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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FOSAMPRENAVIR IN DRUG SUBSTANCE BY RP-HPLC METHOD

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ABSTRACT

Analytical method was developed for the estimation of Fosamprenavir drug substance by liquid chromatography. The chromatographic separation was achieved on C18 column (Symmetry 75*4.6mm, 5um) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1% v/v Trifluoro acetic acid in Water: Acetonitrile. The flow rate was 1.0 ml/ minute and ultra violet detector at 260nm. The average retention time for Fosamprenavir found to be 1.93 min the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 50-150µg/ml for Fosamprenavir.

KEYWORDS

Fosamprenavir, Isocratic, HPLC, C18, Trifluoro acetic acid and Methanolandvalidation.

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INTRODUCTION

Fosamprenavir is used for the treatment of HIV-1, but not in combination with low-dose ritonavir or other antiviral drugs. Fosamprenavir (marketed by ViiV Healthcare as the calcium salt under the trade names Lexiva in the U.S. and Telzir in Europe) is a drug for the treatment of HIV infections. It is a pro-drug of the protease inhibitor and antiretroviral drug amprenavir. The human body metabolizes fosamprenavir in order to form amprenavir, which is the active ingredient. fosamprenavir a slow-release version of amprenavir

and thus reducing the number of pills required versus standard amprenavir.

Fosamprenaviris chemically designated as 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methyl-1, 3-thiazole-5-carboxylic acid. Its molecular formula is $C_{16}H_{16}N_2O_3S$, and its molecular weight is 316.375. Fosamprenaviris a white-to-off white powder. It is soluble in methanol and practically insoluble in water.

EXPERIMENTAL

Equipments

The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (Symmetry 5µ, 75 mm × 4.6 mm) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance, Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials

Pharmaceutically pure sample of Fosamprenavir were obtained as gift samples from Fortune pharma training institute, srisainagar, KPHB and Hyderabad, India.

HPLC-grade Methanol and Acetonitrile was from qualigensreagents pvt ltd. Trifluoro acetic acid (AR grade) was from SD fine chem.

Chromatographic conditions

The sample separation was achieved on a C18 (5 µ, 75 cm X 4.6 mm i.d.) Symmetry column, aided by mobile phase mixture of 0.1% TFA in water: Acetonitrile (60:40). The flow rate was 1.0 ml/ minute and ultra violet detector at 260nm that was filtered and degassed prior to use, Injection volume is 20 µl and ambient temperatures.

Preparation of mobile phase

Buffer Preparation

Take accurately 1ml of Trifluoro acetic acid in 1000mL of water.

Mobile phase

Then add 60volumes of buffer and 40 volumes of Acetonitrile mixed well and sonicated for 5 min.

Diluent: Buffer: Methanol: 50: 50 v\v.

Preparation of standard stock solution

A 50mg of pure Fosamprenavir were weighed and transferred into 50 ml of volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution containing 1000µg/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 100µg/ml of Fosamprenavir.

Preparation of sample solution

A 50mg of Fosamprenavir sample were weighed and transferred to 50 ml of volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution containing 1000µg/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 100µg/ml of Fosamprenavir.

RESULTS AND DISCUSSIONS

Determination of Working Wavelength (λmax)

10 mg of the Fosamprenavir standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 1.0 ml is pipetted into 100 ml VF and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using methanol as blank. The λmax was found to be 260nm

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1% Trifluoro acetic acid in water: CAN (60:40). The flow rate was 1.0 ml/ minute brought sharp peaks. The chromatogram was shown in Figure No.1.

METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 50-150 µg/ml of Fosamprenavir. From the primary stock solution

0.5ml,0.75ml,1.0ml,1.25ml,1.5 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 50µg/mL, 75µg/mL, 100µg/mL, 125µg/mL and 150 µg/mL of Fosamprenavir. Curve was established with concentration verses peak areas from the above prepared solutions (Table No.1).

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively (Table No.2).

LOD = 3.3 δ/S ……. (1)

LOQ =10 δ/S …….. (2)

Where,

σ = Standard deviation of the response

The slope S may be estimated from the calibration curve of the analyte.

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 100 µg/ml of FOSAMPRENAVIR without changing the parameter of the proposed chromatographic method (Table No.3).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of fosamprenavir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of fosamprenavir. The percentage recovery results obtained are listed in Table No.4.

Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table No.5).

Ruggedness

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD (Relative Standard Deviation) of assay results between two analysts was calculated

This indicates the method was rugged. The results were shown in Table No.6.

