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DEVELOPMENT AND VALIDATION OF FOSTEMSAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV SPECTROPHOTOMETRIC METHOD

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ABSTRACT

The objective of this study is to develop a novel, rapid and sensitive UV spectrophotometeric technique for the determination of Fostemsavir in bulk and pharmaceutical dosage form . The solvent used was phosphate buffer pH 6.8 and the maximum absorbance was recorded at 278 nm. Analytical calibration curves were linear within a concentration range of 7.5 to 45µg/ml and coefficient of correlation 0.999. The percentage RSD was found to be less than 2. The result of analysis has been validated statistically. This method was validated in accordance with ICH requirements which included the parameters like linearity, accuracy ,precision, sensitivity, specificity and robustness. Hence the proposed method can be used for the reliable quantification of Fostemsavir in bulk and tablet formulation. The present outcome of research summarizes that the method is suitable for routine analysis for the determination of Fostemsavir.

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INTRODUCTION[1,2]

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 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. Fostemsavir is the phosphonooxymethyl prodrug of temsavir, a novel HIV-1 attachment inhibitor.It binds to and inhibits the activity of gp120, a subunit within the HIV-1, gp160 envelope glycoprotein that facilitates the attachment of HIV-1 to host cell CD4 receptors - in doing so, temsavir prevents the first step in the HIV-1 viral lifecycle. Fostemsavir is indicated, in combination with other antiretrovirals, for the treatment of multidrug-resistant HIV-1 infection in heavy treatment-experienced adults failing their current antiretroviral therapy due to resistance, intolerance, or safety concerns.Fostemavir is the first attachment inhibitor to receive FDA approval, granted in July 2020 for use in combination with other antiretrovirals in highly treatment-experienced patients with multidrug-resistant HIV-1 infection who are failing their current therapy.The chemical structure of Fostemsavir is shown in (Figure1). The aim of this study is to develop a novel, rapid and sensitive UV spectrophotometeric technique for the determination of Fostemsavir in bulk and pharmaceutical dosage form .

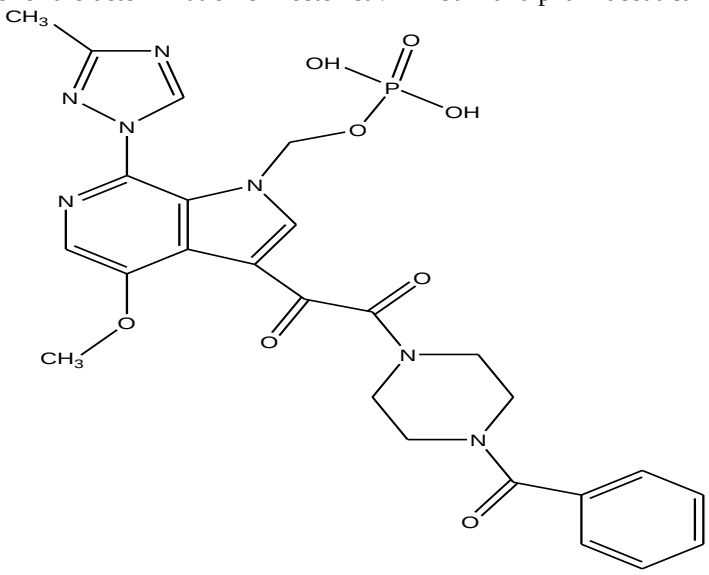

Figure no. 1: Structure of Fostemsavir.

MATERIALS AND METHODS

Chemicals and reagents

Fostemsavir was obtained as a gift sample from Spectrum lab, Hyderabad. All the chemicals used were of analytical grade.

Instrumentation

The instrument used for the study was UV 1800 double beam UV Visible Spectrophotometer with a pair of 10 mm path length matched quartz cells. The analysis was carried out by using UV solutions 2.42 software.

Stock solution preparation

30mg of Fostemsavir was dissolved in Water : Ethanol (60:40v/v) in a 100ml volumetric flask (1000 µg/ mL).

