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Development and Validation of a Diethyl Phosphite Content in Foscarnet Sodium USP by GC MS Technique

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A simple, rapid, selective, and reproducible Gas chromatographic mass spectrometry (GC-MS) method has been developed and validated for the estimation of Diethyl Phosphite content in Foscarnet Sodium USP Drug substance. The drugs were estimated using HP-5, Length-30 M, Internal diameter 0.32 mm; Film thickness 1 µ at a total flow rate of 11.9 ml/min, and column flow of 1.49 ml/min was used for the separation. Flow control mode was pressure. Column oven temperature 70°C and injector temperature 220°C. Oven program modified for proper elution of peak. The linearity range used was 0.025-0.120µg/ml and (Rt) was 6.7 min. The correlation coefficient values were found to be 0.997. Precession studies showed % RSD values less than 15.0% for all the selected concentrations. The percentage recovery of Diethyl phosphite from LOQ to 150% was found in range of 100.7 -116.7%. The content results of Phophite content were within the limits of less than 0.12 ppm. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The developed method was successfully used for the quantitative analysis of commercially available dosage forms.

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Keywords: GC; MS; detector; foscarnet sodium; diethyl phosphate; genotoxic impurity.

1. INTRODUCTION

Foscarnet Sodium [1] is the trisodium salt of a synthetic organic analog of inorganic pyrophosphate with antiviral [2] activity. Foscarnet selectively blocks the pyrophosphate binding site of herpesvirus-specific DNA polymerases at concentrations that do not affect cellular DNA polymerases. This agent does not require phosphorylation by thymidine kinase (TK) or other kinases and therefore is active in vitro against herpes simplex virus (HSV) TK deficient mutants and cytomegalovirus (CMV) UL97 mutants. Because foscarnet crosses the blood brain barrier, it may be used in the treatment of viral infections of the CNS.

Foscarnet is used [3] to treat cytomegalovirus (CMV) retinitis in people with AIDS. Foscarnet is also used to treat the herpes simplex virus (HSV) in people with a weak immune system. Serious side effects may include [4] Anaemia, nausea, and vomiting, disturbances in electrolyte levels and genital ulceration have also been associated with administration of the drug.

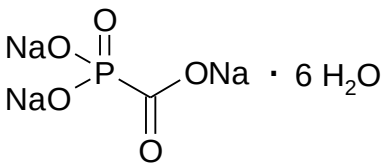


Fig. 1. Structure of Foscarnet sodium

2. MATERIALS AND METHODS

2.1 Instruments

Shimadzu GC2010 Gas Chromatograph with MS detector and Autosampler AOC-20s and Auto injector AOC-20i, Balance (Mettler Toledo).

2.2 Reagents and Materials

Dichloromethane HPLC grade
Di-Sodium hydrogen phosphate dihydrate (Emparta Merck)
Purified water

2.3 Methods

2.3.1 Diluent

Used Dichloromethane as diluent.

2.3.2 Buffer solution

Prepared 1% of Di-Sodium hydrogen phosphate dihydrate in purified water.

2.3.3 Blank preparation

Pipetted 1.5 mL diluent in HS vial and added 17 mL buffer solution mixed well, allowed the layers to settle down. Pipetted out about 1 ml of the lower Dichloromethane layer to an auto sampler vial containing about 150 mg of sodium sulfite. Shook well, decant the supernatant liquid in a vial for analysis.

2.3.4 Standard preparation

Prepared 0.08 ppm of standard solution (Diethyl phosphite) in diluent. Pipetted 1.5 mL of this solution in HS vial and added 17 mL buffer solution mixed well, allowed the layers to settle down. Pipetted out about 1 ml of the lower Dichloromethane layer to an auto sampler vial containing about 150 mg of sodium sulfite. Shook well, decant the supernatant liquid in a vial for analysis.

2.3.5 Test preparation

Weighed 1000 mg of the test sample in the HS vial, added 17 mL buffer solution sonicated to dissolve. Added 1.5 mL diluent and mixed well. Allowed the layers to settle down. Pipetted out about 1 ml of the lower Dichloromethane layer to an auto sampler vial containing about 150 mg of sodium sulfite. Shook well, decant the supernatant liquid in a vial for analysis.

