosamprenavir Calcium in tablet formulation [11]. Further, no official or draft monograph of Fosamprenavir Calcium was published in any of the pharmacopoeia for compendia applications.

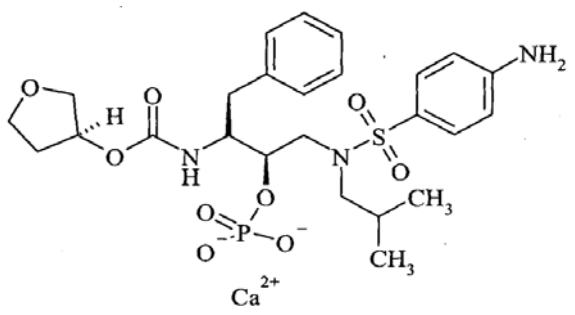

Figure 1: Structure of Fosamprenavir calcium.

It was felt necessary to develop a simple, precise and rapid UV and HPTLC methods for the quantitative estimation of Fosamprenavir calcium in pharmaceutical products. The current research work deals with the development of UV and HPTLC methods and their validation as per International Conference on Harmonisation (ICH) guidelines [12-14].

Materials and Methods

Chemicals and reagents

Fosamprenavir calcium working standard was received as a sample from Mylan Laboratories Pvt. Ltd., Hyderabad (India). Acetonitrile used was of HPLC grade (Qualigens, Mumbai). Millipore water was used throughout analysis. Pharmaceutical dosage form (Fosamprenavir Calcium tablets containing 100 mg Fosamprenavir) was prepared in laboratory using Lactose Monohydrate, Microcrystalline Cellulose, Starch and Magnesium Stearate. Water was obtained from a Milli-Q UF-Plus apparatus (Millipore) and was used to prepare all solutions for the method. Other chemicals used were analytical or HPLC-grade.

A UV spectrophotometric method

Instruments: Shimadzu UV - 1700 UV/VISIBLE spectrophotometer with UV probe 2.10 software and 1 cm matched quartz cells were used for absorbance measurements. The Balance

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(Mettler Toledo XP205, Mumbai) was used for weighing and an Ultrasonicator (ENERTECH Electronics Pvt. Ltd., Mumbai) was used for sonication. Thermo Scientific Forma 3960 Series environmental chamber was used for stress testing.

Preparation of standard stock solution: Accurately weighed Fosamprenavir Calcium standard drug equivalent to 100 mg of Fosamprenavir was transferred into a 100 mL volumetric flask and dissolved in 0.5 N HCl. The volume was made up to 100 mL with 0.5 N HCl to give the solution containing 1000 µg/mL of Fosamprenavir.

Selection of λ_{max} selection of wavelength for analysis: The standard stock solution was further diluted with 0.5 N HCl to get a 20 µg/mL of concentration. The solution was scanned between 200 and 400 nm using 0.5 N HCl as blank.

Preparation of the sample solution: The tablets of Fosamprenavir Calcium were not available in Indian market; hence tablets manufactured in laboratory were assayed. These were labeled to contain 100 mg of Fosamprenavir per tablet. Twenty tablets containing 100 mg of Fosamprenavir per tablet were accurately weighed and powdered. The powder equivalent to 100 mg of Fosamprenavir was weighed and transferred to a 100 mL volumetric flask; 10 mL 0.5 N HCl was added and sonicated for 10 min. The volume was adjusted to 100 mL with 0.5 N HCl. The solution was filtered through Whatman filter paper No. 01. From this filtrate, 2 mL was transferred to a 100 mL volumetric flask and diluted with 0.5 N HCl to 100 mL.

Assay of fosamprenavir in tablet formulation: Six replicate solutions of sample were prepared and the absorbances of sample solutions were measured at 262.8 nm using 0.5 N HCl as blank. The concentration of sample solution was estimated by comparing the absorbance of sample solution with that of standard and amount of the drug in tablet was determined.

Method validation: The developed UV method was validated as per ICH guidelines for following parameters [12-15].

Linearity: Aliquots of standard stock solution were further diluted with 0.5 N HCl to obtain the solutions of concentration 2-90 µg/mL. The absorbances were measured at 262.8 nm against 0.5 N HCl as blank. Correlation coefficient (r^2) of the line constructed by plotting absorbance against corresponding concentration was determined.

