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Stability Indicating Rp-Hplc Method for Quantification of Impurities in Fosamprenavir Calcium Drug Substance

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Abstract:

The present paper describes the development of a reverse phase chromatographic (RPLC) method for Fosamprenavir in the presence of its impurities and degradation products. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The drug was found to be stable in other stress conditions studied. Successful separation of the drug from theprocess impurities and degradation products formed under stress conditions were achieved on an Xbridge Phenyl (250 x 4.6 mm) 5 µm column. The gradient LC method employs solution A and solution B as mobile phase. The solution A contains aqueous 0.1% TFA in Water and solution B contains acetonitrile as Gradient mode. The HPLC method was developed and validated with respect to linearity, accuracy, precision, specificity and ruggedness.

Key words: Fosamprenavir, Gradient, HPLC, Phenyl, TFA, Acetonitrile, Methanol and validation.

INTRODUCTION

Fosamprenavir is a drug for the treatment of HIV infections. It is a pro-drug of the protease inhibitor and antiretroviral drugamprenavir. The FDA approved it October 20, 2003, while the EMA approved it on July 12, 2004. The human body metabolizes fosamprenavir in order to form amprenavir, which is the active ingredient. That metabolization increases the duration that amprenavir is available, making fosamprenavir a slow-release version of amprenavir and thus reducing the number of pills required versus standard amprenavir.

Structure:

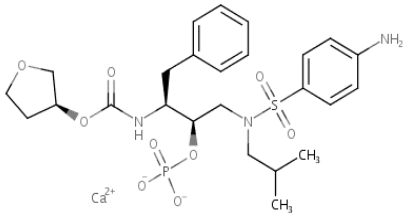

Fig 1: Structure of Fosamprenavir

IUPAC Name: {[(2*R*, 3S)-1-[*N*-(2-methylpropyl) (4-aminobenzene) sulfonamide]-3-({[(3*S*)-oxolan-3-yloxy] carbonyl} amino)-4-phenylbutan-2-yl] oxy} phosphoric acid

Molecular formula : $C_{25}H_{36}N_3O_9PS$
Molecular Weight : 585.61 g/mol

Organic impurities can arise during the manufacturing process and storage of the drugsubstances and the criteria for their acceptance up to certain limits are based on pharmaceuticalstudies or known safety data. As per regulatory guidelines, the pharmaceutical studies using asample of the isolated impurities can be considered for safety assessment. During thedevelopment of an analytical procedure, the LC method was developed for the determination ofFosamprenavir and the impurities arising during its manufacturing. In the present study, we describea reverse phase column liquid chromatography method for the separation and quantification ofprocess and degradation impurities of Fosamprenavir sodium. The accuracy, precision, limit ofdetection (LOD), limit of quantification (LOQ) and robustness of the method were determined inaccordance with ICH guidelines. This paper reports, a rapid, efficient, simple and validated LCmethod for separation of potential impurities and degradation products.

EXPERIMENTAL:

Reference standard of Fosamprenavirand impuritiesprocured as a gift samples .All reagents used were ofanalytical reagent grade unless stated otherwise. Milli Q water, HPLC-grade Acetonitrile,
HPLC-grade Trifluoro acetic acid (TFA) was purchased from SDFCL.

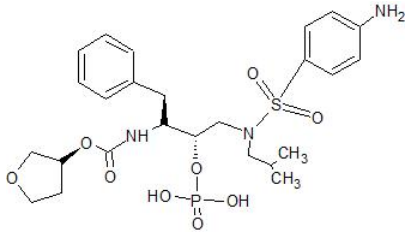

Fig 2a: Structure of Isomer

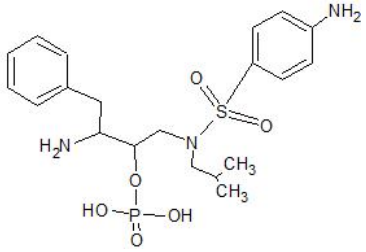

Fig 2b: Structure of Amino Impurity

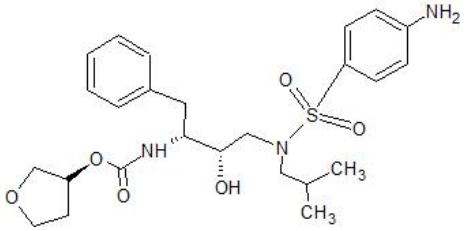

Fig 2c: Structure of Amprenavir Imp

Equipments: The chromatographic technique performed on a waters 2695 with 2996 detector and Empower2 software, reversed phase Phenyl column (Xbridge 5µ, 250 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.

