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Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Fosamprenavir Calcium in Pure and Pharmaceutical Dosage Forms

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The main objective is to develop a simple, specific, accurate, precise and stability-indicating RP-HPLC method for the estimation of fosamprenavir calcium in pure and tablet dosage form. The optimized method uses a reverse phase column, Inertsil C8- 3 (250 mm × 4.6 mm × 5 µm) HPLC column in isocratic mode employing buffer (ammonium formate and triethylamine pH 3.2 ± 0.05) and solvent mixture (methanol:acetonitrile 60:40) in the ratio 60:40 (v/v) with a flow rate of 1.0 mL min^{-1}. Detector wavelength was monitored at 266 nm and column temperature was maintained at 30 °C.The developed method resulted in fosamprenavir calcium eluting at 4.1 min. The developed method was validated according to International Conference on Harmonization (ICH) guidelines. Fosamprenavir calcium exhibited linearity in the range of 22.4-134.4 µg/mL. The relative standard deviation for both intra-day and inter-day precision was found to be not more than 1 %. Percentage mean recovery was found to be in the range of 100 to 101 %, during accuracy studies. Degradation studies are performed under various conditions like acid, alkali, peroxide and photolytic. Where better resolution was achieved for the analyte peak from the degradants and in each condition it was found that purity threshold value was greater than the angle value hence the peak is said to be pure. These results are proved that the method would have great value when used for the analysis of commercial samples economically.

Keywords: RP-HPLC, Fosamprenavir calcium, Method development, Validation, Degradation studies.

INTRODUCTION

Human immunodeficiency infection is a manageable but not a curable disease. This is because of the advent of highly active antiretroviral therapy, where the patients are treated with a cocktail drugs which are designed in order to minimize their viral loads to significant low levels[1]. IUPAC name of fosamprenavir calcium, (1*S*,2*R*)-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-1-(phenylmethyl)-2-(phosphonooxy)-propyl]carbamic acid C-[(3*S*)-tetrahydro-3-furanyl]ester calcium salt (Fig. 1), is the phosphate ester prodrug of HIV PI amprenavir[2-4]. fosamprenavir was first approved by the Food and Drug Administration in 2003[5] and then by the European Medicines Agency in 2004[6]. Fosamprenavir is classified as class II according to the BCS classification system[7]. Literature survey revealed that one dissolution method[8], electro chemical evaluation method was reported[9] and a stability indicating HPLC method was reported[10]. No official methods are available in pharmacopoeia.

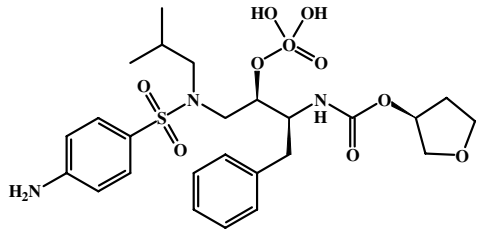

Fig. 1. Chemical structure of fosamprenavir calcium

Hence an attempt was made to develop an accurate, rapid, specific and stability-indicating method for the determination of assay of fosamprenavir calcium economically. This method is also used for the quantitative analysis of tablet dosage forms. Current method proved that the noninterference of excipient's and degradants from the analyte peak of interest.

EXPERIMENTAL

Fosamprenavir calcium working standard and tablets were received from Taj Pharmaceuticals, India. HPLC grade acetonitrile was purchased from Merck, Darmstadt, Germany. All the other chemicals used are of analytical grade purchased from Rankem, Mumbai, India. Milli 'Q' water was used throughout the analysis.

The HPLC system used for analysis was a Waters alliance HPLC with 2695 separation module equipped with quaternary gradient pumps, an inbuilt auto injector, a 270852 thermostatic compartment and a 2487 UV detector. Empower2 software was used for data acquisition and system suitability calculations.

Chromatographic conditions: The chromatographic separation was achieved on an Inertsil C8- 3 (250 mm × 4.6 mm × 5 µm) HPLC column in isocratic mode employing buffer (ammonium formate and triethylamine pH 3.2 ± 0.05) and solvent mixture (methanol:acetonitrile 60:40) in the ratio 60:40 (v/v) with a flow rate of 1 mL min^{-1}. Detector wavelength was monitored at 266 nm and column temperature was maintained at 30 °C. The injection volume limited to 10 µL.

