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ESTIMATION OF FOSTEMSAVIR USING A NOVEL HPLC ANALYTICAL METHOD

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ABSTRACT: Fostemsavir was quantified using a simple and reliable HPLC technique developed and validated. PhenomenexC18 column was used to achieve chromatographic segregation (150 x 4.6 mm, 5m). The mobile phase was a 55:45 mixture of 0.01N disodium orthophosphate and acetonitrile pumped through the column at 1.0ml/min. The temperature was maintained at 30 °C and the buffer used was 0.01N sodium dihydrogen phosphate. The wavelength of the ideal choice was 278.0nm. Fostemsavir's retention time was discovered to be 2.471 minutes. Fostemsavir's percent RSD was found to be 0.7 percent. For Fostemsavir, the percentage recovery was 100.07 percent. Fostemsavir's regression equation yielded LOD and LOQ values of 0.18 and 0.54, respectively. Fostemsavir's regression equation is y = 26381x + 8398.8. Since run time and retention time were minimized, the system developed was simple and cost-effective, and it could be used on a daily basis for quality control.

INTRODUCTION: Fostemsavir is chemically known as ({3 - [2 - (4 - benzoylpiperazin-1-yl) - 2-oxoacetyl] – 4 – methoxy – 7 - (3 – methyl - 1H-1, 2, 4-triazol-1-yl) - 1H-pyrrolo [2, 3-c]pyridin-1-yl}methoxy) phosphonic acid **Fig. 1** with molecular formula ($C_{25}H_{26}N_7O_8P$) and molecular weight 583.498g/mol. It is soluble to more than 250 mg/mL in aqueous solutions with a pH higher than 3.7. In terms of physical state, it is solid. Rukobia is the brand name for fostemsavir. The phosphono-oxymethyl prodrug of temsavir, a novel HIV-1 attachment inhibitor, is fostemsavir. It is an HIV-1 antiretroviral therapy. The active moiety of fostemsavir is temsavir, which is a first-in-class HIV-1 attachment inhibitor that binds to the viral envelope glycoprotein 120.

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In HIV-1–infected persons, long-term antiretroviral treatment, the longer life expectancy that comes with it and/or some coinfections may increase the risk of chronic liver and kidney disease [1]. Fostemsavir is a prodrug of temsavir, a first-in-class HIV-1 attachment inhibitor that binds to gp120 and blocks attachment to the CD4 receptor on the target cell, preventing entry and infection. Fostemsavir is a novel temsavir prodrug that prevents gp120 from binding to CD4 receptors on host cells, preventing infection while leaving host cells unharmed [2]. The chemical structure of Fostemsavir is depicted in **Fig. 1.**

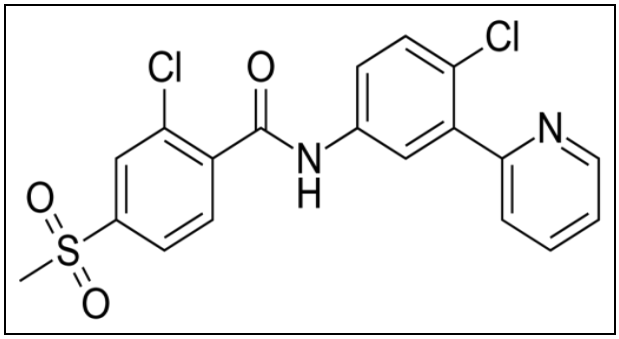

FIG. 1: STRUCTURE OF FOSTEMSAVIR

MATERIALS AND METHODS: Acetonitrile, Methanol, Phosphate buffer, Ortho-phosphoric acid, Potassium dihydrogen orthophosphate buffer, Distilled water, Fostemsavir pure drug (API), Fostemsavir tablets (Rukobia). Rankem laboratories supplied all of the chemicals and solvents listed above.

Chromatographic Conditions: For chromato-graphic separation, a WATERS HPLC 2695 SYSTEM with quaternary pumps and an Autosampler was used. Empower 2 Software was used to integrate a Photo Diode Array detector. The diluent was chosen based on the drugs' solubility.