HPLC-gradeAcetonitrile was from qualigens reagents pvt ltd. Trifluoro acetic acid (AR grade) was from sd fine chem.

Chromatographic conditions The sample separation was achieved on a Phenyl (5 µ, 25 cm X 4.6 mm i.d.) column, aided by mobile phase mixture of 0.1%v/v Trifluoro acetic acid in water: Acetonitrile as Gradient Method. The flow rate was 1.0 ml/ minute and ultra violet detector at 265nm,Injection volume is 10 µl and ambient temperatures.

Table 1: Gradient method

Time	Flow	A	B
1.0	1.0	62	38
2.0	1.0	62	38
7.0	1.0	30	70
9.0	1.0	30	70
10.00	1.0	62	38
15.00	1.0	62	38

Preparation of mobile phase:

Buffer Preparation: Taken accurately 1ml of Trifluoro acetic acid in 1000mL of water

Mobile phase-A: Buffer

Mobile phase-B: Acetonitrile

Sample solution preparation:

A 10mg of pure Fosamprenavir were weighed and transferred to 10ml of volumetric flask and dissolved in Diluents. The flask was shaken and volume was made up to mark with diluent solution concentration is 1.0mg/ml.

Impurities solution preparation (0.1%)

Stock Solution-1:10mg of pure impuritieswere weighed and transferred to 100ml of volumetric flask and dissolved in Diluent. The flask was shaken and volume was made up to mark with diluents to give a stock solution-1 containing 0.1mg/ml.

Stock Solution-2: From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 10µg/ml of Impurities.

From above solution again 1.0ml pipette out into 10ml volumetric flask and volume was made up to mark with diluents to give a solution containing 0.001mg/ml.

METHOD VALIDATION

System suitability

The system suitability was evaluated by injecting a impurities spiked solution with fosamprenavir at 0.1 % level with respect to test concentration. The system suitability criteria like USP tailing factor, USP Resolution and USP theoretical plates summarized in table 2. The corresponding chromatogram is shown in fig.3.

Specificity

The specificity of the LC method was evaluated to ensure that there was no interference from the other Fosamprenavir related substances. Fosamprenavir and each impurity solution were prepared individually at a concentration of 0.1mg/ml and a solution of all impurities spiked with Fosamprenavir was also prepared. All these solutions were analyzed using the PDA detector as per the HPLC method. The representative chromatogram for Specificity is shown in Chromatograms. Stress studies were performed for Fosamprenavir drug substance to provide an indication of the stability indicating property and specificity of the proposed method. Degradation was attempted to stress condition of heat, acid, base, oxidation and photolytic degradation to evaluate the ability of the proposed method to separate Fosamprenavir sodium from its degradation product.

Precision

System precision

The System precision of the related substance method was checked by injecting six replicating injections of 0.1% Reference solution including Fosamprenavir, Amino Imp, Isomer Imp and Amprenavir Imp with respect to the Fosamprenavir analyte concentration. The % RSD of the area for each impurity (Amino, Isomer and Amprenavir) and Fosamprenavir was calculated.

Method precision

The Method precision of the related substance method was checked by injecting six individual preparations of Fosamprenavir (1.00 mg/mL) spiked with 0.1% of Amino Imp and 0.1% of Isomer Imp, 0.1% Amprenavir Imp with respect to the Fosamprenavir analyte concentration. The % RSD of the recovery for each impurity (Amino, Isomer, and Amprenavir) was calculated.

Limit of detection and limit of quantification

The LOD and LOQ were determined by measuring the magnitude of analytical background. The LOD and LOQ were determined from S\N ratio method. The LOD and LOQ for Amino Imp, Isomer Imp, and Amprenavir Imp were determined by injecting a series of dilute solutions with known concentrations.

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The linearity of the HPLC method was demonstrated by analyzing the solutions ranging from LOQ to 150% of the specification limit.

