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Determination and Validation of RP-HPLC Method for the Estimation of Fosamprenavir in Tablet Dosage Form

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Abstract

A new simple, accurate, profitable, rapid-fire and precise rear phase high performance liquid chromatographic system has been developed for the validated of Fosamprenavir in bulk form and its pharmaceutical lozenge form. Chromatographic separation was carried out on Zorbax $C18(4.6mm$ X $250mm,\ 5m,\ Make\ X\ terra)$ column using a admixture of Acetonitrile Methanol Water (503020 v/ v) as the mobile phase at an inflow rate of $1.0\,ml/min$, the discovery was carried out at 265nm. The retention time of the Fosamprenavir was set up to be $5.462 \pm 0.02\ min$. The system was validated according to ICH guidelines for linearity, perceptivity, delicacy, perfection, particularity and robustness. The response was set up to be direct in the medicine attention range of $50-90\,mcg/ml$ for Fosamprenavir. The correlation measure was set up to be 0.999. The LOD and LOQ for Fosamprenavir were set up to be $1.6\,\mu g/ml$ and $4.8\,\mu g/ml$ independently. The proposed system was set up to be good chance recovery for Fosamprenavir, which indicates that the proposed system is largely accurate. The system perfection for the determination of assay was below 2.0 RSD. The system is useful in the quality control of bulk and retailed pharmaceutical phrasings

Keywords: Fosamprenavir; RP-HPLC; Method Development; Validation; ICH Guidelines

Abbreviations: HPLC: High-Pressure Liquid Chromatography; GC: Gas Chromatography; LC: Liquid Chromatography; RSD: Relative Standard Deviation.

Introduction

Chromatography is a technique for the separation of a mixture. The admixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The colorful ingredients of the admixture trip at different pets, causing them to separate. The separation is grounded on discriminational partitioning between the mobile and stationary phases. Subtle differences in a compounds partition measure result in discriminational retention on the stationary phase and therefore affect the separation [1].

Chromatography may be preliminary or logical. The purpose of preliminary chromatography is to separate the factors of a admixture for after use, and is therefore a form of sanctification. Analytical chromatography is done typically with lower quantities of material and is for establishing the presence or measuring the relative proportions of analytes in an admixture. The two aren't mutually exclusive [2].

Chromatography is grounded on the principle where moites in mixture applied onto the surface or into the solid, and fluid stationary phase is separating from each other while moving with the aid of a mobile phase. factors effective on this separation process molecular characteristics related to adsorption(liquid-solid), partition (liquid-solid) and affinity or differences among their molecular weights [3,4]. Because of these differences, some factors of the admixture stay longer in the stationary phase, and they move sluggishly in the chromatography system, while others pass fleetly into mobile phase, and leave the system briskly [5,6].

Grounded on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a " solid " phase or " a subcaste of a liquid adsorbed on the solid support surface"
- Mobile phase: This phase is always composed of "liquid" or a "gassy element".
- Separated moites

The type of commerce between stationary phase, mobile phase and substances contained in the admixture is the introductory element effective on separation of motes from each other. Chromatography styles grounded on partition are veritably effective on separation, and identification of small moites as amino acids, carbohydrates and adipose acids still, affinity chromatography's (i.e. ion- exchange chromatography) are more effective in the separation of macromolecules like nucleic acids, and proteins. Paper chromatography is used in the separation of proteins, and in studies related to synthesis of protein; gas-liquid chromatography is employed in the separation of alcohol, Esther, lipid, and amino groups, and observation of enzymatic relations, while molecular- sieve chromatography is employed especially for the determination of molecular weights of proteins. Agarose- gel chromatography is used for the sanctification of RNA, DNA patches, and contagions [7,8].

Stationary phase in chromatography is a solid phase or a liquid phase carpeted on the face of a solid phase. Mobile phase flowing over the stationary phase is a gassy or liquid phase. However, and if its gas also it's called gas chromatography (GC), If mobile phase is liquid it's nominated as liquid chromatography (LC). Gas chromatography is applied for feasts, and fusions of unpredictable liquids, and solid material. Liquid chromatography is used especially for thermal unstable and non-volatile samples [9,10].

The purpose of applying chromatography which is used as a system of quantitative analysis piecemeal from its separation is to achieve a satisfactory separation within a suitable time interval colorful chromatography styles have been developed to that end. Some of them include column chromatography, thin-layer chromatography(TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel saturation chromatography, high-pressure liquid chromatography, and affinity chromatography [11,12].

