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# NOVEL RPHPLC METHOD FOR ESTIMATION OF FOSAMPRENAVIR CALCIUM IN SUSPENSION DOSAGE FORM

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**ABSTRACT**

A robust Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed for the estimation of Fosamprenavir calcium in pharmaceutical dosage forms. Chromatographic separation was performed on an Agilent Zorbax SB-C18 column (4.6x250mm, 5µm), with a mobile phase comprising methanol, acetonitrile, and water in the ratio of 40:40:20/v, at a flow rate of 1.0 ml/min, and detection at 249 nm. System suitability tests demonstrated excellent performance, with theoretical plates at 6341 and a tailing factor of 1.78. The method showed high accuracy with a percentage recovery of 100.6%, precision with a %RSD of 0.93%, and strong linearity with a correlation coefficient of 0.999. The limits of detection and quantification were 0.507 µg/ml and 1.538 µg/ml, respectively, indicating high sensitivity. This method is suitable for routine analysis and quality control of Fosamprenavir calcium in oral suspension dosage forms.

**Key words**: Fosamprenavir calcium, RP-HPLC, Pharmaceutical Analysis, Accuracy, Sensitivity.

## INTRODUCTION

Pharmaceutical analysis is a critical field that encompasses the procedures necessary to determine the identity, strength, quality, and purity of pharmaceutical compounds [1-3]. This discipline also involves the analysis of raw materials and intermediates during the manufacturing process of drugs. One of the key techniques used in pharmaceutical analysis is chromatography, which is based on the difference in the rate at which the components of a mixture move through a porous medium (stationary phase) under the influence of a solvent or gas (mobile phase). Among the various chromatographic methods [4], High-Performance Liquid Chromatography (HPLC) stands out as a modern, reliable, and reproducible technique for the standardization of both single and compound formulations.

HPLC is a versatile instrument widely used in pharmaceutical analysis due to its ability to provide precise and accurate identification of different components within a sample. The initial identification is typically made by comparing the retention time (Rt) of different analytes with those of known standards [5]. However, since many substances can have similar Rt values, various chromatographic parameters—such as different mobile phases, columns, and flow rates—are employed to confirm the identity of the compounds.

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Another common method for compound identification is spiking, which involves adding a pure compound to the original sample and observing an increase in peak height or area.

The goals of analytical methods in HPLC include achieving peak resolution, precision, specificity, and sensitivity. For instance, pharmaceutical methods for potency assays of an active pharmaceutical ingredient (API) generally require a resolution greater than 1.5 from the closest eluting components, precision of retention time and peak area with a relative standard deviation (RSD) of less than 1–2%, and linearity in the range of 50–150% of the label claim.

Fosamprenavir is an antiviral medication used to prevent the multiplication of the human immunodeficiency virus (HIV) in the body [6]. It is prescribed for the treatment of HIV, the virus responsible for acquired immunodeficiency syndrome (AIDS), and is suitable for use in adults and children aged four weeks and older. While Fosamprenavir is not a cure for HIV or AIDS, it is used in combination with other antiretroviral agents for the treatment of HIV-1 infection. However, it is contraindicated in patients with a history of clinically significant hypersensitivity to any components of the product or to amprenavir [7-12]. The aim of this study is to develop and validate a new Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantitative estimation of Fosamprenavir calcium in oral suspension dosage forms, following the guidelines set forth by the International Council for Harmonisation (ICH) Q2 (R1).

## METHODOLOGY

### Chemicals grade

The chemicals used in this study were HPLC grades.

### Selection of Initial Conditions for Method Development

The test procedure includes the selection of solvent which was done by solubility check and it was found to be methanol (10ml).0.1mg of each drug taken and checked for the solubility in methanol and water and acetonitrile [13].

### Wavelength selection by UV Spectroscopy

Accurately weighed and transferred about 25mg of ATV working standard into a 25mL volumetric flask separately, then add to it 5mL of methanol and sonicate it for 10 minutes to dissolve and make up with diluents-2. This produced standard stock solution (1mg/mL). Further diluent 5mL of above solution was transferred into 25mL volumetric flask and volume was made up with diluents-3. This produced solutions of concentration 200µg/mL. Finally diluted 0.5mL of above solution to 10mL using methanol and mixed well. The concentration of the working solution thus produced was 10µg/mL. The working standard solutions of ATV (10µg/mL) were scanned over the range of 200-400nm using blank. By observing the overlain spectra of standard solutions λ 265nm was taken for trials to develop HPLC method [8].

