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Stability indicating RP-HPLC method development and validation of foscarnet in bulk and pharmaceutical dosage form

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ABSTRACT

Foscarnet, phosphonoformic acid, is an antiviral drug used to treat herpes viruses. It is used to treat in HIV rescue therapy. For this drug we developed a reverse-phase high performance liquid chromatographic technique and valid in keeping with the ICH pointers. For this purpose ODS C_{18} (4.6 x 150mm, 5µm) column was used. As a mobile part, a combination of Phosphate buffer, Acetonitrile and Water with a pH 4.0 were used which was adjusted with orthophosphoric acid to the quantitative relation of 40:60%v/v. ultraviolet detection was done at 225nm. Stress degradation conditions were established for Foscarnet by subjecting it to acid, base, oxidation, UV, water and thermal stress. The stress samples were assayed and the mass balance was compared against a qualified reference standard. The developed technique was valid as per ICH norms. The established technique was with success used for the regular analysis of commercially accessible indefinite quantity kind. The flow rate was 1.1 millilitre min^{-1} and therefore the retention time was 3.885 min. The activity curve was found to be linear upon the concentration ranges of 0.25-1.5ppm. The LOQ and LOD values were found to be 3.98 and 1.32. The proportion of recovery and low constant of variance confirms that the tactic is appropriate for the estimation of Foscarnet drug in pharmaceutical indefinite quantity kind.

Key words: Foscarnet, RP-HPLC, Phosphate buffer and Acetonitrile.

INTRODUCTION

Foscarnet chemically phosphonoformic acid and is shown in figure-1.1. [1-5] It is an antiviral drug used to treat herpes viruses. It can be used to treat in HIV rescue therapy. Foscarnet is selectively inhibits the pyrophosphate binding site on viral DNA polymerases. [6-9]

Literature survey reveals that only a few strategies are out there for its analysis. [10] Therefore our commit to establish a new technique for analysis in pharmaceutical dose forms, when an in depth study, set to develop a new RP-HPLC technique and its validation consistent with ICH norms.[11-13] For the determination of this technique we tend to used Phosphate buffer and Acetonitrile with a pH scale 4.0 adjusted with ortho phosphoric acid within the magnitude relation of 40:60v/v. The column ODS C18 was used, at a flow of 1.1 ml/min. and ultraviolet detection done at 225nm.

MATERIALS AND METHODS

2.1. Chromatographic parameters and Apparatus:

A Waters HPLC with Auto sampler, Empower 2.0 software, ODS C_{18} column and UV detector was used in the study. A Hecht Assistent pH meter, Afcoset digital balance and ambient column oven were the other instruments used for this study.

2.2. Drug samples:
The Foscarnet drug used for estimation for this study was procured from injection. The label claim was 24mg/ml.

2.3. Reagents and solutions:
HPLC grade Acetonitrile, a GR grade/Merck potassium di hydrogen phosphate, HPLC grade water and Foscarnet drug was utilized in the study. a combination of potassium di hydrogen ortho phosphate buffer and Acetonitrile in the quantitative relation of 40:60%v/v was used as a mobile section at a pH 4.0 adjusted with Ortho phosphoric acid and also used as a diluent for getting ready the working solution of drug. The mobile section was degassed in ultrasonic water bath for 5 minutes and filtered under vacuum filtration.

2.4. Preparation of the Foscarnet standard & sample solution:
2.4.1. Buffer:
Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate, 1ml of TEA was added, finally make up the volume with water and pH adjusted to 4.0 with dil. OPA.

2.4.2. Mobile phase:
Buffer and Acetonitrile taken in the ratio 40:60.

2.4.3. Standard solution preparation: (Foscarnet 120µg/ml)
Accurately Weighed and transferred 12mg Foscarnet working Standard into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solution, 1 ml was pipetted out in to a 10ml Volumetric flask and then make up to the final volume with diluent.

2.4.4. Sample solution preparation:
1ml from the formulation was taken into a 10ml volumetric flask and made up to the mark with diluents. From the above sample stock solution 0.5ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Label Claim: 24mg/ml of Foscarnet in injection bottle.

3. METHOD DEVLOPMENT:
Trials were performed for the method development and the best peak with least fronting factor was found to be with RT= 3.885min and shown in figure-3.1.

4. METHOD VALIDATION:
4.1.Precision:
The standard solution was injected and measured the area for all injections in HPLC. The %RSD for the area of replicate injections was found to be within the specified limits and the result were depicted in table-4.1.1.