1. Column chromatography
2. Ion-exchange chromatography
3. Gel-permeation (molecular sieve) chromatography
4. Affinity chromatography
5. Paper chromatography
6. Thin-layer chromatography
7. Gas chromatography
8. Dye-ligand chromatography
9. Hydrophobic interaction chromatography
10. Pseudo affinity chromatography
11. High-pressure liquid chromatography (HPLC)

Instrumentation of HPLC

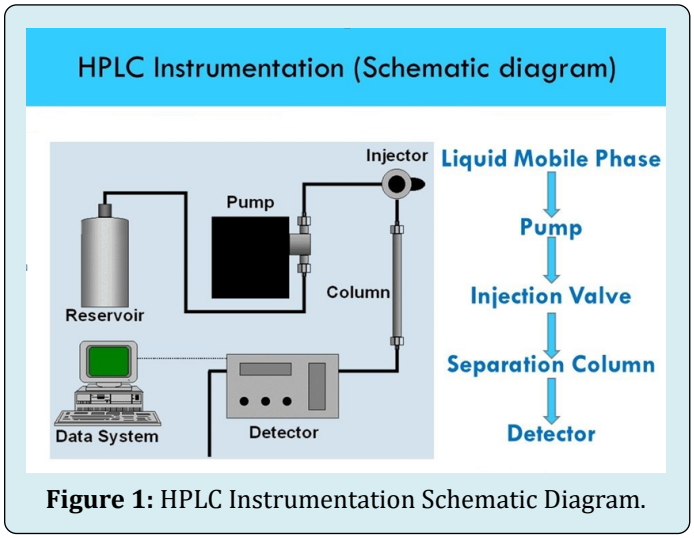

Figure 1: HPLC Instrumentation Schematic Diagram.

As shown in the schematic illustration in Figure 1 over, HPLC instrumentation [13,14] includes a pump, injector, column, sensor and integrator or accession and display system. The Main Part of the system is column in this separation occurs

Experimental Methods

Instruments Used:

S. No	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detectors.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Lab man

Table 1: Instruments Used.

Chemicals Used:

S. No.	Chemical	Brand names
1	Fosamprenavir (Pure)	Sura labs
2	Water and Methanol for HPLC	Lichrosolv (Merck)
3	Acetonitrile for HPLC	Merck

Table 2: Chemicals Used.

HPLC Method Development (Trails)

Preparation of standard solution: Accurately weigh and transfer 10mg of Fosamprenavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make up the volume up to mark with the Methanol [15]. Further pipette 0.7ml of the above Fosamprenavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note that the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Water and Methanol: Phosphate buffer with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer $(pH-4.0)$ $(35:65\% v/v)$ respectively [16].

Optimization of Column: The method was performed with various columns like C18 column, X- bridge column, X terra, and C18 column. Symmetry ODS $C18(4.6mm \text{ X } 150mm,\ 5\mu m,\ Make:\ X\ terra)$ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow [17].

Optimized Chromatographic Conditions:

Instrument used	Waters HPLC with auto sampler and PDA detector 996 model.
Mobile phase ratio	Acetonitrile: Methanol: Water (50:30:20% v/v)
Column	Zorbax C18 (4.6mm x 250mm, 5mm, Make: X terra)
Column temperature	35^0C
Wavelength	265nm
Flow rate	1ml/min
Injection volume	20µl
Run time	10min

Table 3: Optimized Chromatographic Conditions.

Validation

Preparation of Mobile Phase: Accurately measured 500ml (50%) of Acetonitrile, 300ml (30%) of Methanol and 200ml of HPLC Grade water (20%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µm filter under vacuum filtration [18].

Diluent Preparation: The Mobile phase was used as the diluent.

Validation Parameters

System Suitability: Accurately weigh and transfer 10mg of Fosamprenavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make the volume up to the mark

with the same solvent (Stock solution). Further pipette 0.7ml of the above Fosamprenavir stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: The standard result was fitted for five times and measured the area for all five injections in HPLC. The RSD for the area of five replicate injections was set up to be within the specified limits.