### Estimation of Fosamprenavir Calcium In Oral Suspension

**Preparation of Mobile Phase:** The Mobile phase was prepared by mixing water, methanol and acetonitrile in the proportions of (10:20:20) respectively

**Preparation of diluent-1:** Use degassed methanol as diluent-1

**Preparation of diluents-2:** Prepare a required volume of degassed mixture of 0.025 N HCl solutions and methanol in ratio of 1:1 v/v

**Preparation of diluents-3:** Prepare a required volume of degassed mixture of 0.025 N HCl as diluent -3.

### ASSAY

### Preparation of Fosamprenavir Calcium Standard & Sample Solution

### Preparation of Standard Solution

Accurately Weigh and transfer about 25mg of Fosamprenavir Calcium working standards into a 25ml clean, dry volumetric flask, add about 5ml of diluent-1, sonicate to dissolve at room temperature and dilute to volume with diluent-2 and mix. Further dilute 5ml of this solution to 25 ml with diluent -3 and mix. Filter the solution through membrane filter (millipose pvf 0.45m/ nylon 0.45 or whatmann GF/C 1.2 m or suitable)

### Preparation of Sample Solution

Measure accurately about 20ml of the oral suspension and transfer into 500ml clean & dry volumetric flask, add about 50 ml of diluents-3 and sonicate for about 15 minutes to mix the drug completely at room temperature with intermittent shaking. Add about 300 ml of diluent-1 and sonicate for about 20 minutes at room temperature with intermittent shaking dilute with diluent -2 upto 1cm below the mark, allow cooling at room temp and makeup with diluent -3 and mix. Centrifuge the solutions at 10,000 rpm for 5 mins. Further dilute 5ml of this solution to 50 ml with dilutent -3 and mix. Filter the solution through 0.45 m membrane filter.

### Procedure

20 µL of the standard and sample solutions were injected into the chromatographic system and areas for the Fosamprenavir Calcium peaks were measured.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{LC} \times 100$$

**Method validation**

As per ICH guidelines [14], validation parameters such as System Suitability, Accuracy (Recovery), Precision, Linearity, Robustness, Limit of Detection, Limit of Quantification were done

**RESULTS**

**Selection of wavelength**

The sensitivity of the HPLC method depends upon the proper selection of wavelength. The detection wavelength is 249 nm. Fosamprenavir Calcium was selected was the drug showed optimal absorbance at that wavelength

**ASSAY**

The assay limits for Fosamprenavir Calcium were 90-110% and the results obtained for Fosamprenavir Calcium was found to be 100.63%. Hence the results were within the limits

**Method validation**

**System suitability**

Tailing factor obtained from the standard injection is 1.78. Theoretical plates obtained from the standard injection-1 are 6341.

**Accuracy**

The accuracy studies were shown as % recovery for Fosamprenavir calcium at 50%, 100%, and 150% at limits of % recovered should be in range of 98=102% the results obtained for Fosamprenavir calcium were found to be within limits. Hence the method was found to be accurate. The accuracy studies showed % recovery of the Fosamprenavir calcium.is 99.7%. The limits of % recovery of drugs was 98-102% and from the above results it indicates that the method was accurate and also revealed that the commonly used excipients present in the pharmaceutical formulation do not interfere in the proposed method.

**Precision**

The % RSD of peak area for Fosamprenavir calcium was found to be 0.93 which is below 2.0% indicates that the system gives precise result.

**Linearity:**

The linearity range was found to be 150-600 (µg/ml) for Fosamprenavir calcium. Calibration curve was plotted and correlation co-efficient for the drug found to be 0.999. Hence the results obtained were within the limits. The linearity chromatograms recorded were shown in the graph.

