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AFFORDABLE REVERSE PHASE FLUID CHROMATOGRAPHIC STRATEGY FOR THE ASSESSMENT OF FOSAMPRENAVIR IN TABLETS

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ABSTRACT
A straightforward, exact, fast and affordable reverse phase fluid chromatographic strategy has been produced for the assessment of fosamprenavir in mass and in tablets, utilizing versatile stage containing 20mM Ammonium acetate buffer (pH-3.0) and acetonitrile [50:50 %v/v] at a stream pace of 1.0ml/min. An Phenomenex Luna C18 (250 x 4.6mm i.d, 5µ) column was utilized as a stationary phase. Quantitation was performed utilizing diode exhibit identifier at 225nm and the run time noticed was 4.0min. The retention time of the medication was discovered to be 1.598min. The linearity was seen in the scope of 5.0-15µg/ml with connection coefficient 0.9974. The rate measure of fosamprenavir was 99.5 to 101.2%.The created technique was approved to decide its exactness and accuracy via completing recuperation considers and intra-day and between day examines. Consequently, the current strategy is basic, exact, precise and fast for the assessment of fosamprenavir in tablet measurement structure.
Keywords: Fosamprenavir; Reversed-Phase High-Performance Liquid Chromatographic Method; Tablet; Validation.

INTRODUCTION:

Fosamprenavir[1,2] (**Figure 1**), {[(2*R*,3*S*)-1-[*N*-(2-methyl-propyl)(4-amino-benzene)--sulfonamido]-3-({[(3*S*)-oxolan-3-yloxy]-carbonyl}-amino)-4-phenyl-butan-2-yl]-oxy}-phosphonic acid, an oral protease inhibitor used clinically for the treatment of HIV-1 infections.

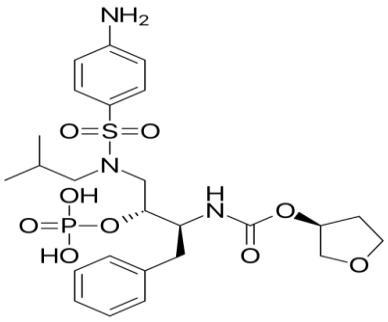

Figure 1: Structure of fosamprenavir

Notwithstanding, till date, no soundness technique has been accounted for the assessment of fosamprenavir in dosage forms[3-6]. In this way, the mark of the current research is to develop a RP- HPLC method to decide the steadiness of fosamprenavir as indicated by the International Conference of Harmonization rules [ICH, Q1A (R2)][7].

EXPERIMENTAL:

Instrumentation: The current HPLC examination of fosamprenavir was performed on Waters fluid chromatographic framework [Model 2695] furnished with quaternary siphon, UV-Visible detector, and column stove and auto sampler. The yield signal was checked and coordinated utilizing Waters (Alliance) Empower 2 programming. A Phenomenex Luna C18 (250 x 4.6mm i.d, 5µ) column was as fixed phase in the current investigation. An electronic insightful gauging balance (0.1mg affectability, Shimadzu AY 220), advanced pH meter (DELUX model 101) and a sonicator (sonica, model 2200 MH) were likewise utilized in this investigation. The dish sets utilized were of 'A' grade, and were cleaned altogether with chromic acid and twofold refined water and later dried in hot air stove preceding use.

Materials and Reagents: Fosamprenavir drug was made accessible from Merck Ltd. India (99.8%) as complimentary sample and its market details in the brand name of Lexiva (700mg), fabricated by Alkem Laboratories Ltd was obtained from Local pharmacy store. Potassium dihydrogen phosphate, dipotasssium hydrogen phosphates were gotten from Qualigens fine synthetics, India Ltd. Orthophosphoric acid and Acetonitrile were acquired from Rankem labs, India. All synthetic substances and reagent were utilized as HPLC evaluation and Milli-Q-water was utilized all through the

analysis.

Mobile Phase arrangement: The mobile phase utilized in this investigation was a combination of 20mM Ammonium acetate buffer (pH 3.0) and acetonitrile 50:50 %v/v.

Arrangement of Diluent: Buffer and methanol in the proportion of 50:50%v/v was used as diluent in the current examine

Preparation of standard stock arrangement: Weigh and move 100mg of fosamprenavir working standard into 100mL volumetric carafe add 60mL of diluents and sonicate to break down and weaken to volume with diluent (Stock arrangement).

Preparation of working standard arrangements: Transfer aliquots of the above standard stock arrangement into arrangement of various 100mL volumetric flasks and diluted to volume with mobile phase to acquire fixation scope of 5.0-15µg/ml individually.

Preparation of pharmaceutical plans: For examination of business definitions twenty tablets of Lexiva by Alkem Laboratories Ltd (Label ensure: 700mg of fosamprenavir) acquired from local Apollo pharmacy were measured and finely powdered. A decisively measured measure of fine powder equivalent to 100mg of fosamprenavir was moved into 100mL volumetric container containing 60mL of diluent, sonicated for 10mins to separate and finally debilitated to volume with diluent. The test stock was then filtered through 0.45µfilter. From this aliquots were moved and debilitated with portable stage; to get an obsession in the extent of linearity as of late chose (5.0-15.0µg/mL). A while later, these plans were then implanted in three-fold into the recently referenced HPLC structure.