A 50:50 mixture of water and methanol was used. The absorbances of Fostemsavir solution were measured using a UV-VIS spectrophotometer PG Instruments T60 with a special bandwidth of 2mm and 10mm and matched quartz cells, as well as UV win 6 Software and a UV-VIS spectrophotometer PG Instruments T60 with a special bandwidth of 2mm and 10mm and matched quartz cells [3].

A pH meter was used to determine the pH of the solutions (BVK enterprises, India). A BVK enterprises ultrasonicator was used. On an electronic balance, all analytical measurements were made (Denver).

Standard stock solutions' preparation: 30mg of Fostemsavir was measured correctly and transferred to a 50ml volumetric flask. The flask was then sonicated for 10 minutes with three-quarters of the diluents applied. Normal stock solution (600g/ml of Fostemsavir) was then made up with diluents and labeled [4].

Preparation of Standard Working Solutions (100% Solution): 1 mL of each stock solution was pipetted into a 10 mL volumetric flask, which was then filled with diluent (60 g/mL Fostemsavir).

Preparation of Sample Stock Solutions: After weighing five tablets, the average weight of each was determined. One tablet's weight was put in a 100ml volumetric flask, along with 50ml of diluent, and the flask was sonicated for 25 min.

After that, the amount was diluted with diluent and purified through HPLC filters (600g/ml Fostemsavir).

Preparation of Sample Working Solutions (100% Solution): A 10ml volumetric flask was filled with 0.1ml of diluted sample stock solution and diluent (60g/ml of Fostemsavir).

Preparation of Buffer: 0.01N KH_2PO_4 Buffer: In a 1000ml volumetric flask, 1.36gm potassium dihydrogen Orthophosphate was correctly measured, and 900ml milli-Q water was added degassed to sonicate until the amount was made up with water to change the pH to 5.4 with dilute Orthophosphoric acid solution.

0.1%OPA Buffer: HPLC grade water was used to dilute 1ml of orthophosphoric acid to 1000ml. 0.1% formic acid buffer: HPLC grade water was used to dilute 1ml of formic acid solution to 1000ml. 0.01N NA2HPO4 buffer.

In a 1000ml volumetric flask, 1.42gm of disodium phosphate or sodium hydrogen phosphate was precisely measured, and 900ml milli-Q water was added and degassed to sonicate. Water was used to make up the volume.

Method Validation: As mentioned in the ICH guidelines, the established method was validated for linearity, accuracy, precision, robustness, the limit of detection, and system suitability parameters [5].

Linearity: From the stock solution, chromatograms of six linear concentrations were made 15, 30, 45, 60, 75, and 90 g/ml.

The calibration curve was constructed using the mean peak areas at their respective concentrations, and the individual mean peak areas were determined from chromatograms [6].

The drug's correlation coefficient was found to be 0.999. The linearity data for fostemsavir were tabulated in **Table 1,** and a calibration plot was shown in **Fig. 2.**

TABLE 1: LINEARITY TABLE FOR FOSTEMSAVIR

Conc (µg/mL)	Peak area
15	394023
30	821034
45	1190162
60	1593367
75	2014985
90	2355269

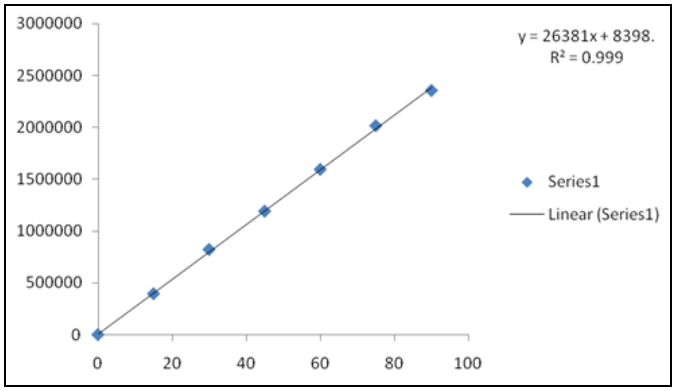

FIG. 2: CALIBRATION CURVE OF FOSTEMSAVIR

Accuracy: The method's accuracy was determined by calculating the percentage recovery of Fostemsavir using the standard addition method (standard stock solution spiked into the placebo). Each stage should have a percentage recovery of 98.0 to 102 [7]. We calculated the standard deviation, average area, and percent RSD. Fostemsavir's percent RSD was found to be 0.3 percent. The precision of the device passed this process because the precision limit was less than "2." The findings of the recovery studies were shown in **Table 2.**