**Table: 1 Observation of Assay Results**

| S.No | Name | RT | Area | USP plate count | USP tailing |
|---|---|---|---|---|---|
| **1** | **FSP** | **3.52** | **4306198** | **5437** | **1.38** |

**Table: 2 Observation of Assay Results**

| Formulation | Label claim | % assay |
|---|---|---|
| LEXIVA | 50 | 100.63% |

**Table: 3 Accuracy (recovery of Fosamprenavir calcium)**

| Concentration | Peak Response | Average Peak area | Amount Added | Amount Found | % Recovery | Mean Recovery |
|---|---|---|---|---|---|---|
| **50%** | 5613787 | 5616407 | **300** | 305.1 | 101.70 | 99.7% |
| | 5610866 | | | | | |
| | 5624568 | | | | | |
| **100%** | 9928627 | 9924076 | **400** | 397.12 | 99.28 | |
| | 9915165 | | | | | |
| | 9928437 | | | | | |
| **150%** | 15384338 | 15340585 | **500** | 504.05 | 99.28 | |
| | 15288620 | | | | | |
| | 15348798 | | | | | |

**Table: 4 Limit of Detection and Limit of quantification of Fosamprenavir calcium**

| Drug | Concentration (µg/ml) |
|---|---|
| LOD | **0.507** |
| LOQ | **1.538** |