Acceptance Criteria: The % RSD should not be more than 2%
4.2. Accuracy:
Injected the standard solutions of Accuracy -50%, 100% and 150% and calculated the Amount found, Amount added for Foscarnet and the individual recovery and mean recovery values and the result were depicted in table-4.2.1.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

4.3. Recovery studies:
To determine the accuracy and precision of the proposed method recovery studies were carried out. A fixed amount of sample was taken and standard drug was added at 50%, 100% and 150% levels. The results were analyzed and the results were within the limits.

4.4. Linearity and Calibration curve:
operating dilutions of Foscarnet within the vary of 0.25-1.5ppm was ready by taking appropriate aliquots of operating commonplace solutions of drug in several 10ml volumetric flask and diluting up to the mark with mobile section. 20µl amount of every dilution was injected in to the column at a rate of 1.1ml/min. the drug within the rinse was monitored at 225nm and also the corresponding recordings were recorded. From these the mean peak areas were calculated and a plot of concentration vs peak areas was created and shown in figure-4.4.1. The regression of the plot was computed by least sq. regression methodology. The slope and intercept worth for standardization curve was y=20866x+8315 (R^2=0.999) based and also the result were portrayed in table-4.4.1.

4.5. Limit of detection and Limit of quantification:
Limit of Detection (LOD) is that the lowest concentration of associate degree analyte in an exceedingly sample that may be detected however not quantified. The LOD won't solely rely upon the procedure of research however additionally on the sort of instrument. In activity, detection limit is that the injected quantity that ends up in a peak with a height a minimum of double or thrice as high as baseline amplitude.

The LOD for Foscarnet was found to be 1.32

Limit of Quantification (LOQ) is outlined as lowest concentration of analyte in an exceedingly sample that may be determined with acceptable preciseness and accuracy and reliableness by a given methodology underneath declared experimental conditions. In activity, limit of quantification is that the injected quantity that ends up in a peak with a height, 10 times as high as bottom line amplitude.

The LOQ for Foscarnet was found to be 3.98

4.6. Robustness:
Robustness is set by creating deliberate amendments within the natural action conditions like change in rate, mobile section composition and temperature and evaluated for the impact on the strategy. It had been discovered from the chromatograms that the results were at intervals the boundaries. This means that the strategy developed is powerful.

5. STABILITY INDICATING ANALYTICAL METHODS:
Stability Indicating Studies are quantitative analytical procedure used to determine the amount of the Active Pharmaceutical Ingredients present in the degradation products. These methods can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference". This can be obtained by following process and results were shown in table-5.1.

5.1 Oxidation:
To 1 ml of stock solution of Foscarnet, 1 ml of 20% hydrogen peroxide (H2O2) was added and the solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 120µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded and results were shown in table-5.1.

5.2 Acid Degradation Studies:
To 1 ml of stock ssolution of Foscarnet, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 120µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded and results were shown in table-5.1.

5.3 Alkali Degradation Studies:
To 1 ml of stock solution Foscarnet, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 120µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample and and results were shown in table-5.1.

5.4 Dry Heat Degradation Studies:
The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 120µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample and results were shown in table-5.1.

5.5 Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the 100µg/ml&10µg/ml&25µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 120µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample and results were shown in table-5.1.

5.6 Neutral Degradation Studies:
Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 120µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample and results were shown in table-5.1.

RESULTS AND DISCUSSION

A simple, fast and precise methodology has been developed and valid for the drug Foscarnet. The estimation was dispensed with a mix of Phosphate buffer and Acetonitrile with a pH 4.0 adjusted with orthophosphoric acid within the quantitative relation of 40:60%v/v. preciseness of the ways were studied by creating recurrent injections of the samples and system preciseness values were determined. The retention time was 3.885 min. The standardization curve was linear over the concentration vary of 0.25-1.5ppm. The LOD and LOQ values were found to be 1.32 and 3.98. Stress degradation studies were established for Foscarnet by subjecting it to acid, base, oxidation, UV, water and thermal stress. The stress samples were assayed and results shown were within the range when compared against a reference standard. The high share of recovery and low share constant of variance make sure the quality of the strategy. Therefore it had been all over that the RP-HPLC methodology developed was considerably suit for routine analysis and the result were portrayed in table-6.1.