Accuracy

The accuracy of the method for all the related substances was determined by analyzing Fosamprenavir sample solutions spiked with all the related substances at three different concentration levels of 50, 100 and 150% of each in triplicate at the specified limit. The percentage of recoveries for the impurities was calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between Fosamprenavir and Isomer Imp was recorded. The parameters selected were flow rate and wavelength (± 2 nm)

Solution stability and mobile stability

To determine the stability of sample solution, the sample solutions of Fosamprenavir spiked with related substances at specified level were prepared and analyzed immediately after preparation and after 24h, The results from these studies indicated, the sample solution was stable at room temperature for at least 24 h.

RESULTS AND DISCUSSION:

Optimization of chromatographic conditions

The main objective of the chromatographic method was to separate Fosamprenavir from Amino Imp, Isomer Imp, and Amprenavir Imp. During the evaluation of pH study, no effect was observed in elution order and retention times towards acidic side. Elution of impurities required higher ratios of organic modifier, hence 0.1% TFA was chosen as buffer solution to rule out precipitation of aqueous salt buffers with combination of higher organic modifier ratios. During the evaluation of various column chemistries, Phenyl was observed to give better resolution. Conditions were optimized as mentioned under section "Chromatographic Conditions". In optimized chromatographic conditions Fosamprenavir, Amino Imp, Isomer Imp, Amprenavir Imp were separated with a resolution greater than 1.5, typical relative retention times were approximately 3.729min, 5.553min, 8.586min with respect to Fosamprenavir eluted at 6.232min(Figure 2). The system suitability results are given in Table 1 and the developed LC method was found to be specific for Fosamprenavir and all of its impurities namely Amino Imp, Isomer Imp, Amprenavir Imp (Figure 3).

Validation of the Optimized Method

System Suitability

The system suitability was evaluated by injecting a impurities spiked solution with fosamprenavir at 0.1 % level with respect to test concentration.

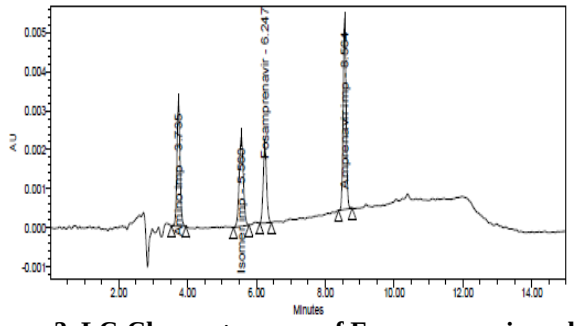

Figure 3. LC-Chromatograms of Fosamprenavir and its impurities.

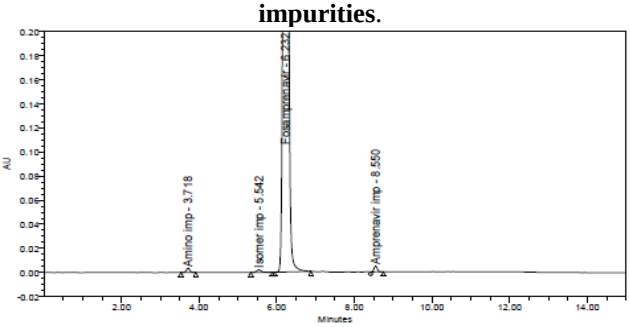

Figure 4. LC-Chromatogram of Fosamprenavir and its impurities.

Precision

System Precision

The Precision was determined at the Specification level concentrations for Amino Imp, Isomer Imp, Amprenavir Imp and Fosamprenavir, the %RSD was found below 2.0 %.

Table 2: System Suitability

Parameter	Amino	Isomer	Amprenavir	Fosamprenavir	Acceptance criteria
Retention time	3.719	5.538	8.551	6.231	+-10
Theoretical plates	8020	9894	47434	17817	>2000
Tailing factor	1.08	1.02	1.04	1.03	<1.50
Resolution between Isomer and Fosamprenavir	**3.32**				<1.50

Table 3: Summary of method precision Data

S.NO	Sample name	Amino impurity Area	Isomer impurity Area	Fosamprenavir impurity	Amprenavir impurity
1.	Injection1	20048	19478	13598	29215
2.	Injection 2	19786	19476	13789	28515
3.	Injection 3	20399	19660	14053	28737
4.	Injection 4	19903	19656	13509	28567
5.	Injection 5	19896	19890	13864	28585
6	Injection 6	20182	19838	13689	28790
Mean		20033.2	19704	13780.8	28638.8
%RSD		1.25	0.84	1.47	0.41

Method Precision

The Precision was determined at the Specification level concentrations for Amino Imp, Isomer Imp, and Amprenavir Imp were spiked to Fosamprenavir Calculated % recoveries of impurities.