Working standard preparation

By diluting the stock solution with Phosphate buffer pH 6.8, a working standard solution (100 g/ mL) was prepared.

Method Validation[3]

Validation of the method was performed out as per the ICH guidelines Q2 (R1) (ICH, 2005) and the parameters were evaluated accordingly:

Linearity[4]

In a series of 10 ml volumetric flasks, different aliquots of fostemsvir in the range of 7.5–45 ml were transferred. A spectrophotometer was used to scan the solutions in the UV range of 200–400 nm. At 278 nm, the spectrum was recorded. The linearity was determined by plotting concentration against corresponding absorbance.The regression equation (y=0.010x+0.007) were determined after plotting absorbance versus concentration to create the calibration curves.

Preparation of calibration curve

From this stock solution, appropriate dilutions were mad to get working standard in the range of 7.5-45 µg/ml and absorbance was taken at λmax 278 nm . Averages of such 6 sets of values were taken for standard calibration curve. illustrates overlay spectrum of fostemsavir at different concentrations.

Accuracy[5]

The accuracy of the planned UV method was evaluated using recovery studies after standard addition of analyte of interest. Three different solutions of Fostemsavir were prepared in triplicate at level of 50%, 100% and 150% of its predefined concentration (15, 30, 45 µg/mL) and accuracy was determined based on percent recovery.

Precision[6]

The precision of an analytical method refers to the degree of agreement (degree of scatter) between a set of measurements obtained from different samplings of the same homogeneous sample under the same set of conditions.Intraday precision was determined by analyzing 15, 30 and 45 µg/ml of Fostemsavir solutions for three times in the same day. Interday precision was determined by analyzing 15, 30 and 45 µg/ml of Fostemsavir solutions daily for 3 days over the period of week.

Specificity[7]

The ability of a system to accurately quantify a compound in the presence of other components including impurities, degradation products, and matrix components is referred to as specificity. The proposed method's specificity was tested by analysing a placebo solution made with pharmaceutical formulation excipients. Thus, the inert component mixture was prepared in their normal tablet concentrations, and the method was used to see whether any component of the formulation could produce a response or a read with an absorption band close to the drug.

Senstivity [8]

The Limit of Detection (LOD) of the UV method developed was calculated by using following formula:

$$\mathbf{LOD=3.3\times SD/S}$$

Where, SD= Standard deviation of Y-intercept S= Slope.

The Limit of Quantiation (LOQ) of the UV method developed was calculated by using following formula:

$$\mathbf{LOQ= 10\times SD/S}$$

Where, SD= Standard deviation of Y-intercept S= Slope

Robustness [9]

The method's robustness was tested by calculating absorbance at two different wavelengths. The percentage RSD, which is less than 2%, was used to represent the result. The results for robustness is shown in (Table 6).

Assay [10]

The average weight was calculated after powdering twenty tablets (RUKOBIA 600 mg). The concentration of Fostemsavir was measured at 278 nm after a quantity equivalent to 10 mg of drug was dissolved in Phosphate buffer pH 6.8 and volume was made up to get a working concentration of 30µg/ml each.

RESULTS AND DISCUSSIONS:

Wavelength Selection For Fostemsavir:

The preestabished wavelength of maximum absorption (λmax) was 289 nm. The overlain spectrum for Fostemsavir was shown in (Figure 2).