Table No.1: Linearity

S.No	Level	Concentration (mg/mL)	Peak area
1	50%	0.05	1329518
2	75%	0.075	2057388
3	100%	0.10	2729278
4	125%	0.125	3443750
5	150%	0.150	4141254

Table No.2: LOD and LOQ values Calculated from calibration curve

S.No		mg
1	**LOD**	0.002
2	**LOQ**	0.006

Table No.3: Summary of peak areas for method precision

S.No	Sample No	Retention time	Peak area	% Assay
1	1	1.932	2729443	100.5
2	2	1.932	2721509	100.4
3	3	1.934	2737361	100.6
4	4	1.932	2712871	100.1
5	5	1.935	2738365	100.6
6	6	1.936	2726629	100.6
7	Mean	1.934	2727696	100.4
8	%RSD	0.09	0.36	0.20

Table No.4: Recovery data

LEVEL	S.No	% Recovery of Fosamprenavir	Average
50	1	100.2	99.9%
	2	99.5	
	3	100	
100	1	100.5	100.5%
	2	100.4	
	3	100.6	
150	1	101.1	100.7%
	2	100.6	
	3	100.3	

Table No.5: Results of Robustness study

S.No	parameter	Rt of Fosamprenavir	Theoretical plates	Asymmetry
1	Decreased flow rate (0.9ml/min)	2.219	2342	1.28
2	Increased flow rate (1.1ml/min)	1.769	2212	1.26
3	Wave Length 258nm	1.936	2248	1.28
4	262	1.934	2266	1.27

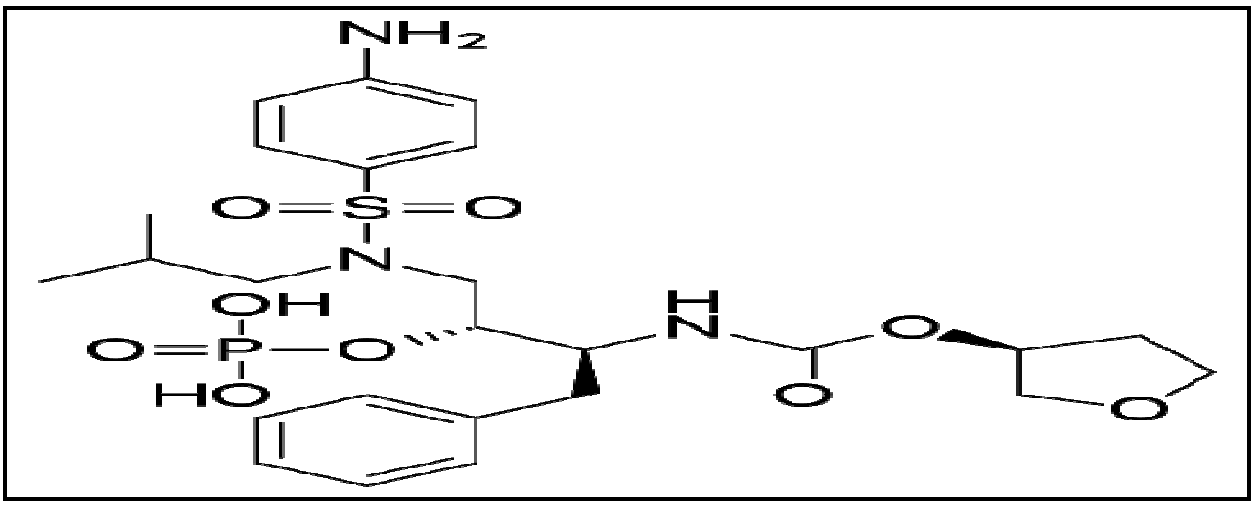
Table No.6: Results of Ruggedness

S.No			%Assay	%RSD
1	Analyst-1	Fosamprenavir	100.6	0.14%
2	Analyst-2		100.8	

Table No.7: Validation parameters of evaluated method

S. No	Parameter	Limit	Value Obtained
1	Linearity concentrations Range (mg/mL) Correlation coefficient	NLT 0.990	0.05 to 0.15 mg\ml 0.9999
2	Method precision (Repeatability) (%RSD, n = 6)	98.0 to 102.0 %	100.1 to 100.6 %
3	ACCURACY(%Recovery)	98-102%	99.9 to 100.7%
4	Robustness	It should be meet System suitability criteria	Complies
	Flow Variation(0.9mL to 1.1mL/min)		
	Wavelength Variation (258nm to 262nm)		
5	Ruggedness (Intermediate Precision) (%RSD analyst to analyst variation)	NMT 2%	0.14%