Table-4.1.1: Precision Results

S.No.	Peak area	%Assay	Day_day Precision
1	2537131	100.70	2615883
2	2501488	99.29	2624325
3	2501991	99.31	2621540
4	2528632	100.37	2657001
5	2542849	100.93	2626148
6	2511733	99.70	2638906
AVG	2520637	100.05	2630634
SD	18016.6	0.72	14992
%RSD	0.71	0.71	0.57

Table-4.2.1: Accuracy Results

%Concentration	50%	100%	150%
Trail-I	99.86	100.26	100.76
Trail-II	100.14	99.83	100.42
Trail-III	100.56	100.65	99.74
AVG (%Recovery)	100.19	100.25	100.31
SD	0.36	0.41	0.52
%RSD	0.35	0.41	0.52

Table-4.4.1: Linearity Results:

S.No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm(Foscarnet)	% Linearity Level
1	0.25	10	30	25
2	0.5	10	60	50
3	0.75	10	90	75
4	1	10	**120**	100
5	1.25	10	150	125
6	1.5	10	180	150

Table-5.1: Stress Stability Studies:

Parameter	Area under curve	Peak purity
Acid degradation	2404924	9259
Base degradation	2422371	93.27
Peroxide degradation	2445718	94.16
Thermal degradation	2471553	95.16
UV degradation	2546322	98.04
Water degradation	2579219	99.3

Table-6.1: Results and Discussion

S. No.	Parameter	Acceptance criteria	Observed value
1	Accuracy	95-105%	100.25%
2	Precision	RSD within 2%	0.71%
3	Linearity	R^2 not less than 0.99	R^2=0.999
4	LOD	S/N=3	1.32
5	LOQ	S/N=10	3.98

Figure -1.1: Structure of Foscarnet

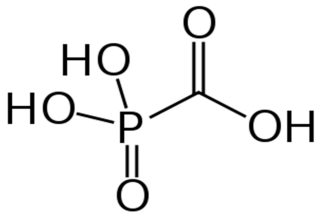


Figure – 3.1: Method Devolvement Chromatogram

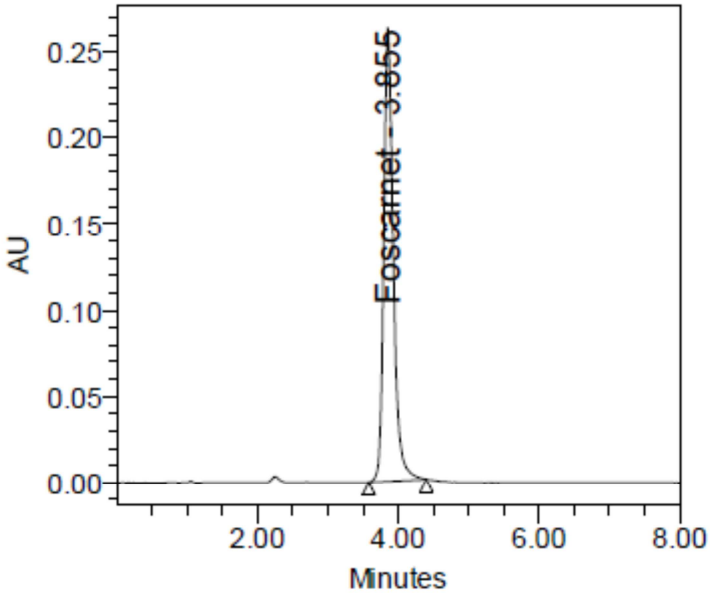
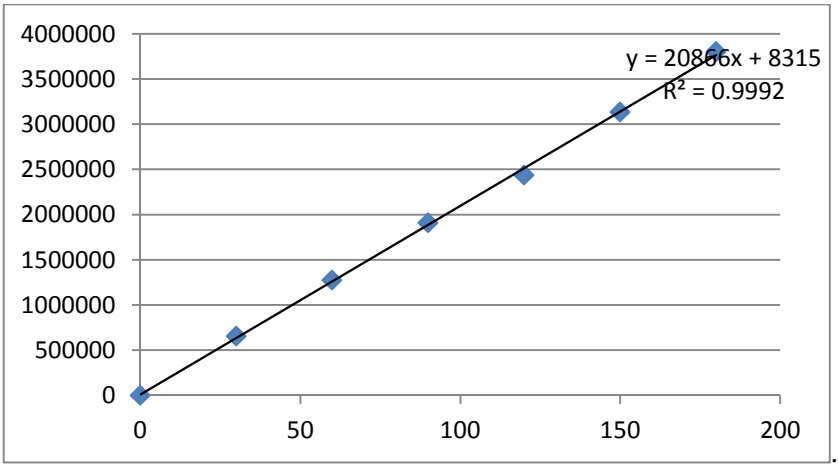


Figure -4.4.1: Linearity graph



X-Axis = Concentration, Y-Axis = Peak area

CONCLUSION

The projected study describes new and easy RP-HPLC methodology for the estimation of Foscarnet. The strategy valid was found to be straightforward, correct and precise. Thus the projected study methodology may be used for quantification of Foscarnet in bulk and pharmaceutical indefinite quantity kind. . Stability indicating studies of Foscarnet were also undertaken in the present study. The method was found to be very specific and there was no interference of any degraded compounds after forced degradation studies. The degraded products were successfully resolved and the method can be employed for determination of degraded products.

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