3. METHOD DEVELOPMENT

Because in foscarnet sodium Diethyl phosphite impurity(Genotoxic impurity) in liquid state and no chromophore found in structure hence Gas Chromatography Mass spectrometer method used for detection. In GC MS, non-polar stationary phases such as phenyl ie. (***5***%-phenyl)-methylpolysiloxane are used. Other parameters such as column compartment temperature, Mobile phase flow play a significant role during this evolution in terms of stationary and mobile phases. During stationary phase screening, HP-5, Length-30 M, Internal diameter 0.32 mm; Film thickness 1 µ and HP-5, Length-60 M, Internal diameter 0.32 mm; Film thickness 0.25 µ availability in 30 M and 60 M lengths.

When the HP-5, Length-30 M, Internal diameter 0.32 mm; Film thickness 1 µ as utilized, superior impurity separation, peak sharpness, and system suitability were discovered.

Table 1. Content of Diethyl phosphite impurity chromatographic condition for GC

Component	Specification
Instrument	Shimadzu GC2010 or equivalent gas Chromatograph with MS detector and Auto sampler AOC-20s and Auto injector AOC-20i
Column	HP-5, Length-30 M, Internal diameter 0.32 mm; Film thickness 1 µ or equivalent
Carrier gas	Helium
Column Oven Temp.	70°C
Injection Temp.	220°C
Injection Mode	Split
Flow Control Mode	Pressure
Pressure	3.0 psi
Total Flow	11.9 mL/min
Column Flow	1.49 mL/min
Linear Velocity	44.7 cm/sec
Purge Flow	3.0 mL/min
Split Ratio	5.0
High Pressure Injection	Off
Carrier Gas Saver	Off
Splitter Hold	Off
External Wait	No
Equilibrium Time	0.5 min.
Injection Volume	2.0 µL

Table 2. Oven program

Temp. (°C)	Hold Time (min)	Rate (°C/min)
70	2	10
230	12	-

Table 3. Autosampler parameters

Parameter	
# of Rinses with Pre solvent	5
# of Rinses with Solvent (Post)	5
# of Rinses with Sample	2
Plunger Speed (Suction)	High
Viscosity Comp. Time	0.2 sec
Plunger Speed (Injection)	High
Syringe Insertion Speed	High
Injection Mode	Normal
Pumping Times	5
Inj. Port Dwell Time	0.3 sec
Terminal Air Gap	No
Plunger Washing Speed	High
Washing Volume	8 µL
Syringe Suction Position	0.0 mm
Syringe Injection Position	0.0 mm
Solvent Selection	only A

Table 4. MS parameters

SIM Mode		Scan Mode (Only for m/z identification purpose)	
Ionization Mode	: EI	Ionization Mode	: EI
Ion Source Temperature	: 250°C	Ion Source Temperature	: 250°C
Interface Temperature	: 250°C	Interface Temperature	: 250°C
Solvent Cut Time	: 3.0 min	Solvent Cut Time	: 6.0 min
Detector Gain Mode	: Relative	Detector Gain Mode	: Relative
to the tuning result		to the tuning result	
Detector Gain	: 0.50 kv	Detector Gain	: 0.50 kv
Threshold		Threshold	
Start Time	: 3.00 min	Start Time	: 3.00 min
End Time	: 12.00 min	End Time	: 12.00 min
ACQ Mode	: SIM	ACQ Mode	: Scan
Event Time	: 0.30 sec	Event Time	: 0.30 sec
Ch1 m/z	: 111.00	Start m/z	: 35
Ch2 m/z	: 83.00	End m/z	: 450

Here column oven program kept from temp 70°C-230°C, pressure flow with column flow is 1.49 kept. The total analysis time is 12 minutes. Different trial runs of standard preparation are used to select the optimal gradient program, flow rate, and column oven temperature.

Mass parameters such as EI ionization mode with ion source temperature 250°C and interface temperature 250°C. ACQ mode as SIM and star time from 3 and end time 12 min. channel 1 m/z as 111 and channel 2 m/z 83.

The concentration limit in ppm of genotoxic impurity (Diethyl phosphite) in drug substance derived from the TTC (Threshold of Toxicological Concern) can be calculated based on the expected daily dose to the patient using the equation:

Concentration limit (ppm) = TTC [µg/day] /dose (g/day] = 1.5/12.6 = 0.119 ppm

The chromatographic conditions are detailed in Table 1.