TABLE 2: ACCURACY TABLE OF FOSTEMSAVIR

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	30	29.99	99.95	100.07%
	30	30.01	100.04	
	30	30.20	100.66	
100%	60	59.16	98.60	
	60	60.01	100.01	
	60	59.23	98.71	
150%	90	90.52	100.58	
	90	91.25	101.39	
	90	90.58	100.64	

Precision: Six replicates of samples with concentrations of 15, 30, 45, 60, 75, and 90g/ml were analyzed on the same day and on six separate days, respectively, to determine intraday and interday precision [8]. Fostemsavir had a 0.3 percent RSD. Since the Precision limit was less than "2," the system precision passed. The results of intra-day and inter-day results were discussed **Table 3.**

TABLE 3: PRESCISION STUDY OF FOSTEMSAVIR

Concentration	Intraday	Interday Study	Repeatibility
Mean	1537016	1592509	1544819
S.D	4690.7	11437.9	10896.7
%RSD	0.3	0.7	0.7

Repeatability: Six working sample solutions with the same concentrations were prepared and each injection from each working sample solution was given and obtained [9].

The percent RSD, average area, and standard deviation of fostemsavir were calculated and found to be 0.7 percent. Since the precision limit was less than "2," the system precision passed this test.

LOD and LOQ: The method's limit of detection and quantitation were calculated to be 0.18 and 0.54, respectively. The chromatogram of the standard's LOD and LOQ was shown in **Fig. 3** and **4.**

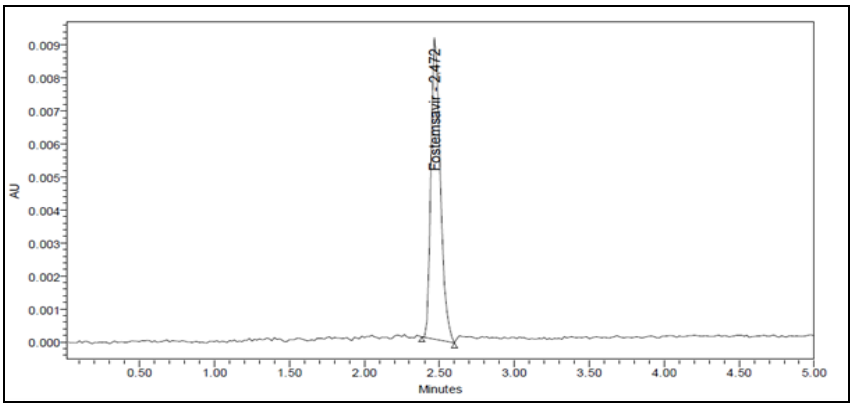

FIG. 3: LOD CHROMATOGRAM OF STANDARD

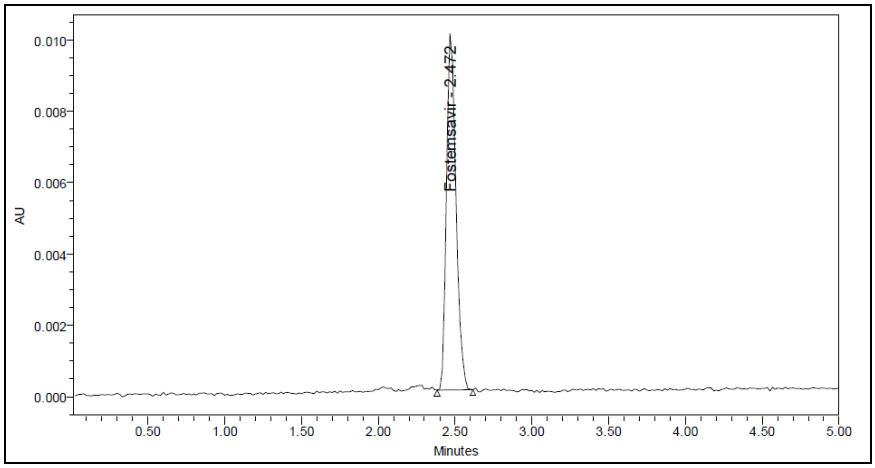

FIG. 4: LOQ CHROMATOGRAM OF STANDARD

Robustness: The temperature, mobile phase composition, flow rate were all adjusted slightly from the optimized chromatographic conditions to achieve robustness. Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (60B:40A), mobile phase plus (50B:50A), temperature minus (25°C), and temperature plus (35°C) were all preserved and injected in a replica manner. The suitability parameters of the device were not greatly affected, and all of the parameters passed the experiment. The percent RSD was within the appropriate range. The outcomes were reported in **Table 4.**