Preparation of diluents: Buffer preparation: 3.14 g of ammonium formate in 1000 mL water adjusted pH 3.2 with formic acid. Diluent 1: buffer: acetonitrile 300:700. Diluent 2: buffer: solvent mixture 600: 400

Standard solution preparation: Weighed accurately and transferred 56 mg of fosamprenavir calcium (eq. to 50 mg fosamprenavir) working standard into 100 mL volumetric flask, diluent-1 and diluted to volume with diluent-1. (Stock solution). Further diluted 4 mL of standard stock solution into 25 mL volumetric flask and dilute to volume with diluent-2. Filtered the solution through 0.45 µm PVDF syringe filter.

Sample solution preparation: Transferred eq. to 4245 mg fosamprenavir into 500 mL volumetric flask, add 50 mL of diluent buffer, sonicated for 30 min, add 350 mL of diluent-1 sonicated for 40 min, diluent-1, after complete dispersion made up to the volume with diluent-1. Centrifuge and further diluted 4 mL of the supernatant to 200 mL with diluent-2.

Assay of fosamprenavir calcium (mg per tablet) was calculated using this formula =

$$\frac{A_t \times W_S \times 4 \times 500 \times 200 \times P \times 585.608}{A_s \times 50 \times 25 \times W_T \times 4 \times 100 \times 623.7} \times A_{WT}$$

where, A_t = Average peak area of fosamprenavir from test solution. A_s = Average peak area of fosamprenavir from standard solution. W_S = Weight of the fosamprenavir calcium standard taken, in mg. for standard solution. P = Potency of fosamprenavir calcium standard in %, on as is basis. W_T = Weight of sample taken, in mg, for test solution. A_{WT} = Average weight of fosamprenavir calcium, in mg. 623.7 = Molecular weight of fosamprenavir calcium. 585.608 = Molecular weight of fosamprenavir.

Assay of fosamprenavir calcium (% of label claim) =

$$\frac{\text{Reported assay value in mg/tablet}}{\text{Label claim of fosamprenavir (mg per unit dose)}} \times 100$$

Validation of the method: The developed HPLC method for the estimation of fosamprenavir calcium was validated according to the ICH guidelines[11,12]. The developed method was validated with the following parameters.

Precision: The system precision was determined by injecting six replicate standard injections and the % RSD was calculated. The method precision of the developed method was determined by injecting of six assay preparations of fosamprenavir calcium test solution with respect to valid working standard. The % RSD for six assay results was calculated. Similarly the intermediate precision was evaluated by performing the method precision on different days, different analysts using different instruments (another HPLC system and column of the same make with different lot number) in the same work place.

Linearity: Linearity is the ability of the method to elicit the test results. The linearity of the developed method was assessed by the construction of a calibration curve or plot between different concentrations of the analyte and peak area. Test solutions were prepared from a stock solution at five different concentration levels covering the range of 25 to 150 % of target assay concentration. Slope, intercept and correlation coefficient was calculated.

Accuracy/recovery: Accuracy is the closeness of the agreement between the actual value and the mean analytical value. The accuracy of the developed method was evaluated in triplicate at three different concentration levels *i.e.*, 50, 100 and 150 % of the analyte concentration, the percentage recovery and relative standard deviation at each level was determined.

Robustness: Robustness of the method was evaluated by making small deliberal changes in the choromatographic conditions such as flow rate, column temperature and system suitability parameters (*i.e.*, tailing factor, plate count and retention time *etc*.) were evaluated. The flow rate variation was evaluated at 0.9 mL/min and 1.1 mL/min and column temperature effect was evaluated at 25 and 35 °C.