Specificity: The specificity of the method was determined by comparing the spectra of tablet solution with that of standard solution.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Repeatability: Repeatability of the method was determined by analyzing six solutions of concentration 20 µg/mL of drug. The area of each solution was measured.

Intraday and interday precision: Intraday precision was determined by analyzing the drug at three different concentrations and each concentration for three times, on the same day. Interday precision was determined similarly, but the analysis being carried out daily, for three consecutive days.

Accuracy (Recovery studies): To ensure accuracy of the method, recovery studies were performed by standard addition method at 80 %, 100 %, and 120 % level to preanalyzed samples and subsequent solutions were reanalyzed. At each level, three determinations were performed. The absorbances were measured at 262.8 nm using 0.5 N HCl as blank and the amount of drug recovered from the formulation were calculated.

Robustness: The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ± 2 nm. For changes of conditions, the sample was assayed in triplicate.

Ruggedness: To determine ruggedness, two different analysts performed assay of tablets in similar operational and environmental conditions using developed method.

Limit of detection and limit of quantitation: The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by using the formula, LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is residual standard deviation of regression line and S is slope of corresponding regression line.

Solution stability: The stability of the sample solution was tested at intervals of 1, 6 and 24 h. The concentration of sample solution was estimated by comparing the absorbance of sample solution with that of standard.

HPTLC method

HPTLC instrumentation and chromatographic conditions: HPTLC plates pre-coated with silica gel GF_{254} on aluminum plate, (20.0 x 10.0 cm), Merck, were used for the analysis. Densitometry was carried out with a CAMAG TLC Scanner 3, fitted with win CATS 1.4.0 planar chromatography manager software. Samples were applied to the HPTLC plates using the spray-on technique of CAMAG Linomat 5 under nitrogen gas flow and developed in a CAMAG 20.0 x 10.0 cm twin trough chambers. Fosamprenavir calcium reference standard solution was prepared using methanol as solvent. Solutions of 0.2 µL was applied to the HPTLC plates as spot bands of 8mm using LINOMAT V. The development chamber was left for saturation with mobile phase (methanol) vapors for 5 minutes before each run. Development of the plate was carried out by the ascending technique to a migration distance of 8 cm. After development, the plates were air dried. All the analyses were carried out at room temperature. Densitometry scanning was done in absorbance mode at 270 nm using a deuterium lamp.

Preparation of standard solution: Fosamprenavir calcium (equivalent to 10 mg fosamprenavir) was accurately weighed and transferred into 100 mL volumetric flasks, and dissolved in 10 mL of methanol. This solution was further diluted upto the mark with methanol to get the final concentration of 100µg/mL.

Preparation of test solution: Ten tablets were accurately weighed, their average weight was determined and they were finely powdered. The powder equivalent to 100 mg of Fosamprenavir was transferred

to a 100 mL volumetric flask. About 70 mL of methanol was added and sonicated for 10 minutes. The solution was diluted to volume with methanol. The solution was filtered through 0.45 µm nylon filter. The aliquot of 5.0 mL from this solution was transferred into a 50 mL of volumetric flask and diluted to volume with the methanol.

Analysis of pharmaceutical dosage form: 3 µL of sample solution was applied on the TLC plates followed by the development and measured at 270 nm. The analysis was repeated for six times. The concentration of sample was determined using linear regression equation of calibration graph and amount of drug in tablet was determined.

Method validation: The HPTLC method was validated as per the ICH guidelines [12-15].

Linearity: The working standard solution was spotted on the HPTLC plate to obtain final concentration range of 200–900 ng/spot. Each concentration was spotted six times on the HPTLC plate. The plate was developed using methanol as mobile phase and scanned. The peak areas were plotted against the corresponding concentrations to obtain the calibration graph. Linear calibration curve was generated using linear-regression analysis.

Specificity: Specificity of the method was determined by comparing the chromatogram of sample with the chromatograms of standard.

Precision: Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of (300 ng/spot) of the drug in hexaplicate on the same day. Intermediate precision of the method was checked by repeating studies on two different days. The % RSD of twelve determinations was calculated.