TABLE 4: ROBUSTNESS STUDY OF FOSTEMSAVIR

S. no.	Condition	% RSD of Fostemsavir
1	Flow rate (-) 0.9ml/min	0.5
2	Flow rate (+) 1.1ml/min	1.7
3	Mobile phase (-) 60B:40A	0.2
4	Mobile phase (+) 50B:50A	0.6
5	Temperature (-) 25°C	1.6
6	Temperature (+) 35°C	0.5

Specificity: Specificity refers to an analytical technique's ability to ensure that the target peak(s) elute as distinct responses even when excipients, impurities, or degradation compounds are present. Specificity was calculated using the drug's resolution factor, the peak from the closest resolving peak and all other peaks [10]. A placebo (without Fostemsavir) was made to assess process specificity with the excipients needed for commercial production and compared to the appropriate drug standard. The placebo and standard chromatograms were compared for retention time, resolution factor, and purity. The chromatograms were shown in order **Fig. 5** and **6.**

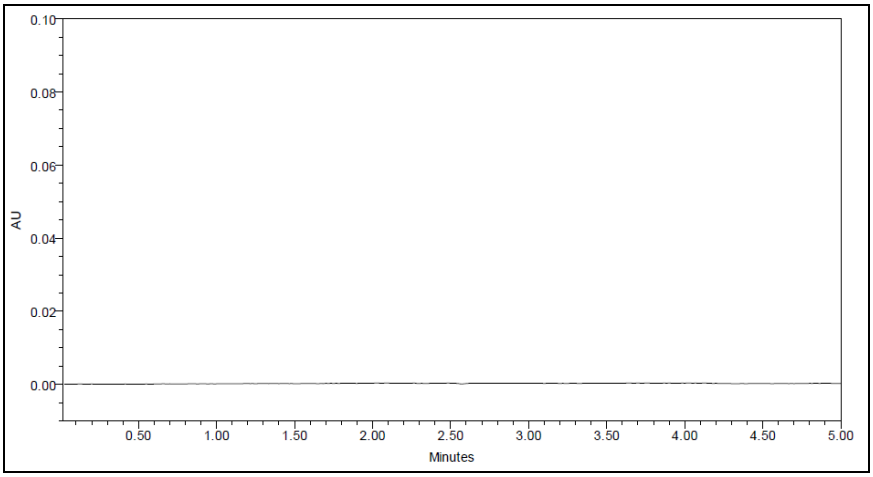

FIG. 5: CHROMATOGRAM OF BLANK

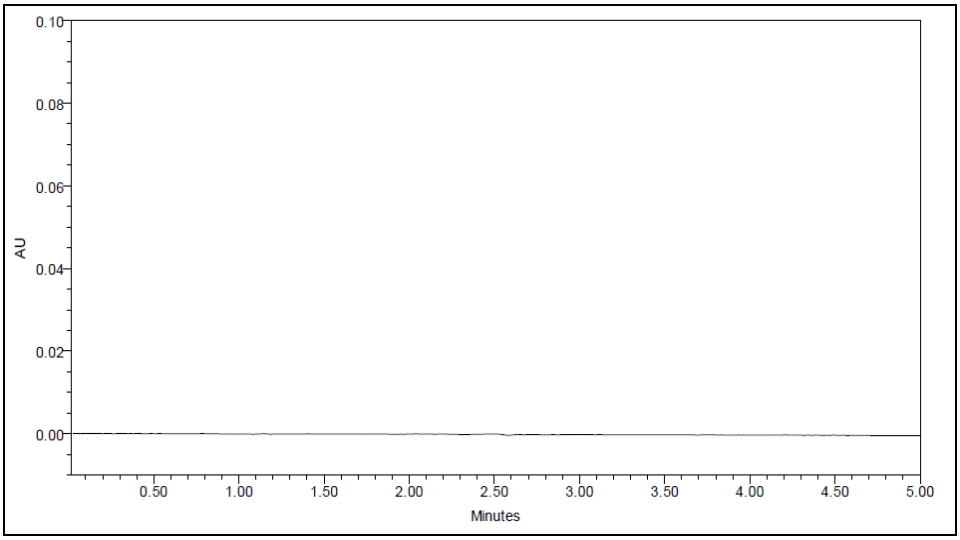

FIG. 6: CHROMATOGRAM OF PLACEBO

System Suitability Parameters: A device suitability test (SST) was conducted to ensure the validity of the analytical technique used. For the device suitability test, percent relative standard deviation (percent RSD) of the region, RSD of retention time (RT), theoretical plates, USP tailing factor, and resolution were chosen [11]. Six times a regular solution was injected to evaluate the parameters. The system suitability findings were summarised in **Table 5,** and the chromatogram was optimized in **Fig. 7.**