Specificity Study of Drug

Preparation of Standard Solution: Directly weigh and transfer 10mg of Fosamprenavir working standard into a 10ml of clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it fully and make volume up to the mark with the same solvent [19] (Stock solution) Further pipette 0.7ml of the above Fosamprenavir stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 0.7ml of the below Fosamprenavir stock results into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution: Take average weight of Tablet and crush in a mortar by using pestle and weight 10mg original weight of Fosamprenavir sample into a 10ml clean dry volumetric flask and add 7ml of Diluent and sonicate to dissolve it fully and make volume up to the mark with the same solvent [20,21]. Further pipette 0.7ml of Fosamprenavir above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{Sample\ area}{Standard\ area} X \frac{Weight\ of\ standard}{Dilution\ of\ standard} X \frac{Dilution\ of\ sample}{Weight\ of\ sample} X \frac{Purity}{100} X \frac{Weight\ of\ tablet}{Label\ claim} X 100$$

Results and Discussion

Mobile phase ratio	Acetonitrile: Methanol: Water $(50:30:20\%\ v/v)$
Column	Zorbax $C18(4.6mm\ x\ 250mm,\ 5\mu m,\ Make:\ X\ terra)$
Column temperature	$35^0 C$
Wavelength	$265nm$
Flow rate	$1ml/\min$
Injection volume	$20\mu l$
Run time	10min

Table 4: Optimized Chromatogram (Standard).

S. No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Fosamprenavir	5.462	1052689	75421	1.62	9625

Table 5: Optimized Chromatogram (Standard) Observation.

Observation: In this trial it shows proper peak, tailing, plate count and baseline in the chromatogram. So it's optimized chromatogram.

S. No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Fosamprenavir	5.466	1068547	76584	1.63	9658

Table 6: Optimized Chromatogram (Standard).

Acceptance Criteria:

- Theoretical plates must be not less than 2000.
- Tailing factor must be not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Fosamprenavir	5.474	1052658	75842	9658	1.63
2	Fosamprenavir	5.466	1058475	75481	9758	1.62

3	Fosamprenavir	5.474	1059854	75162	9685	1.63
4	Fosamprenavir	5.452	1054786	75241	9635	1.62
5	Fosamprenavir	5.446	1052642	75468	9649	1.63
Mean	-	-	1055683	-	-	-
Std. Dev.	-	-	3331.494	-	-	-
% RSD	-	-	0.315577	-	-	-

Table 7: Results of system suitability for Fosamprenavir.

Specificity

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Fosamprenavir	5.427	1052689	75421	1.63	9674	1
2	Fosamprenavir	5.43	1052854	75462	1.62	9657	2
3	Fosamprenavir	5.443	1055365	75489	1.62	9625	3

Table 8: Peak results for assay standard.

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Fosamprenavir	5.453	1065851	76854	1.63	9785	1
2	Fosamprenavir	5.462	1065482	76352	1.64	9786	2
3	Fosamprenavir	5.466	1063544	76586	1.63	9795	3

Table 9: Peak results for Assay sample.

$$\%ASSAY = \frac{Sample\ area}{Standard\ area} X \frac{Weight\ of\ standard}{Dilution\ of\ standard} X \frac{Dilution\ of\ sample}{Weight\ of\ sample} X \frac{Purity}{100} X \frac{Weight\ of\ tablet}{Label\ claim} X 100$$

The % purity of Fosamprenavir in pharmaceutical dosage form was found to be 99.58%.

Linearity

Chromatographic Data for Linearity Study:

Concentration mg/ml	Average Peak Area
50	786789
60	945685
70	1102689
80	1265241
90	1405476

Table 10: Chromatographic Data for Linearity Study.

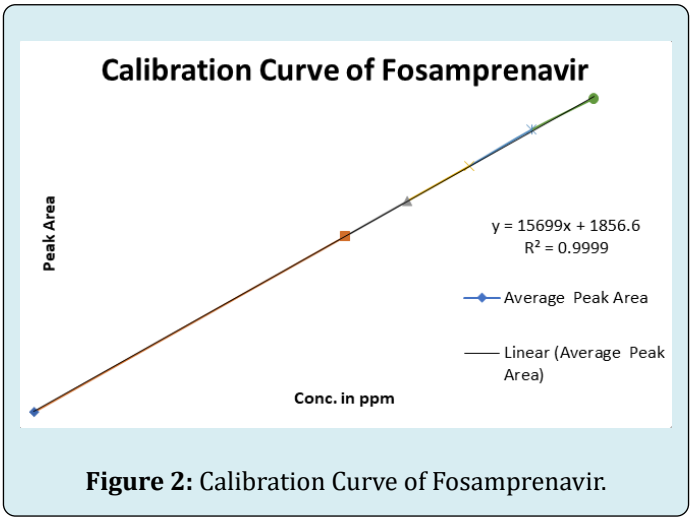

Figure 2: Calibration Curve of Fosamprenavir.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Fosamprenavir	5.419	1052658	76231	9658	1.63
2	Fosamprenavir	5.405	1056854	75898	9667	1.62
3	Fosamprenavir	5.478	1052468	75452	9652	1.63
4	Fosamprenavir	5.466	1052774	75468	9635	1.62
5	Fosamprenavir	5.466	1055245	76214	9658	1.63
Mean	-	-	1054000	-	-	-
Std.dev	-	-	1958.724	-	-	-
%RSD	-	-	0.185837	-	-	-