4. RESULTS AND DISCUSSION

The IP, BP, USP, and Q2 (R1) [5-8] of the ICH guideline were used in this validation and development study. For the finalization of the specified limit based on treatment duration and dose, the ICH guideline M7 (R1)[9] was used. The validation parameters for Analytical method [10-15] are explored in more detail below.

4.1 Specificity

By injecting Blank (diluent), standard (0.08 ppm Diethyl phosphite), and sample solution, the selectivity research parameter was done (666666 ppm). The chromatograms are analyzed at the same chromatograph having a mass detector as the method specifies. Table 5 contains the specificity data, as well as a chromatogram in Fig. 2. Blank (diluent) has no effect on the retention period of the Diethyl phosphite peak. All recognized and unknown peaks in the sample solution are well isolated from one another.

4.2 Limit of Detection (LOD) and Limit of Quantitation(LOQ)

The signal-to-noise ratio approach was used to calculate the LOD and LOQ conc. of Diethyl phosphite impurity in Foscarnet sodium. Injecting various concentration levels (between 10 and 100 percent) of standard solutions of Diethyl phosphite at limit level concentrations to determine the projected LOD and LOQ concentrations. 0.025 ppm was the predicted LOQ concentration value for Diethyl phosphite impurity. The LOD concentration is calculated by multiplying the predicated LOQ concentration by a factor of 0.33. Table 6 shows the predicted LOD and LOQ values.

4.3 Linearity and Range

The capacity of a method to produce test findings that are proportionate to the concentration of analyte in a given test sample is known as linearity. Standard solutions of Diethyl phosphite impurity with LOQ Level to 150 percent specified limit (including 30 50, 80, 100, 120, and 150 percent) of concentration were used in the linearity investigation.

Table 5. Data of Specificity of Diethyl phosphite in Foscarnet sodium

Impurities Name	Individual solution	Spiked test preparation
	Retention time (minutes)	Retention time (minutes)
Diethyl phosphite	6.713	6.668
Ethanol	Not detected	Not detected
Impurity D	12.903	12.898