*RSD = Relative standard deviation



Structure of Fosamprenavir

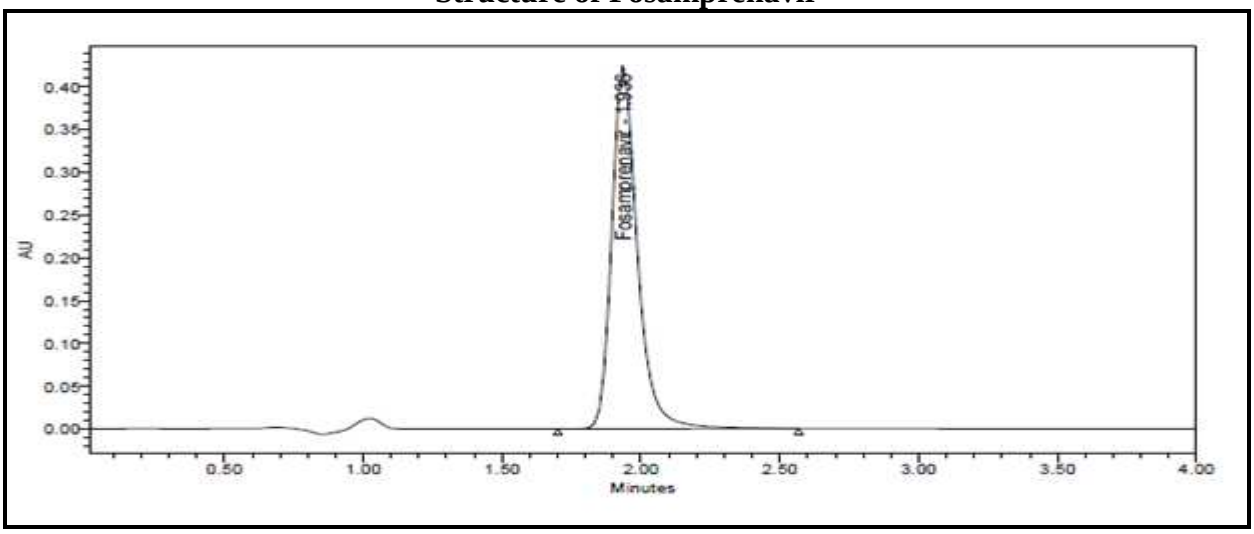


Figure No.1: Chromatogram of Fosamprenavir

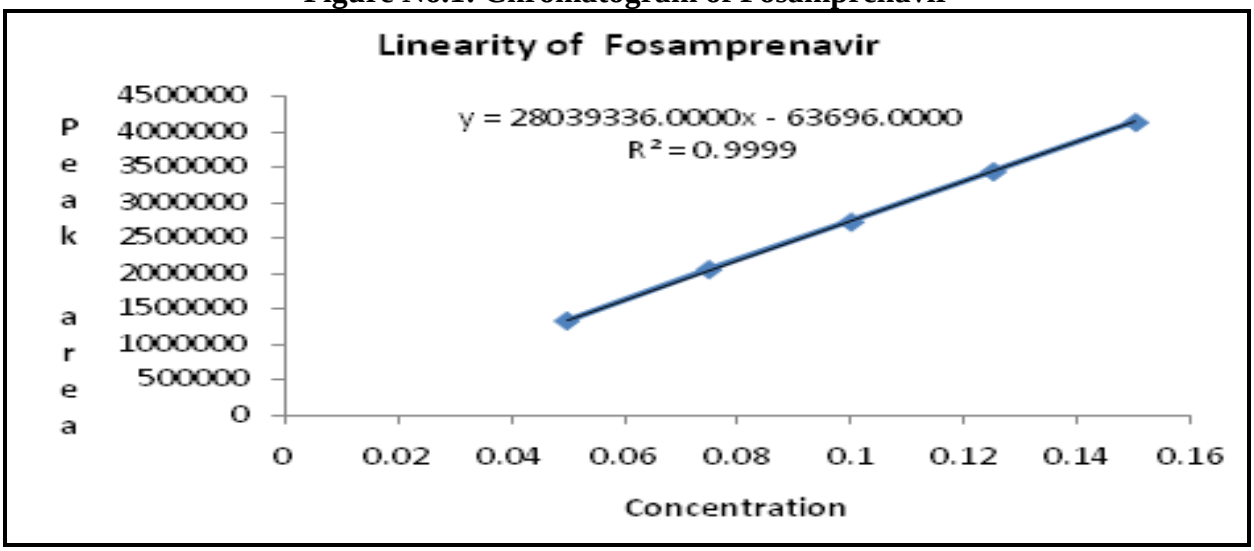


Figure No.2: Linearity (calibration) curve of Fosamprenavir

CONCLUSION

From the above results analytical method was concluded that, estimation of FOSAMPRENAVIR was found to be simple, precise, accurate and high resolution and shorter run time makes this method more acceptable and cost effective and it can be use for regular analysis in institutions, quality control department in industries, approved testing laboratories.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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