Specificity (forced degradation studies): In order to evaluate the stability indicating power of the developed method forced degradation studies were conducted. Degradation studies were performed under acidic, alkali, peroxide and thermal conditions according to the ICH guidelines. Study included: light, 1.2 million lux h and an integrated near-ultraviolet energy of not less than 200 Wh/m^2 for two days in a photo stability chamber, acid hydrolysis (5 N HCl, 30 min of heating at 80 °C), base hydrolysis (5 N NaOH, 30 min of heating at 80 °C) and oxidation (5 % H_2O_2, 60 min of heating at room temperature). Purity angle and threshold values for each condition were evaluated.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: Several experiments were conducted in order to achieve best separation of fosamprenavir calcium, excipients and degradation products. The developed HPLC method was able to separate the analyte peak from the excipients and degradation products effectively with good resolution in short period of time, economically with acceptable system suitability parameters. The results of the developed assay method were presented in Table-1.

The chromatographic separation was achieved on an Inertsil C8- 3 (250 mm × 4.6 mm × 5 µm) HPLC column in isocratic mode employing buffer (ammonium formate and triethylamine pH 3.2 ± 0.05) and solvent mixture (methanol: acetonitrile) in the ratio 60:40 (v/v) with a flow rate of 1.0 mL min^{-1}. Detector wavelength was monitored at 266 nm and column temperature was maintained at 30 °C. Upon application of the proposed method, well separated sharp peak was obtained for fosamprenavir calcium at retention time of 4.169.

TABLE-1
ASSAY RESULTS OF FOSAMPRENAVIR CALCIUM

Sample area	2209538
Standard area	2221472
Standard weight (mg)	55.8
Sample weight (mg)	4245
Label claim (mg)	700
Average weight (mg)	1415
Standard purity (%)	98.0
Assay (%)	97.3

The representative chromatogram of fosamprenavir calcium was given in Fig. 2.

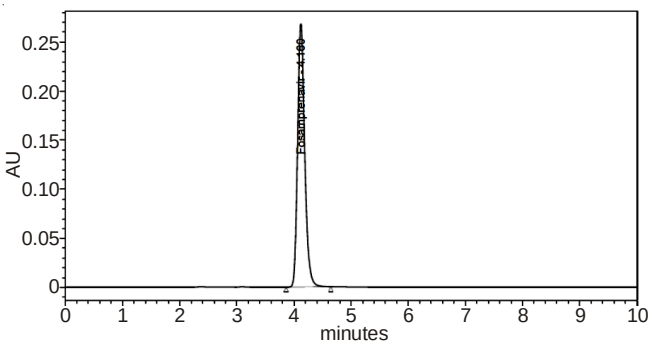

Fig. 2. Typical chromatogram showing peaks of fosamprenavir calcium

Method validation: The method was validated for specificity, linearity, limit of detection and quantitation, accuracy and precision.

Specificity: Forced degradation studies were performed to establish the specificity. Degradation studies are performed under acidic, alkali, UV and oxidative conditions. Acidic and alkaline samples were neutralized prior to final dilution. The representative chromatograms of acidic, basic, oxidative and thermal stress tests for fosamprenavir calcium were given in Figs. 3-6, respectively. In each degradation study it was observed that purity angle is less than the threshold value, it indicated the no interference of degradants with the drug peaks so the peak was said to be pure. Degradation studies reveal that the developed method was stability indicating. The results are represented in Table-2.