Table 4: Summary of method precision Data

Sample No	% of Amino	% of Isomer	% of Amprenavir
1	0.101	0.10	0.101
2	0.101	0.101	0.101
3	0.102	0.10	0.102
4	0.098	0.10	0.098
5	0.099	0.098	0.101
6	0.102	0.099	0.099
Mean	0.100	0.099	0.100
%RSD	**1.71**	**1.71**	**1.71**

Precision at LOQ

Table 5. LOQ precision data for Fosamprenavir and its Impurities.

Injection No.	Amino	Isomer	Fosamprenavir	Amprenavir
1	2618	3634	3670	3103
2	2655	3700	3609	2965
3	2681	3775	3637	2905
4	2547	3792	3676	3052
5	2627	3710	3794	2894
6	2630	3661	3575	2924
Mean	2628	3727.6	3658.2	2948
SD	50.3	54.5	84.5	64.1
%RSD	**1.91**	**1.46**	**2.31**	**2.18**

Limit of detection and limit of quantification:

The values of LOQ and LOD for Fosamprenavir and other impurities was given in table no.6 & 7

Table 6: Summary of Limit of Quantitation (LOQ) for Fosamprenavir and its Impurities

Component	Concentration (mg/mL)	s/n ratio	LOQ (%)
Amino	0.000173	9.0	0.01
Isomer	0.000232	9.7	0.01
Fosamprenavir	0.000278	9.7	0.01
Amprenavir	0.00011	10.7	0.01

Table 7: Summary of Limit of detection for Fosamprenavir and its Impurities

Component	Concentration (mg/mL)	s/n ratio	LOD (%)
Amino	0.000060	2.8	0.003
Isomer	0.000076	2.8	0.004
Fosamprenavir	0.000091	3.1	0.005
Amprenavir	0.000036	3.7	0.002

Linearity:

Linear calibration plot for the related substance method was obtained over the calibration ranges tested, *i.e.*, LOQ to 150% to the test concentration for impurities and Fosamprenavir. The correlation co-efficient obtained was greater than 0.9990. The above result show that an excellent correlation between the peak area and the concentration of all impurities.