CHROMATOGRAPIC PROVISIONS: The separation was accomplished utilizing Phenomenex Luna C18 (250 x 4.6mm i.d, 5µ) column at a detection wavelength of 225nm utilizing the mobile phase established of 20mM Ammonium acetate buffer (pH-3.0) and acetonitrile 50:50 %v/v at the flow rate of 1.0 ml/min and injection volume of 20µl.

RESULTS AND DISCUSSION: The current procedure method was executed according to the ICH guidelines [7].

System Suitability: The framework reasonableness was inspected by playing out the assessment and checking for changes in maintenance time, peak area and peak deviation. Five implantations of the standard plan of fosamprenavir (10µg/mL) were mixed thus.

The framework appropriateness was insisted by calculating the general standard deviation regards for boundaries like maintenance time, peak area, peak imbalance, speculative plates, plates per meter and stature indistinguishable from the theoretical plate. It was seen that every one of the qualities are inside the cutoff points (**Table 1**).

Table 1: System suitability data of fosamprenavir

Parameter	Fosamprenavir
Retention time	1.958
Theoretical plates	2221
Tailing factor	1.354
% RSD	0.13

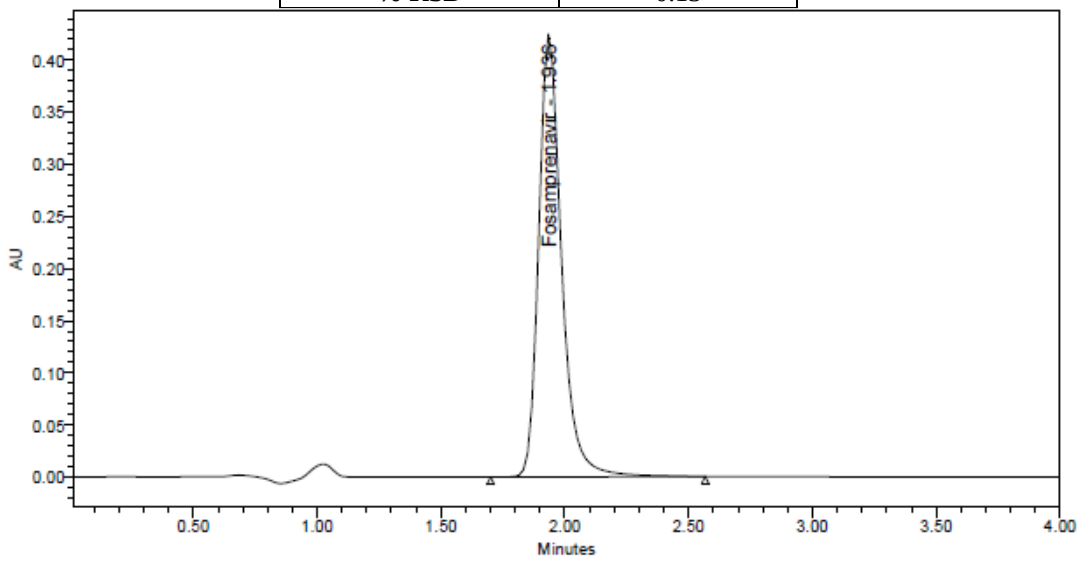

Figure 2: Distinctive Chromatogram of Standard

Explicitness: The particularity of the projected RP-HPLC method was set up by infusing and assessing clear and fake treatment arrangements by misuse of the recommended improved chromatographical conditions. It has been seen that there no endogenous peaks for diluent and blank treatment at the retention time of fosamprenavir along these lines, delivering proposed RP-HPLC technique more particular and explicit.

Linearity: The linearity of the current technique was destitute somewhere around implanting six unmistakable convergences of working standard arrangements orchestrated from fosamprenavir stock plan ran from half to 150% o in

three-fold into the HPLC framework. An alignment bend was created by plotting the area recorded against each fixation and a relapse condition was figured (**Figure 3**) and the eventual outcomes of this assessment were found in **Table 2**. The decided connection coefficient was 0.9974 where the slope is 28223 and y-intercept was - 37373. The connection coefficient showed the phenomenal linearity of the proposed methodology.