TABLE 5: OPTIMIZED CHROMATOGRAPHIC CONDITION OF FOSTEMSAVIR

Mobile phase	**55% OPA: 45% Na_2HPO_4**
Flow rate	1.0ml/min
Column	Phenomenex150 (4.6 x 150mm, 5µm)
Detector wavelength	278nm
Column temperature	30°C
Injection volume	10µL
Run time	6 min
Results	Fostemsavir peak has good resolution, tailing factor, theoretical plate count, and resolution

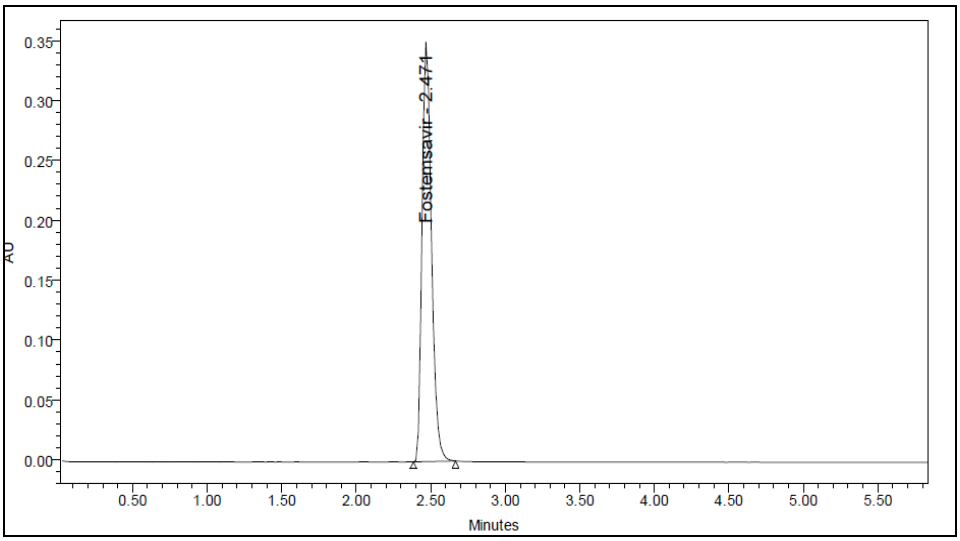

FIG. 7: OPTIMIZED CHROMATOGRAM

Assay: Fostemsavir 600mg is claimed on the label of Rukobia. The above formulation was used for the assay. Fostemsavir had an average percent assay of 100.21 percent. The assay conditions are shown in the diagram **Table 6.**

TABLE 6: ASSAY DATA OF FOSTEMSAVIR

S. no.	Standard Area	Sample area	% Assay
1	1532199	1550208	100.56
2	1532994	1543134	100.10
3	1542937	1531787	99.36
4	1535640	1551910	100.67
5	1535665	1558959	101.12
6	1542660	1532913	99.43
Avg	1537016	1544819	100.21
Stdev	4690.7	10896.7	0.707
%RSD	0.3	0.7	0.71

Forced Degradation Studies: 1 ml of Fostemsavir stock solution was subjected to acidic, alkaline, neutral, oxidising, thermal, and photolytic conditions to conduct the forced degradation studies. For acidic degradation, 1 mL of Fostemsavir stock solution was combined with 1 mL of 2N Hydrochloric acid and refluxed for 30 minutes at 600°C. The resulting solution was diluted to make a 60g/ml solution, which was then injected into the system in a 10µl solution. For alkaline degradation, 1 ml of Fostemsavir stock solution was mixed with 1 ml of 2N sodium hydroxide and refluxed at 600°C for 30 min.