Accuracy: Accuracy of the method was determined by standard addition method in which the known amount of standard Fosamprenavir calcium solutions were added to pre-analyzed sample solution. These amounts corresponded to 80, 100, and 120 % of the sample solution. The amounts of Fosamprenavir were estimated by applying these values to the regression equation of the calibration curve. Accuracy study was performed for three times, and % recovery of Fosamprenavir calcium was calculated.

Limit of detection and limit of quantitation: The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by using the formula, LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is residual standard deviation of regression line and S is slope of corresponding regression line.

Solution stability: The sample solution was stored at room temperature. The aliquots of sample solution were tested after 1, 6, and 24 h of storage. The concentration of sample was determined using linear regression equation of calibration graph. The assay was performed in triplicate.

Results and Discussion

UV spectrophotometric method

Selection of wavelength for analysis: The UV spectrum of Fosamprenavir Calcium had shown λ_{max}, at 262.8 nm. Hence, it was selected for the analysis (Figure 2).

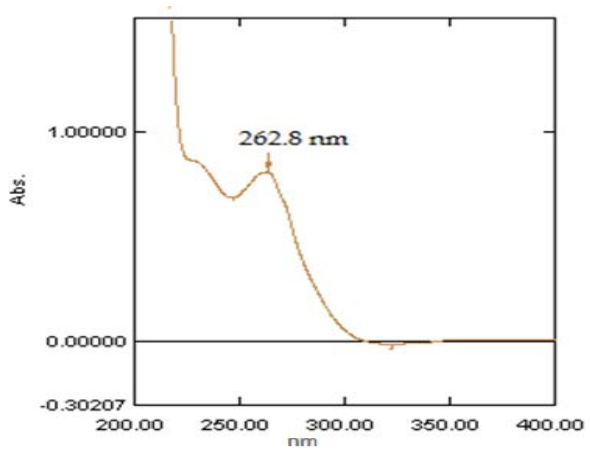

Figure 2: Typical UV spectrum of standard Fosamprenavir Calcium.

Table 1: Assay of tablet formulation of Fosamprenavir Calcium by UV method.

Labeled claim (mg)	Amount Found* ± SD (mg)	% Assay*	%RSD
100	99.13 ± 1.02	99.13	1.03

*Mean of six determinations.

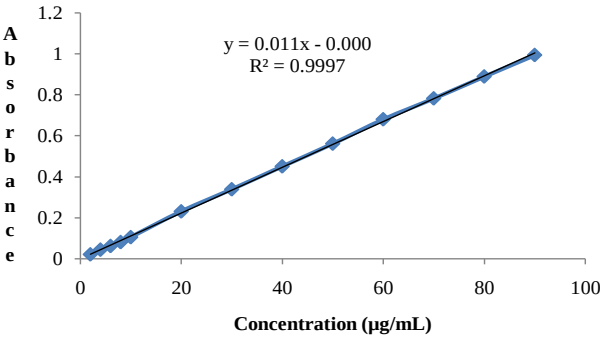

Figure 3: Linearity plot of Fosamprenavir Calcium by UV spectrophotometric method.

Assay of fosamprenavir in tablet formulation: The amount of Fosamprenavir present in formulation was calculated by comparing with the absorbance of standard solution. The results obtained are shown in Table 1.

Validation of UV method

Linearity: The drug showed linearity in the concentration range of 2-90 µg/mL (Figure 3). Linear regression data is shown in Table 2.

Specificity: The spectra obtained from tablet solutions were identical with that obtained from standard solution containing an equivalent concentration of Fosamprenavir. This showed that there was no any interference from excipients. Therefore, it could be said that developed method is highly specific.

Precision: The method was found to be precise as the % RSD values for repeatability, intraday and interday precision were found to be less than 2 %. The results are summarized in Table 3 and Table 4 respectively.

Recovery studies: The method was found to be accurate, indicated

Table 2: Linear regression data of Fosamprenavir Calcium UV Method.

Sr. No.	Parameters	Results
1	λmax (nm)	262.8 nm
2	Beer's law limit (µg/mL)	2-90
3	Correlation coefficient	0.9997
4	Slope (*m*)	0.011
5	Y - Intercept (*c*)	0.000

Table 3: Result of Repeatability studies of Fosamprenavir Calcium by UV method.