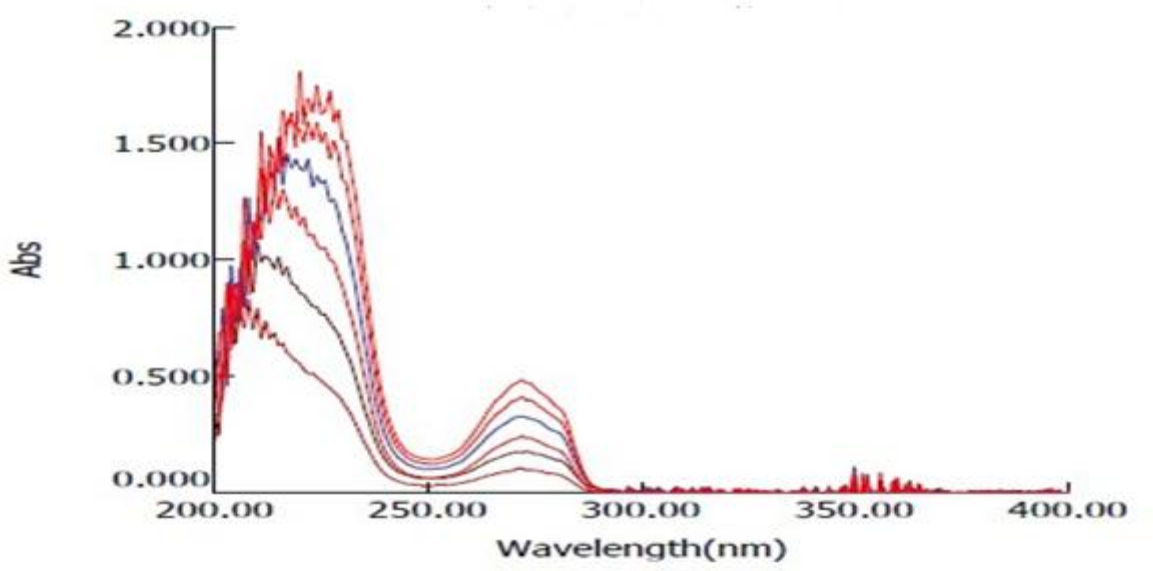

Figure no. 2: UV-visible spectrum.

Linearity

The linearity data for fostemsavir was tabulated in (Table 1), and a calibration plot was shown in (Figure 3).

Table No. 1: Linearity Study.

S.No.	Concentration(µg/ml)	Absorbance
1.	7.5	0.096
2.	15	0.164
3.	22.5	0.247
4.	30	0.324
5.	37.5	0.407
6.	45	0.479

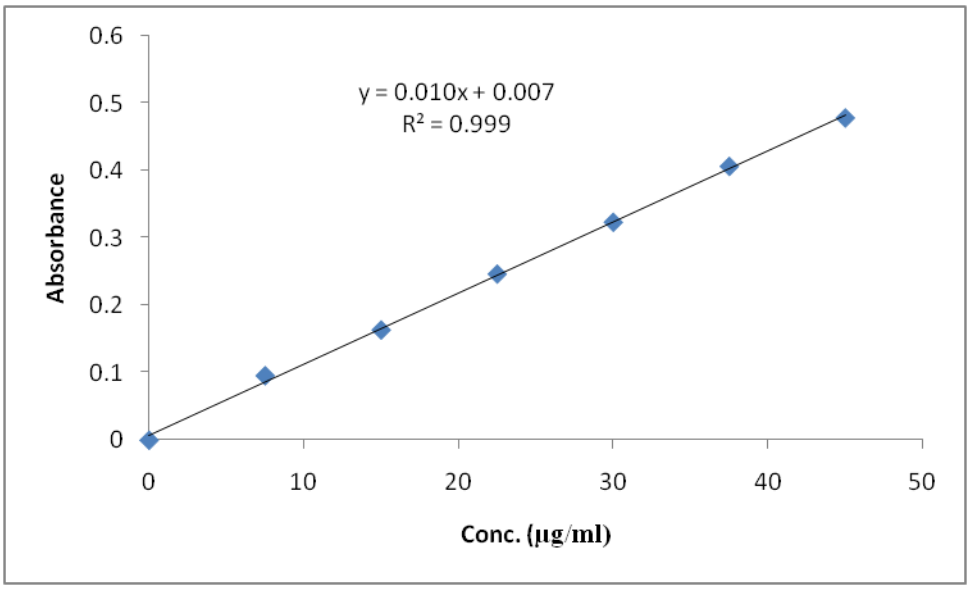

Figure no. 3: Calibration curve of Fostemsavir.