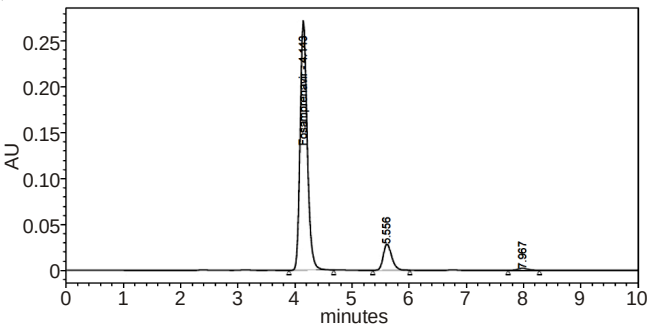

Fig. 3. Acid stress treated sample analytes

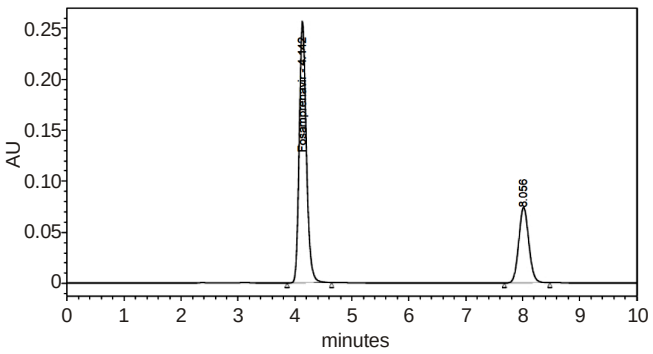

Fig. 4. Chromatogram showing basic stress

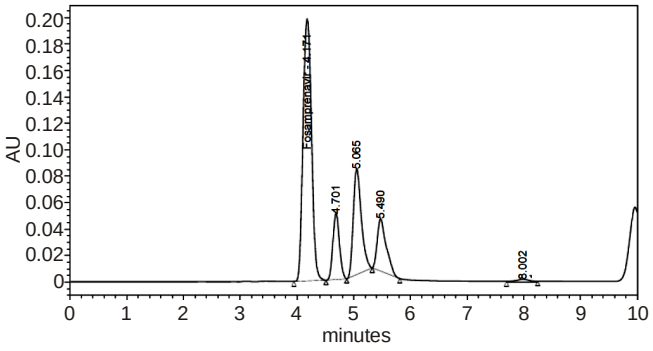

Fig. 5. Oxidative stress based degradation

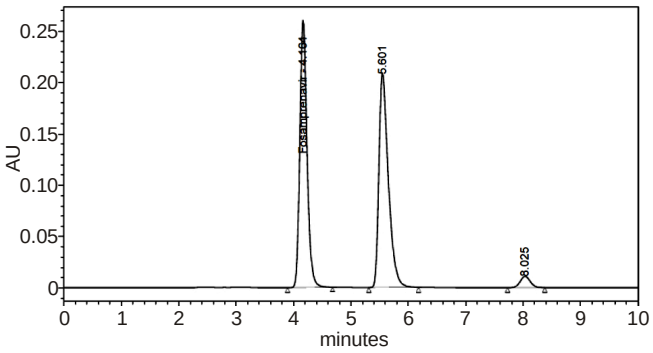

Fig. 6. Chromatogram showing photolytic stress

Precision: The relative standard deviation for both intra-day and inter-day precision was observed that not more than 2 %. The percent assay for precision study was observed between 101.0 to 103 %. The results are presented in Tables 3 and 4.

Linearity: The developed method was found to be linear from 22.4 to 134.4 mg/mL. The linear regression equation and correlation coefficient were y = 25,970x – 22,108, 0.999, respectively. The results of the linearity were proved that good correlation existed between the peak area and concentration of the analyte. Linearity graph for fosamprenavir was given in Figs. 7 and 8. The results are represented in Tables 5 and 6.

TABLE-2
RESULTS OF DEGRADATION STUDIES FOR FOSAMPRENAVIR CALCIUM

S. No.	Sample weight (mg)	Sample area	Assay (%)	Degradation (%)	Purity angle	Purity threshold
Acid	4246.0	2098560	92.4	4.9	0.114	0.232
Alkali	4244.1	1943349	85.6	11.7	0.232	0.546
Peroxide	4245.8	1990854	87.6	9.7	0.223	0.643
UV/Photolytic	4245.5	1965065	86.5	10.8	0.104	0.294

TABLE-3
INTRA-DAY PRECISION RESULTS OF FOSAMPRENAVIR CALCIUM

S. No.	Sample weight (mg)	Sample area	Assay (%)
1	4245.4	2290546	102.7
2	4244.2	2289650	103.0
3	4244.8	2278659	102.0
4	4244.9	2299378	103.0
5	4244.1	2284033	101.0
6	4244.7	2345304	103.8
	Average assay		102.5
	SD		0.8093
	RSD (%)		0.8

TABLE-4
INTER-DAY PRECISION RESULTS OF FOSAMPRENAVIR CALCIUM

S. No.	Sample weight (mg)	Sample area	Assay (%)
1	4245.0	2254640	99.0
2	4245.4	2232044	98.0
3	4245.2	2260950	100.0
4	4245.6	2299023	101.0
5	4245.0	2241023	99.0
6	4244.9	2254659	99.0
	Average assay		99.3
	SD		1.0328
	RSD (%)		1.0