Concentration applied (µg/mL)	Concentration found* ± SD (µg/mL)	% RSD
20	19.71 ± 0.161	0.82

*Mean of six determinations.

Table 4: Result of Intraday and Interday precision studies of Fosamprenavir Calcium by UV method.

Concentration applied (µg/mL)	Intra-day precision		Inter-day precision	
	Concentration found* ± SD (µg/mL)	%RSD	Concentration found*± SD (µg/mL)	%RSD
10	9.99 ± 0.132	1.32	10.10 ±0.132	1.31
20	20.03 ± 0.265	1.32	19.77 ±0.304	1.54
30	29.58 ± 0.180	0.61	29.75 ±0.13	0.44

*Mean of three determinations.

Table 5: Results of recovery studies of Fosamprenavir Calcium by UV method.

Level of addition (%)	Amount of std drug added (µg/mL)	Amount recovered ± SD (µg/mL)*	% Recovery	% RSD
80	16	15.73 ± 0.26	98.28	1.68
100	20	19.94 ± 0.20	99.70	1.00
120	24	23.78 ± 0.13	99.07	0.56

*Mean of three determinations.

Table 6: Result of robustness studies of Fosamprenavir Calcium by UV method.

Method wavelength (nm)	Condition (nm)	% Assay* ± SD	% RSD
262.8 nm	260.8	99.57± 0.866	0.870
	264.8	99.13 ± 0.433	0.437

*Mean of three determinations.

by % recoveries ranging from 98.25 % to 99.70 %. The results obtained are shown in Table 5.

Robustness: Assay of Fosamprenavir for deliberate change of condition was within 98.0 – 102.0 % as shown in Table 6, which indicated robustness of the method.

Ruggedness: The results of assay performed by two different analysts, summarized in Table 7, showed the ruggedness of the method.

Limit of detection and limit of quantitation: The LOD and LOQ were found to be 1.95 µg/mL and 5.91 µg/mL, respectively.

Solution stability: These results of stability studies indicated that

Table 7: Result of ruggedness studies of Fosamprenavir Calcium by UV method.

Parameter	Analyst I	Analyst II
Label claim (mg)	100	100
% Assay*± SD	99.21 ±0.884	98.85±0.935
% RSD	0.89	0.95

*Mean of six determinations.

Table 8: Results of Stability studies of Fosamprenavir Calcium by UV method.

Time (h)	% Assay*± SD	% RSD
1	100.43 ± 0.866	0.86
6	99.57 ± 0.866	0.87
24	99.13 ± 0.750	0.76

*Mean of three determinations.

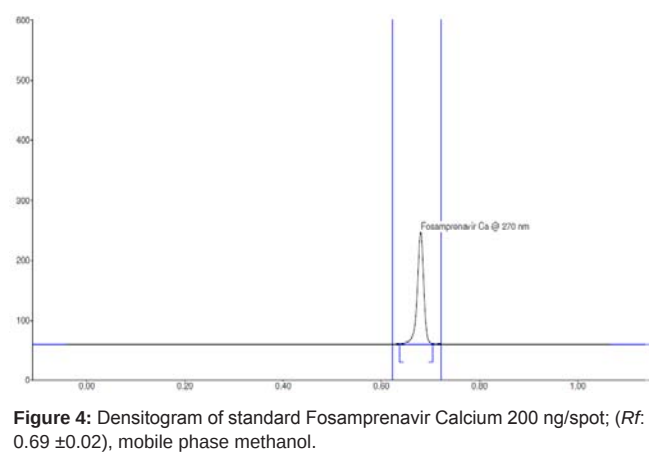

Figure 4: Densitogram of standard Fosamprenavir Calcium 200 ng/spot; (*Rf*: 0.69 ±0.02), mobile phase methanol.

the solution was stable for 24 h at ambient temperature. The assay obtained was 99.13 % after 24 h. The results are shown in Table 8.

HPTLC method

Optimization of the chromatographic conditions: The HPTLC procedure was optimized with a view to develop simple HPTLC method. The standard solution of the drug was spotted on HPTLC plates. Initially, methanol and water were tried in different ratio for developing solvent systems. Methanol was selected as mobile phase and *Rf* was found to be 0.69. It was satisfactorily resolved with *Rf* value at 0.69 ±0.01 (Figure 4). In order to reduce the necklace effect, the TLC chamber was saturated for 5 min using saturation pads. The mobile phase was run upto distance of 8 cm, which takes approximately 10 min for development of HPTLC plate.