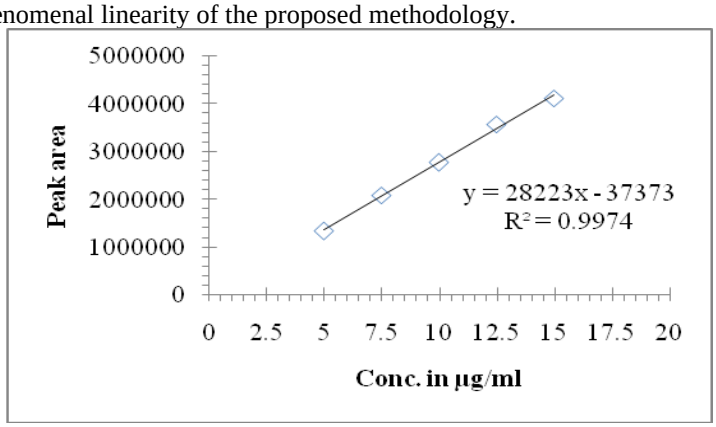

Figure 3: Linearity graph of fosamprenavir

Table 2: Linearity studies of fosamprenavir

% Level (Approx.)	Concentration (µg/mL)	Peak Area Ratio
50	5.0	1337452
75	7.5	2099678
100	10.0	2679898
125	12.5	3585432
150	15.0	4122542
Slope, b		282237.4
Intercept, a		-37373.2
r2		0.9974
LOD (µg/mL)		0.0189
LOQ (µg/mL)		0.063

Sensitivity: The LOD and LOQ for fosamprenavir were found to be 0.0189µg/mL and 0.063µg/mL, respectively (**Table 3**) indicating the adequate sensitivity of the method.

Precision: The exactness of the proposed methodology was mulled over by choosing the one fixed centralization of the fosamprenavir in the specifying for multiple times around a similar time at different interval and decided the motivation to the extent %RSD. The results of the accuracy analyze (**Table 3**) showed the constancy of the system (RSD %< 2.0).

Table 3: Precision data for fosamprenavir

S.No	RT	Area
Injection-1	1.952	2629443
Injection-2	1.954	2631509
Injection-3	1.955	2689261
Injection-4	1.936	2654871
Injection-5	1.955	2638724
Injection-6	1.954	2628514
Mean	**1.954**	2645387
Std. Dev.	**0.0013**	23625.32
***%RSD**	**0.070**	**0.893**

*Mean of six conclusions

Accuracy [Recovery Studies]: The accuracy of the proposed strategy, were finished with a known measure of the unadulterated drug was added to the phony treatment test at the element of half to 150% of the test fixation. The mean recoveries were in extent of 99.6-100.7% which uncovered that there is no impedance from excipients of the procedure (**Table 4**).

Table 4: Accuracy data for fosamprenavir

S.NO	Accuracy level	Injections	Fosamprenavir %recovery
1	**50%**	1	99.9
		2	100.5
		3	100.2
2	**100%**	1	100.9
		2	100.2
		3	100.1
		4	99.9

		5	100.5
		6	100.4
3	**150%**	1	99.6
		2	100.7
		3	99.8

*Mean of three & six conclusions

Robustness: The strength of the made RP-HPLC procedure was made by rolling out slight improvements in chromatographic conditions that remember the change for stream rate. All of the recently referenced factors was changed at two measurements (- 0.1, +0.1ml) at one time concerning enhanced boundaries and the outcomes of these investigations uncovered the unimportant effect on the chromatographic boundaries by slight assortments in chromatographic conditions (**Table 5**).

Table 5: Robustness data for fosamprenavir

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	2.349	2512	1.25
Increased flow rate (1.2ml/min)	1.859	2332	1.32

Ruggedness: A 20µl aliquot of fixation 10µg/ml was infused to consider the unpleasantness of fosamprenavir by two particular sensible logical specialists (Analyst-1and Analyst-2) and the results were recorded and are in the good reach for fosamprenavir. The results exhibited the % RSD was less than 2% separately.

Assay of fosamprenavir in tablet plans: The test for the estimation sorts of fosamprenavir [Lexiva -700mg] was created by mixing the game-plan of test strategy [discussed in the test part] with the present chromatographic condition created to get obsession in the degree of linearity actually chose. All decisions were done in six duplicates and it supposedly was progressively exact and solid (99.99%). The outcomes appeared in **Table 6**.

Table 6: Results of investigation of advertised tablets of fosamprenavir

Formulation in Market	Labeled Amount(mg)	Recovered Amount(Mg)*	%Recovery
Lexiva	700	699.99	99.99

*Mean of three conclusions

CONCLUSIONS:

The RP-HPLC technique created for the quantitative assurance of fosamprenavir in both unadulterated and pharmaceutical dose structures was precise, exact, unequivocal and strength demonstrating. The strategy was completely affirmed showing appealing data brings about understanding to ICH standards. The made RP-HPLC strategy (**Table 7**) is steadiness showing and can be used for the standard assessment of age tests and moreover to check the strength of fosamprenavir tests.

Table 7: Summary of validation data for fosamprenavir

S. No	Parameter	Value Obtained
1	**Linearity concentrations range (µg/mL)** **Correlation coefficient**	5.0 to 15µg/ml 0.9999
2	**Method precision (Repeatability) (%RSD, n = 6)**	< 2.0
3	**Accuracy(%Recovery)**	99.5 to 101.2 %
4.	**Robustness:** **Flow Variation(0.9mL to 1.1mL/min)**	Complies
5.	**Ruggedness:** **(Intermediate Precision) (%RSD analyst to analyst variation)**	0.14%

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