**Figure:1 Structure of Fosamprenavir calcium**

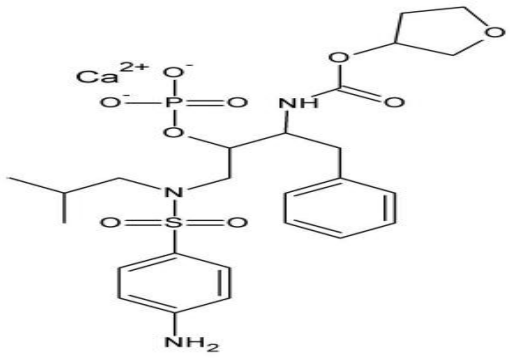



**Figure: 2 UV spectrum of Fosamprenavir calcium**

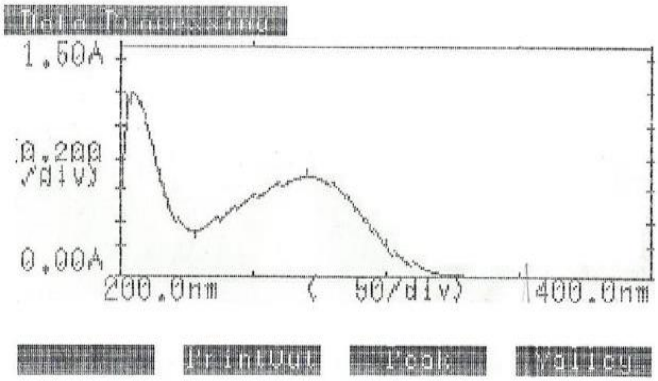



**Figure:3 Standard Chromatograms a. Trial-1; b. Trial-2; c. Trial-3**

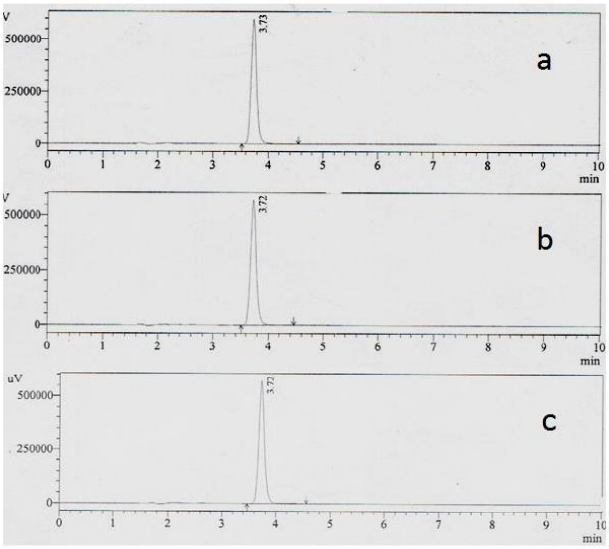



**Figure: 4 Sample Chromatograms a. Trial-1; b. Trial-2; c. Trial-3**

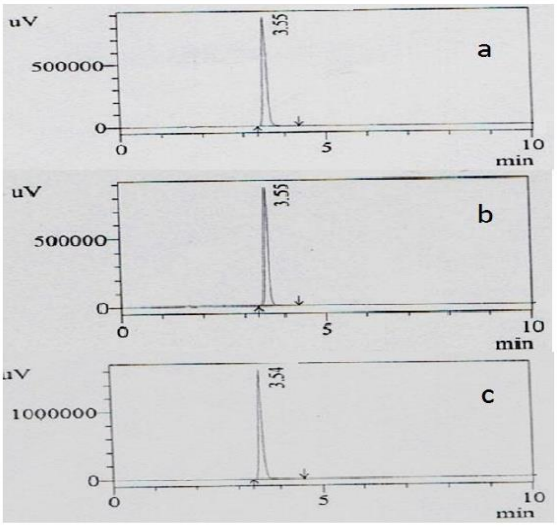



**Figure: 5 System suitability Chromatograms**

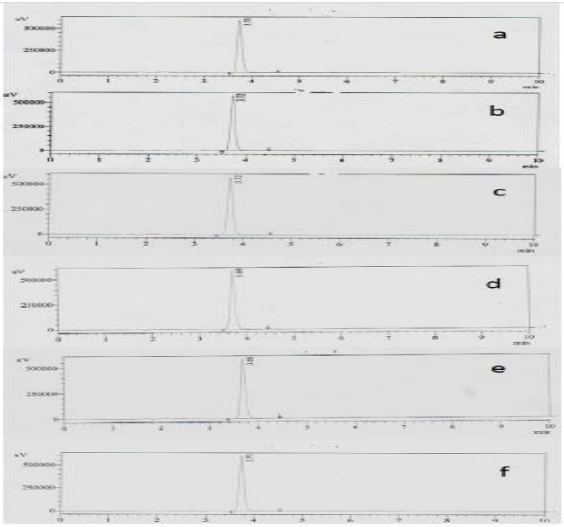



**Figure: 6 Precision Injection Chromatogram a. Trial-1; b. Trial-2; c. Trial-3**

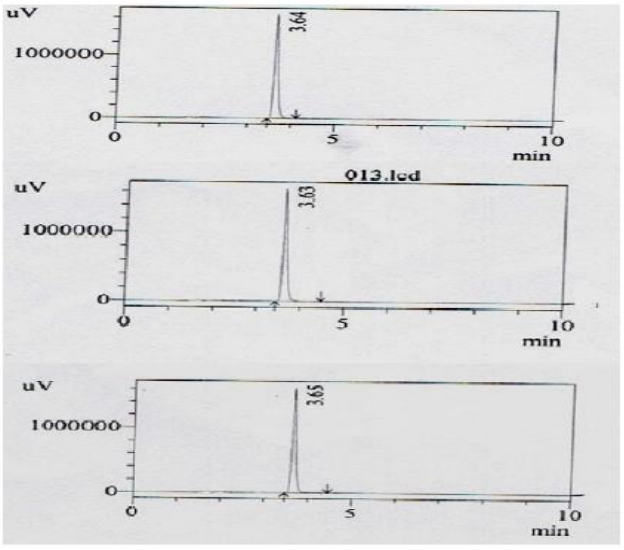


**Figure: 7 Precision Injection Chromatogram a. Trial-4; b. Trial-5; c. Trial-6**

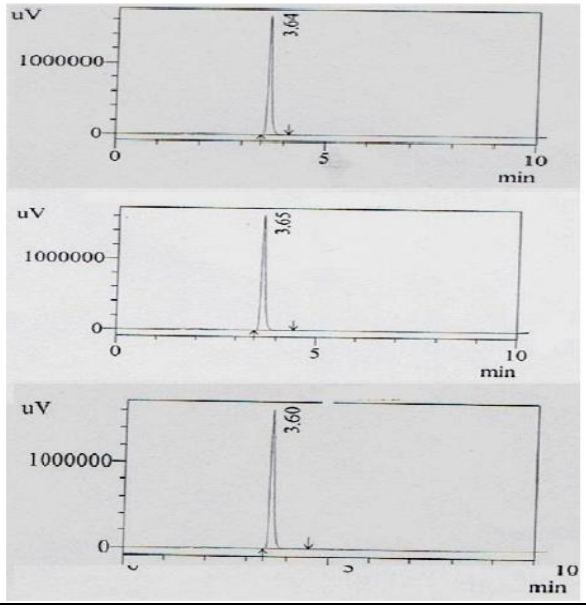
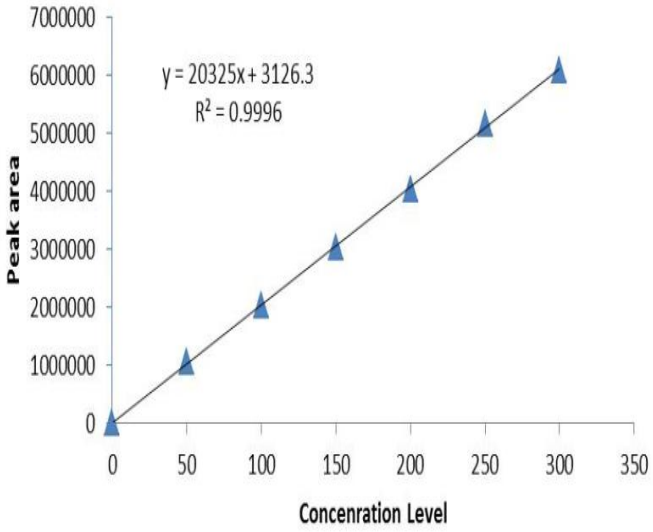



**Figure: 8 Linearity curve graph for Fosamprenavir calcium**




## DISCUSSION

The RP-HPLC method developed for the estimation of Fosamprenavir calcium in pharmaceutical dosage forms demonstrates robust performance across several critical parameters, as summarized in Table 9.1. The chromatographic separation was achieved using an Agilent Zorbax SB-C18 column (4.6x250mm, 5µm), with a mobile phase consisting of a mixture of methanol (MeOH), acetonitrile (ACN), and water (H2O) in the ratio of 40:40:20/v. The flow rate was maintained at 1.0 ml/min, and detection was performed at a wavelength of 249 nm. The system suitability test results are well within the acceptance criteria, indicating the method's reliability and efficiency. The theoretical plates, a measure of column efficiency, were found to be 6341, significantly exceeding the minimum requirement of 3000. This high number of theoretical plates signifies excellent column performance and efficient separation. The tailing factor, which assesses the symmetry of the chromatographic peak, was 1.78, within the acceptable limit of not more than 2. This indicates minimal peak tailing and good peak symmetry. The retention time for Fosamprenavir calcium was recorded at 3.13 minutes, demonstrating the method's suitability