The resulting solution was diluted to make a 60g/ml solution, which was then injected into the system in a 10µl dose. For alkaline degradation, 1 ml of Fostemsavir stock solution was mixed with 1 ml of 2N sodium hydroxide and refluxed at 600°C for 30 minutes. The resulting solution was diluted to a 60g/ml concentration before being injected into the system in 10µl. The drug was neutralized by soaking it in water for 6 h at 60 degrees Fahrenheit. The resulting solution was diluted to a 60g/ml solution for HPLC testing, and 10µl were injected into the device.

For thermal degradation, the standard drug solution was placed in an oven at 105°C for 6h to study dry heat degradation. The regular drug solution was dried heat deteriorated in an oven at 105°C for 6 hours. 1 ml of 20 percent hydrogen peroxide (H_2O_2) was added separately to 1 ml of stock solution of Fostemsavir for oxidation. At 600°C for 30 minutes, the solution was held. The resulting solution was diluted to produce a 60g/ml solution for HPLC testing, and 10l were injected into the device. The drug was also investigated for photostability studies by exposing the 600g/ml solution to UV light for 7 days or 200 Watt-hours/m2 in the photostability chamber. The above-mentioned solutions were then placed under various stress conditions to see if any peaks emerged from the deteriorated excipients. The formulation was subjected to degradation tests. After that, the degraded samples were injected. For injected samples, the assay was determined, and all of the samples passed the degradation limits. **Fig. 8-13** shows typical chromatograms of Fostemsavir degradation activity under various stress conditions and degradation results **Table 7.**