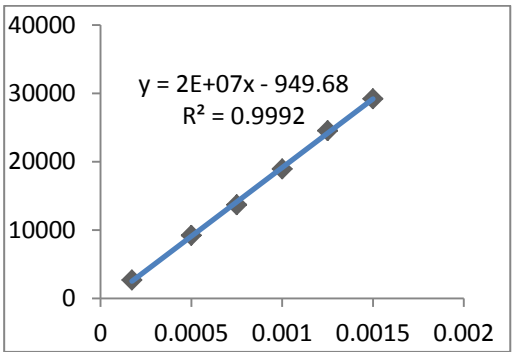

Figure 5a. Linearity plot of Amino impurity

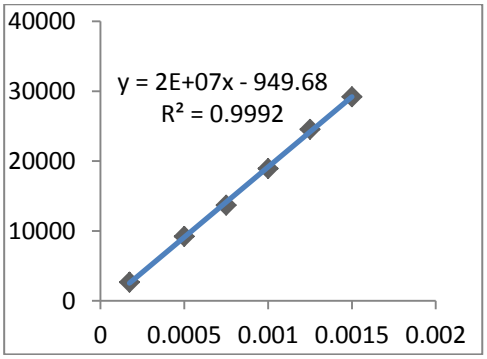

Figure 5b: Linearity Plot for Isomer impurity

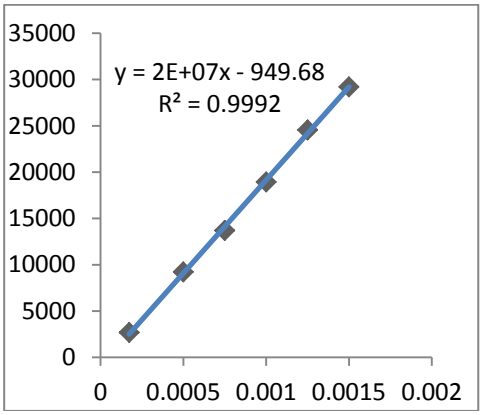

Figure 5c:Linearity Plot for Fosamprenavir

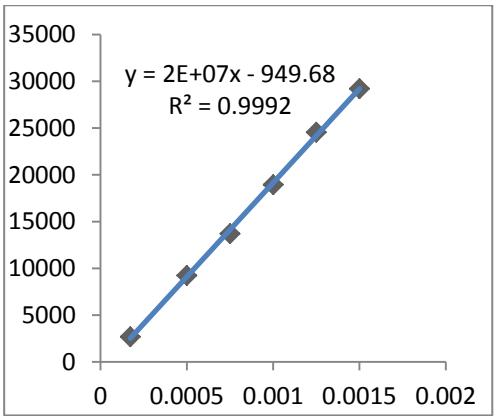

Figure5d: Linearity Plot Amprenavir impurity

Table 8: Linearity for Fosamprenavir and its Impurities

Level	Amino		Isomer		Fosamprenavir		Amprenavir	
	Conc. (mg/mL)	Peak area	Conc. (mg/mL)	Peak area	Conc. (mg/mL)	Peak area	Conc. (mg/mL)	Peak area
LOQ	0.000173	2685	0.000232	2685	0.000298	3267	0.00011	3116
50	0.0005	9229	0.0005	9229	0.0005	5886	0.0005	13659
75	0.00075	13705	0.00075	13705	0.00075	9125	0.00075	20304
100	0.001	18931	0.001	18931	0.001	12828	0.001	27769
125	0.00125	24544	0.00125	24544	0.00125	16698	0.00125	35515
150	0.0015	29197	0.0015	29197	0.0015	20432	0.0015	42829
Correlation coefficient	**0.9996**		**0.9997**		**0.9993**		**0.9995**	

Accuracy

The Accuracy of all these related substances was found to be in between the predefined acceptance criterion of 90.0-110% and the data is given Table 9.

Table 9: Summary of % recoveries of Impurities

Level	%Recovery of Amino	%Recovery of Isomer	%Recovery of Amprenavir
LOQ	100.8	100.6	102.8
	100.9	99.3	104.6
	97.8	101.5	102.0
50 %	100.5	99.8	99.9
	99.8	99.5	101.6
	100.4	99.2	100.4
100 %	100.6	99.5	100.8
	101.3	100.8	100.6
	101.6	99.5	100.8
150 %	100.2	98.1	94.8
	100.3	98.2	94.2
	100.6	99.4	94.8

Robustness

When the chromatographic conditions flow rate and wavelength varied and resolution between the critical pair, *i.e.*Fosamprenavir and Isomer was given in Table No. 10

Table 10: Robustness data for Fosamprenavir and its impurities

Parameter	Resolution between Isomer and Fosamprenavir
Flow rate 1.0 mL/min (Actual)	3.37
Flow rate 0.9 mL/min, (Low flow)	3.17
Flow rate 1.1 mL/min, (High flow)	3.37
Wavelength 265nm (Actual)	3.37
Decreased Wave Length 263nm	3.38
Increased Wave Length 267nm	3.41

Solution stability

There were no significant changes in the amounts of the impurities during solution stability experiment performed using the related substances method. The results from the studies indicated, the sample solution was stable at room temperature for at least 24 hr.

Table 11: Solution Stability

Component	Initial (%w/w)	After 24 hr (%w/w)
Amino	0.1	0.1
Isomer	0.1	0.1
Amprenavir	0.1	0.1

Forced degradation

Degradation was observed in Fosamprenavir sample when subjected to stress conditions. The Summary of Degradation Data given in Table no 12 and Degradation Chromatograms also attached as a Figure 6a to Fig 6e.