Table 11: Results of Repeatability for Fosamprenavir.

Acceptance criteria: %RSD for sample should be NMT 2. The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USPTailing
1	Fosamprenavir	5.484	1075846	76985	9785	1.65
2	Fosamprenavir	5.493	1078254	76854	9748	1.64
3	Fosamprenavir	5.406	1078598	76254	9786	1.65
4	Fosamprenavir	5.419	1075461	76859	9726	1.65
5	Fosamprenavir	5.446	1075236	75898	9742	1.64
6	Fosamprenavir	5.452	1075842	76985	9785	1.65
Mean	-	-	1076540	-	-	-
Std. Dev.	-	-	1483.688	-	-	-
% RSD	-	-	0.13782	-	-	-

Table 12: Results of Intermediate precision for Fosamprenavir.

Acceptance criteria: %RSD of six different sample solutions should not more than 2.

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing
1	Fosamprenavir	5.493	1068545	78574	9865	1.65
2	Fosamprenavir	5.493	1068547	78546	9854	1.64
3	Fosamprenavir	5.478	1063588	78452	9826	1.65
4	Fosamprenavir	5.466	1063542	78542	9824	1.65
5	Fosamprenavir	5.478	1065243	78563	9863	1.66
6	Fosamprenavir	5.419	1065874	78632	9875	1.66
Mean	-	-	1065890	-	-	-
Std. Dev.	-	-	2251.215	-	-	-
% RSD	-	-	0.211205	-	-	-

Table 13: Results of Intermediate precision Analyst 2 for Fosamprenavir.

Acceptance criteria: %RSD of six different sample solutions should not more than 2.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	553829	35	35.159	100.45%	100.29%
100%	1105114	70	70.275	100.39%	
150%	1650868	105	105.039	100.04%	

Table 14: The Accuracy Results for Fosamprenavir.

Acceptance Criteria: The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0ml/min	1052689	5.453	9625	1.62
Less Flow rate of 0.9ml/min	1015241	5.599	9155	1.54
More Flow rate of 1.1ml/min	1023654	4.576	9254	1.56
More Organic phase	1015853	3.827	9147	1.54
Less organic phase	1002514	7.415	9256	1.53

Table 15: Results for Robustness.

Acceptance Criteria: The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Summary

- The analytical method was developed by studying different parameters.
- First the maximum absorbance was found to be at 265nm and the peak purity was excellent.
- Injection volume is selected to be $20\mu l$ which gave a good peak area.
- The column used for study was Zorbax $C18(4.6mm\ x\ 250mm,\ 5\mu m,\ Make:\ X\ terra)$ because it was giving good peak.
- 350C temperatures were found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.
- Mobile phase is Acetonitrile: Methanol: Water $(50:30:20\%\ v/v)$ was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.
- Run time was selected to be 10 min because analyze gave peak around 5.462 ±0.02min respectively and also to reduce the total run time.
- The percent recovery was found to be 98.0-102% was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.
- The analytical method was found linearity over the range 50-90µg/ml of Fosamprenavir of the target concentration.
- The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was full satisfactory.

Conclusion

The HPLC system developed is accurate, precise, repeatable and specific. The system is direct over a wide range and utilizes a mobile phase which can be fluently prepared. The column used is an extensively available reversed phase Zorbax $C18(4.6mm\ x\ 250mm,\ 5\mu m,\ Make:\ X\ terra)$. This system is suitable for the routine quantification of Fosamprenavirin bulk medicine and in the tablet dosage forms.

The Validation of this system was proved to be simple, fast and reliable. The system was validated for its performance parameters Linearity, Repeatability, Accuracy, Precision, Ruggedness, and Robustness etc.

The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution and sample preparation steps and comparative short run time makes the system specific, repeatable and reliable for its intended use in determination of Fosamprenavir in bulk form and pharmaceutical dosage form.

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