Analysis of pharmaceutical dosage form: A single spot at *Rf* value of 0.69 was observed in the chromatogram of the drug samples extracted from tablet. There was no interference from the excipients that are commonly present in the formulations. The drug content was found to be 99.64 %. The results of assay are summarized in Table 9.

Validation of HPTLC method [12-15]**Linearity**: Linear relationship was observed by plotting drug concentration against peak areas. Fosamprenavir Calcium showed linear response in the concentration range of 200–800 ng/spot (Figure 5a and Figure 5b). The corresponding linear regression equation was Y = 747.2 + 4.342 x X with square of correlation coefficient (r^2) of 0.996. Linear regression data is shown in Table 10.

Specificity: No interference of excipients with the fosamprenavir calcium peak was observed. A single peak of fosamprenavir calcium

Table 9: Analysis of Tablet formulation of Fosamprenavir Calcium by HPTLC method.

Labeled claim (mg)	Amount found* ± SD (mg)	% labeled claim*	%RSD
100	99.57 ± 1.05	99.57	1.05

*Mean of six determinations.

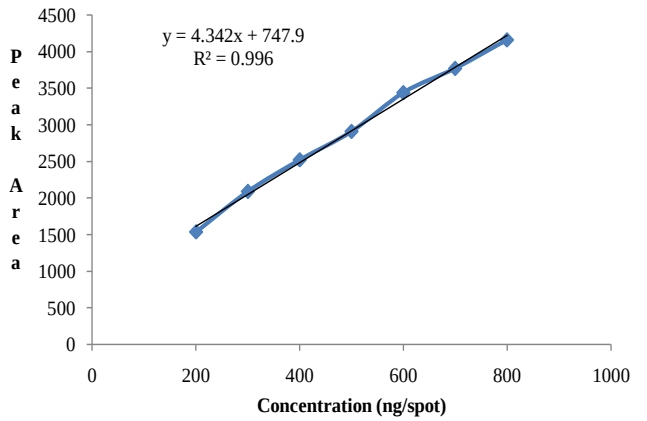

Figure 5a: Plot of Concentration versus Peak area of Fosamprenavir Calcium by HPTLC method.

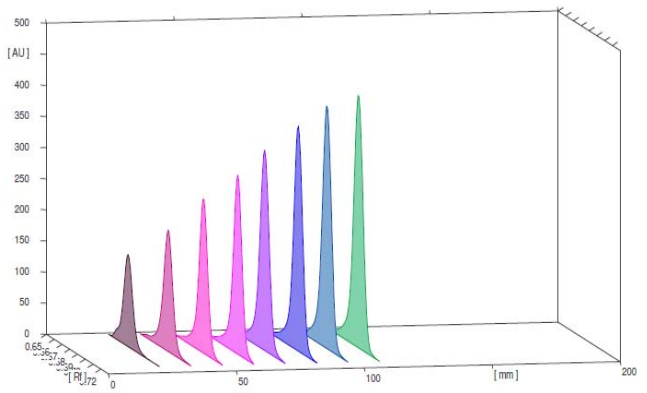

Figure 5b: Three dimensional HPTLC densitogram of linearity bands of Fosamprenavir Calcium.

Table 10: Linear regression data for Fosamprenavir Calcium by HPTLC method.

Sr. No.	Parameters	Results
1	Linearity range	200 - 800 ng/spot
2	Regression equation	Y = 747.9 + 4.342 *X
3	Correlation coefficient	0.996
4	Slope	4.342
5	Y-Intercept	747.9

in tablet solution was observed at R*f* 0.69 (Figure 6a and 6b).

Precision: The results of the repeatability and inter-mediate precision experiments are shown in Table 11. The developed method was found to be precise as the % RSD values for repeatability and intermediate precision studies were < 2 %, respectively.

Accuracy (Recovery study): The results obtained from recovery studies are presented in Table 12. The mean % recovery ranged from 100.15 % to 101.25 % which showed the accuracy of the method.