for rapid analysis. The accuracy of the method, determined by the percentage recovery of Fosamprenavir calcium, was 100.6%. This value is well within the acceptance range of 98-102%, indicating that the method is highly accurate and capable of recovering the analyte quantitatively from the dosage form without interference from excipients. The precision of the method was evaluated by calculating the relative standard deviation (%RSD) of the analyte. The %RSD for Atazanavir sulfate was found to be 0.93%, which is below the acceptance criterion of less than 2%. This low %RSD indicates that the method is highly precise, producing consistent and reproducible results upon repeated analysis. The linearity of the method was assessed by the correlation coefficient ($R^2$), which was found to be 0.999. This value meets the criterion of not more than 0.999, demonstrating a strong linear relationship between the concentration of Fosamprenavir calcium and the corresponding peak area. This confirms that the method can accurately quantify the analyte over a specified range. The limit of detection (LOD) for Fosamprenavir calcium was determined to be 0.507 µg/ml, and the limit of quantification (LOQ) was 1.538 µg/ml. These values indicate the method's sensitivity, with the LOD representing the lowest amount of analyte that can be detected and the LOQ representing the lowest amount that can be quantitatively determined with acceptable precision and accuracy.

## CONCLUSION

The developed HPLC method proved to be precise, specific, accurate, rapid, and cost-effective for estimating Fosamprenavir calcium in oral suspension dosage forms. The recovery rates of the samples across all formulations were consistent with their respective label claims, indicating the method's reliability. This method is well-suited for routine laboratory analysis and quality control of raw materials, formulations, and dissolution studies. Additionally, it can be effectively employed in bioequivalence studies for Fosamprenavir calcium formulations.

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