Accuracy

The findings of the recovery studies were shown in (Table 2).

Table No. 2: Accuracy Studies.

Spiked Conc (µg /mL)	Formulation (µg /mL)	Absorbance	Conc found (µg/ mL)	Recovery (%)	*Mean ± SD, %RSD
	10	0.161	15.4	102.66	
(15)50%	10	0.160	15.3	102	102.44±0.381, 0.37
	10	0.161	15.4	102.66	
	10	0.305	29.9	99.33	
(30)100%	10	0.306	29.8	99.66	99.44±0.190, 0.19
	10	0.305	29.9	99.33	
	10	0.470	46.3	102.88	
(45)150%	10	0.469	46.2	102.66	102.80 ±0.127, 0.123
	10	0.470	46.3	102.88	

*Mean determinations at each level.

Percent recovery for Fostemsavir was found in the range of 99.44 % to 102.8 % which is within the limits (98%-102%) with standard deviation well below 2 indicating accuracy of the method.

Presicion

The results of intra-day and inter-day results were discussed in (Table 3-4).

Table No. 3: Results of Inter-day precision.

Conc.(µg/ml)	Amount found (µg/ml)	*Mean± SD,RSD(%)
15	15.8	106.33±0.33, 0.310
30	31.9	105.33±0.67, 0.636
45	47.1	104.66±0.22, 0.210

Table No. 4: Results of Inter-day precision.

Conc.(µg/ml)	Amount found (µg/ml)	*Mean± SD,RSD(%)
15	15.7	106.66±0.33, 0.314
30	32	104.66±0.66, 0.635
45	47.06	104.58±0.33, 0.321

*Mean determinations at each level

Percentage of RSD for intra-day and inter-day precision studies for Fostemsavir was well within the acceptable range (< 2 %) indicating that the method has excellent repeatability and reproducibility.

Specificity

The results for specificity studies were shown in (Table 5).

Table No. 5 : Specificity Studies.

Test results in the absence of excipient (TA)		Test results in the presence of excipients (TP)		Percentage agreement	Statistical Analysis
Absorbane	Conc.(µg/ml)	Absorbance	Conc.µg/ml)		
0.325	31.8	0.327	32	100.62	Mean= 101.256
0.326	31.9	0.33	32.3	101.25	S.D. = 0.391957
					%RSD =
0.324	31.7	0.329	32.2	101.58	0.387095
0.323	31.6	0.328	32.1	101.58	
0.327	32	0.331	32.4	101.25	

Senstivity

The linearity equation was found to be Y = 0.010X + 0.007. The LOD and LOQ for Fostemsavir were found to be 0.33 µg and 1µg, respectively.

Robustness

The results for robustness were observed in (Table 6).

Table no. 6 : Results for Robustness.

S.No.	276 nm	280 nm
1.	0.322	0.326
2.	0.321	0.321
3.	0.323	0.322
Mean	0.322	0.323
S.D.	0.001	0.002
%RSD	0.3105	0.8191

Assay

The assay results obtained are listed in (Table 7).

Table no. 7 : Analysis of Formulation.

Brand name	Available form	Label claim (mg)	Amount found (mg)	Assay
RUKOBIA	Tablet	600	599.67	98.73

CONCLUSION

The method was successfully validated as per ICH guidelines and can be conveniently employed for routine quality control analysis.The analytical method developed on UV- Visible Spectrophotometer was simple, reliable, accurate and reproducible for the estimation of Fostemsavir in bulk and pharmaceutical dosage forms. Hence present study gives excellent method for determination of Fostemsavir using UV-Vis Spectroscopy. This method is acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries and approved testing laboratories and it is recommended for future research.

ABBREVIATIONS

UV : Ultra Violet
UV-Vis : UltraViolet-Visible
RSD : Relative Standard Deviation
ICH : International Council for Harmonisation
HIV : Human Immunodeficiency Viruses
FDA : Food and Drug Administration
µm : micrometer
nm : nanometer
ml : millilitre
% : Percentage

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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