Limit of detection and limit of quantitation: The LOD and LOQ were found to be 49.10 ng/spot and 148.79 ng/spot respectively.

Solution Stability: There was no indication of degradation in sample solutions of Fosamprenavir Calcium as revealed by % assay of solution stored at different times. The solution was found to be

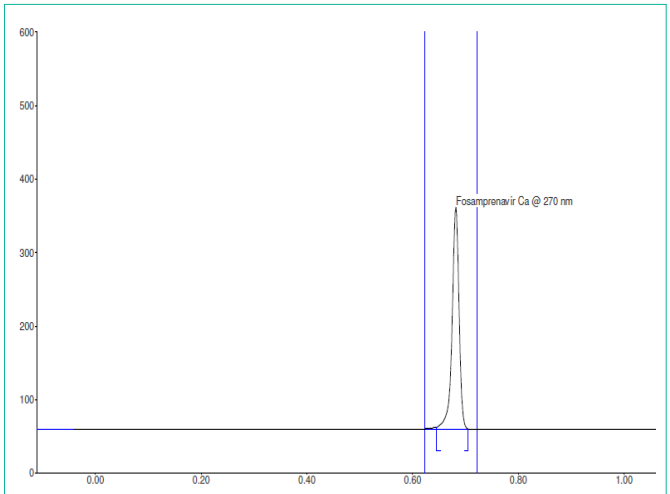

Figure 6a: A typical Densitogram of Fosamprenavir Calcium in tablet solution.

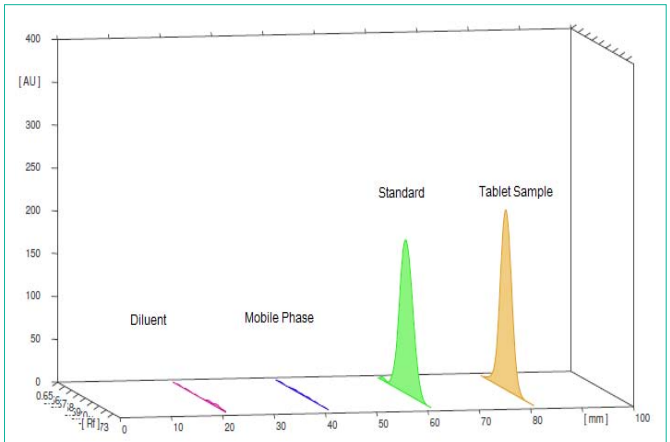

Figure 6b: A Specificity Densitogram of Diluent, Mobile phase, Standard and Tablet sample Fosamprenavir Calcium.

Table 11: Results of Precision Studies of Fosamprenavir Calcium by HPTLC method.

Repeatability (Intraday)		Intermediate precision (Interday)	
% Assay* ± SD (n=6)	% RSD	% Assay* ± SD (n=12)	% RSD
99.51 ± 1.81	1.82	98.26 ± 1.51	1.54

n = number of determinations.

Table 12: Results of recovery studies of Fosamprenavir Calcium by HPTLC method.

Level	Standard Drug Added (ng/spot)	Drug Recovered* ± SD (ng/spot)	%Recovery*	%RSD
80%	240	243.00 ± 1.60	101.25	0.66
100%	300	301.05 ± 0.81	100.35	0.27
120%	360	360.52 ± 3.09	100.15	0.86

*Mean of three determinations.

stable at ambient temperature for 24 h, and no unknown peaks were observed. The results are shown in Table 13.

Conclusion

A simple, rapid and reliable UV and HPTLC method has been developed and successfully validated for estimation of Fosamprenavir

Table 13: Results of Stability studies of Fosamprenavir Calcium by HPTLC method.

Time (h)	% Assay*± SD	% RSD
1	100.04 ±0.45	0.45
6	99.41 ±1.01	1.02
24	98.51 ±0.90	0.91

*Mean of three determinations.

calcium in the tablet dosage form. The results of the validation tests indicated that the method was accurate, precise, robust, and stability indicating. The proposed UV and HPTLC method is suitable for routine determination of Fosamprenavir Calcium in pharmaceutical formulation in quality control laboratories, where economy and time are essential.

Acknowledgments

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