Table 12. Forced degradation studies data

S. No	Degradation Condition	Fosamprenavir %	Major imp Degraded % &RT	%Imp & RT
1	0.1N HCl 24hr	93.77	4.06(2.633)	1.18(2.43RT),0.90(3.2RT)
2	0.1N NaOH 24hr	98.64	0.99(2.595)	0.27(3.19)
3	5% H_2O_2 24h	93.78	2.53(2.52),2.67(2.64)	0.66(3.21)
4	Heat 24 h	99.89	0.03(3.19)	0.02(3.69)
5	UV 24h	99.86	0.04(3.20)	0.03(3.79)

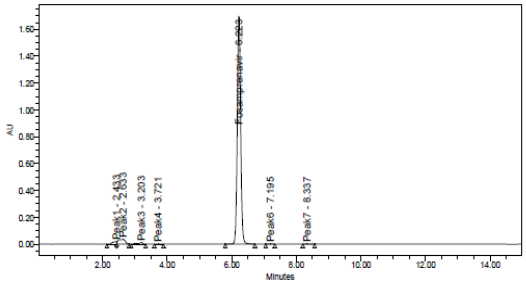

Fig 6a: Chromatogram for Acid Degradation

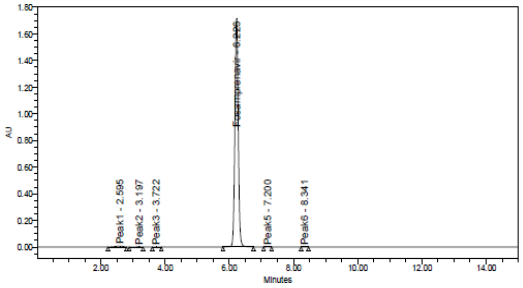

Fig 6b: Chromatogram for Acid Degradation

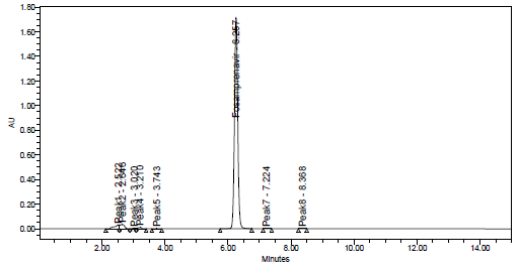

Fig 6c: Chromatogram for H2O2 Degradation

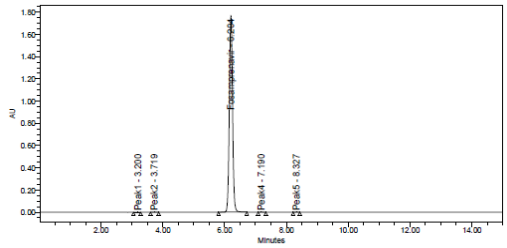

Fig 6d: Chromatogram for Photo Degradation

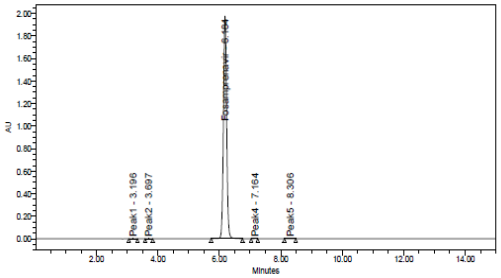

Fig 6e: Chromatogram for Thermal Degradation

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of impurities in FOSAMPRENAVIR was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

REFERENCES:

1. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
2. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
3. http://www.ich.org/
4. http://www.irjponline.com/admin/php/uploads/1029_pdf.pdf
5. http://www.ncbi.nlm.nih.gov/pubmed/20502563
6. http://en.wikipedia.org/wiki/Fosamprenavir
7. Development of High Performance Liquid Chromatography (HPLC) Method for the Estimation of Fosamprenavir Calcium in Pharmaceutical Formulation Pekamwar SS1 *, Bhavar GB2, Aher KB2 and Kakad SJ2
8. Stability-indicating HPLC method for determination of fosamprenavir calcium. Chilukuri M[1], Narayanareddy P[2], Hussianreddy K[3].
9. Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Fosamprenavir Calcium in Pure and Pharmaceutical Dosage Forms, Mohanareddy Chilukuri, Papadasu Narayanareddy , Katreddi Hussianreddy