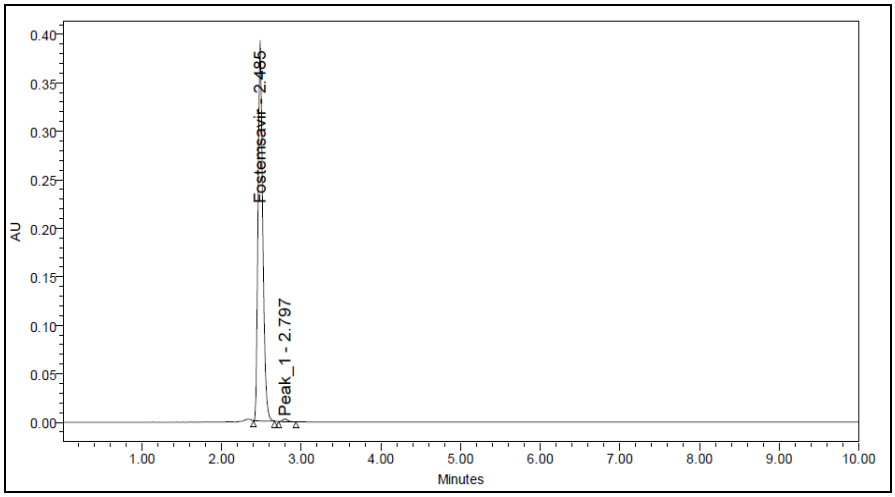

FIG. 8: ACID CHROMATOGRAM OF FOSTEMSAVIR

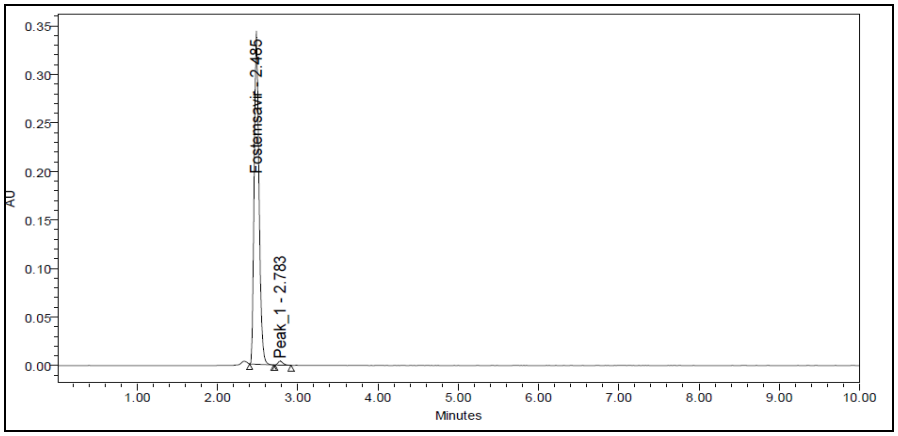

FIG. 9: BASE CHROMATOGRAM OF FOSTEMSAVIR

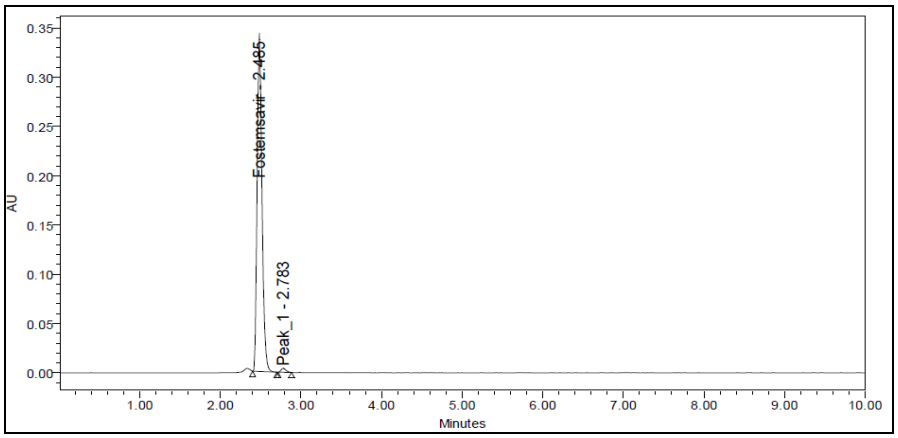

FIG. 10: PEROXIDE CHROMATOGRAM OF FOSTEMSAVIR

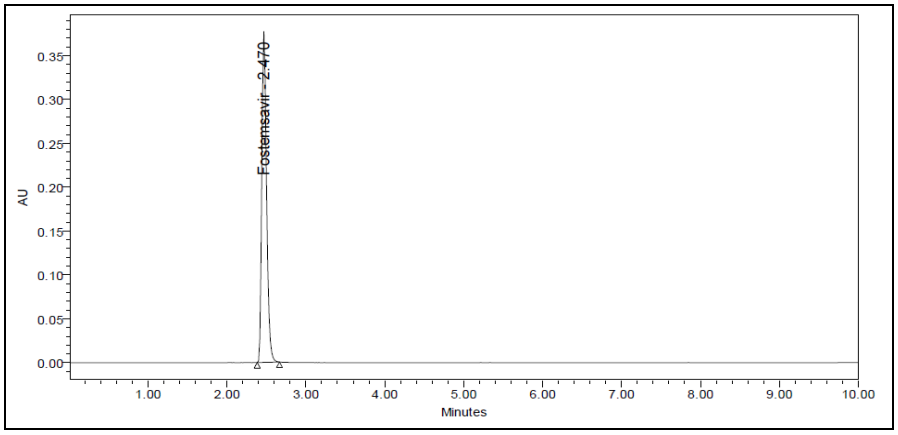

FIG. 11: THERMAL CHROMATOGRAM OF FOSTEMSAVIR

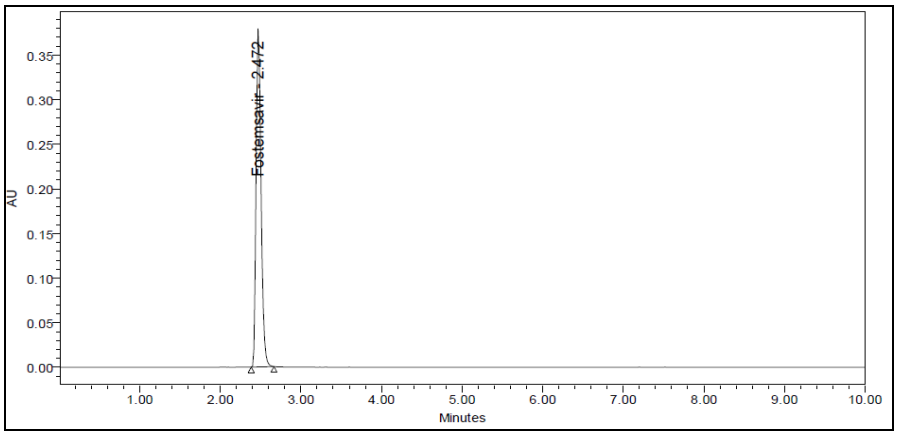

FIG. 12: UV CHROMATOGRAM OF FOSTEMSAVIR

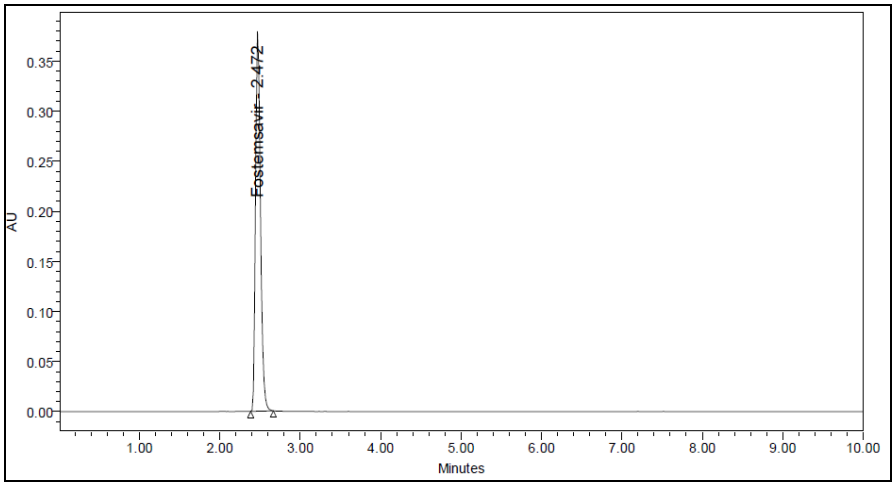

FIG. 13: WATER CHROMATOGRAM OF FOSTEMSAVIR

TABLE 7: DEGRADATION DATA OF FOSTEMSAVIR

S. no.	Degradation Condition	% Drug UnDegraded	% Drug Degraded
1	Acid	94.43	5.57
2	Alkali	94.97	5.03
3	Oxidation	95.50	4.50
4	Thermal	97.46	2.54
5	UV	98.07	1.93
6	Water	98.96	1.04

RESULTS AND DISCUSSIONS: The retention time of fostemsavir was found to be 2.471 min. The RSD for Fostemsavir was found to be 0.3 percent, and the RSD for Fostemsavir was found to be 0.7 percent. Fostemsavir had a 100.07 percent recovery rate. Fostemsavir's regression equation yielded LOD and LOQ values of 0.18 and 0.54, respectively. The regression equation for Fostemsavir is y = 26381x + 8398.8. The system developed was easy and cost-effective, and it could be used in routine quality control tests in industries because the retention time and run time were reduced. The summary was illustrated in **Table 8.**

TABLE 8: SUMMARY

Parameters	Fostemsavir		LIMIT
Linearity Range (µg/ml)	15-90µg/ml		
Regression coefficient	0.999		
Slope(m)	26381		
Intercept(c)	8398.8		R< 1
Regression equation (Y=mx+c)	y = 26381x + 8398.8.		
Assay (% mean assay)	100.21%		90-110%
Specificity	Specific		No interference of any peak
System precision %RSD	0.3		NMT 2.0%
Method precision %RSD	0.7		NMT 2.0%
Accuracy %recovery	100.07%		98-102%
LOD	0.18		NMT 3
LOQ	0.54		NMT 10
Robustness	FM	0.5	%RSD NMT 2.0
	FP	1.7	
	MM	0.2	
	MP	0.6	
	TM	1.6	
	TP	0.5	

CONCLUSION: We developed a novel HPLC analytical method for determining Fostemsavir, which has been validated for linearity, accuracy, precision, robustness, LOD, LOQ, and device suitability. After considering all of the validation criteria, we came to the conclusion that the method

was accurate, linear, effective, robust, and fast for determining Fostemsavir. As a consequence, this method can be successfully used to estimate Fostemsavir.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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