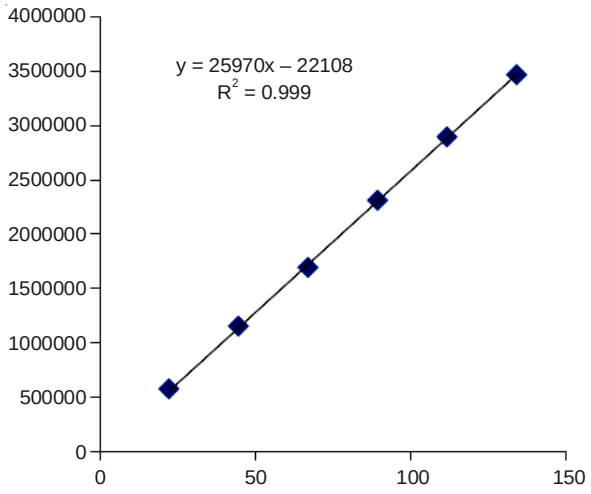

Fig. 7. Calibration curve of fosamprenavir calcium

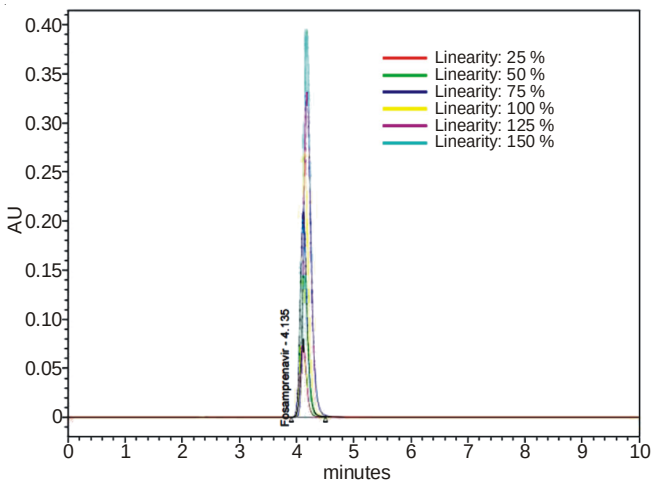

Fig. 8. Linearity overlay of fosamprenavir calcium

TABLE-5
LINEARITY OF THE CHROMATOGRAPHY SYSTEM

Drug	Linearity range (µg/mL)	R^2	Slope	Intercept
Fosamprenavir calcium	22.4-134.4	0.999	25970	22108

TABLE-6
CALIBRATION DATA FOR FOSAMPRENAVIR CALCIUM

Level (%)	Concentration range (µg/mL)	Peak area
25	22.4	569324
50	44.8	1148905
75	67.2	1690546
100	89.6	2309865
125	112.0	2895645
150	134.4	3469590

Accuracy and recovery: Accuracy of fosamprenavir was measured at three levels, 50 100 and 150 %. Accuracy of the method was found to be well within specified limits. The percent RSD values are found to be within 1 %. The percentage recovery of fosamprenavir calcium found in the range of 100.0-102.0 %. The results are presented in Table-7.

TABLE-7
RESULTS OF ACCURACY STUDIES FOR FOSAMPRENAVIR CALCIUM

Concentration level (%)	Area*	Mean recovery (%)*	RSD (%)*
50	1145817	100.9	0.30
100	2283013	100.5	0.10
150	3466916	101.8	0.06

*Mean of three replicates

Robustness: Robustness of the method was studied by applying minor variations in the chromatographic conditions like composition of the flow rate and temperature. System suitability parameters such as number of theoretical plates, retention time, tailing factor and impacts on assay were studied. In all of the varied conditions, the components of the mobile phase were held constant. The selectivity and the performance of the developed method were remain unaffected even after small deliberal changes made in the selected chromatographic condition which proved that the method was robust.

Conclusion

A stability indicating and economic RP-HPLC method was developed and validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, ruggedness, robustness, for the quantitative estimation of fosamprenavir calcium in bulk and tablets. The developed method can be used for the analysis of commercial formulations economically.

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