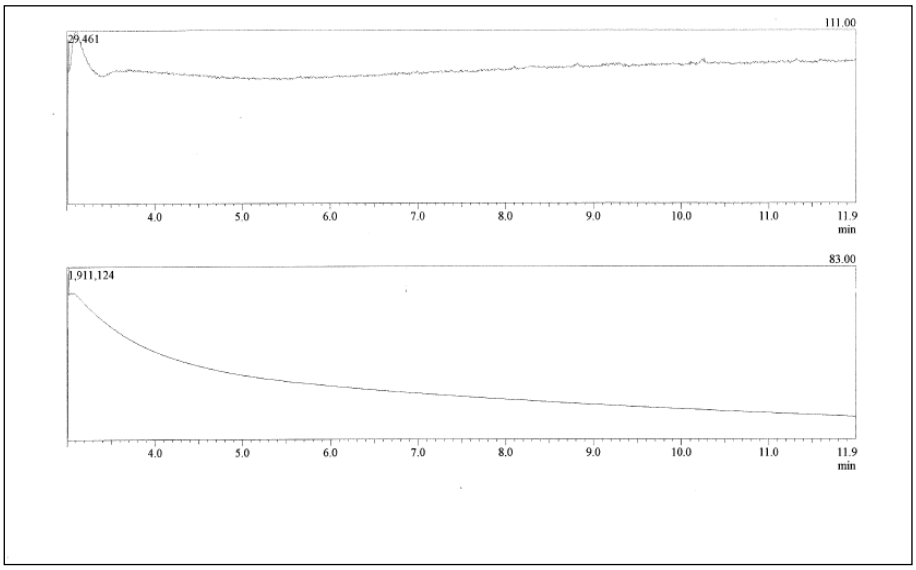


Fig. 2. Specificity: Blank preparation

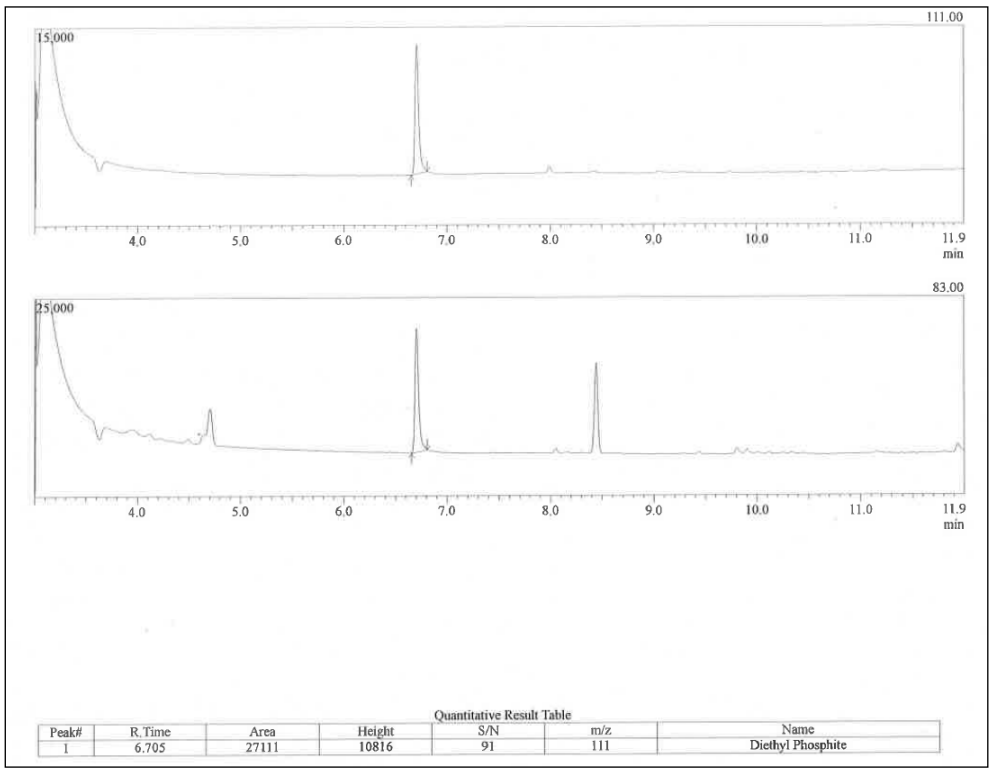
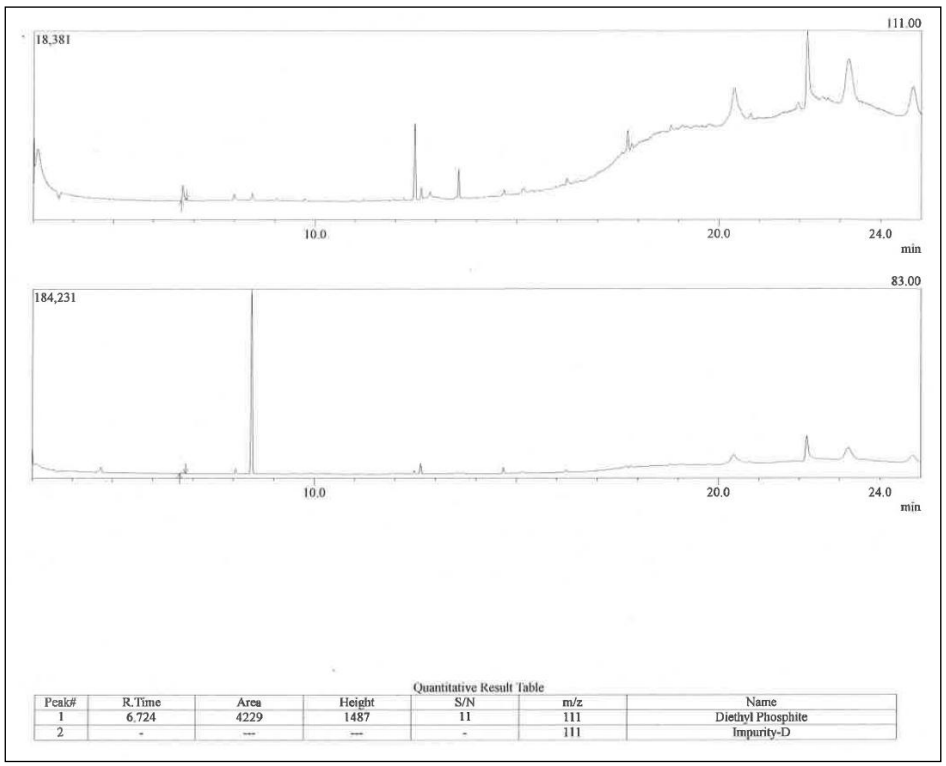


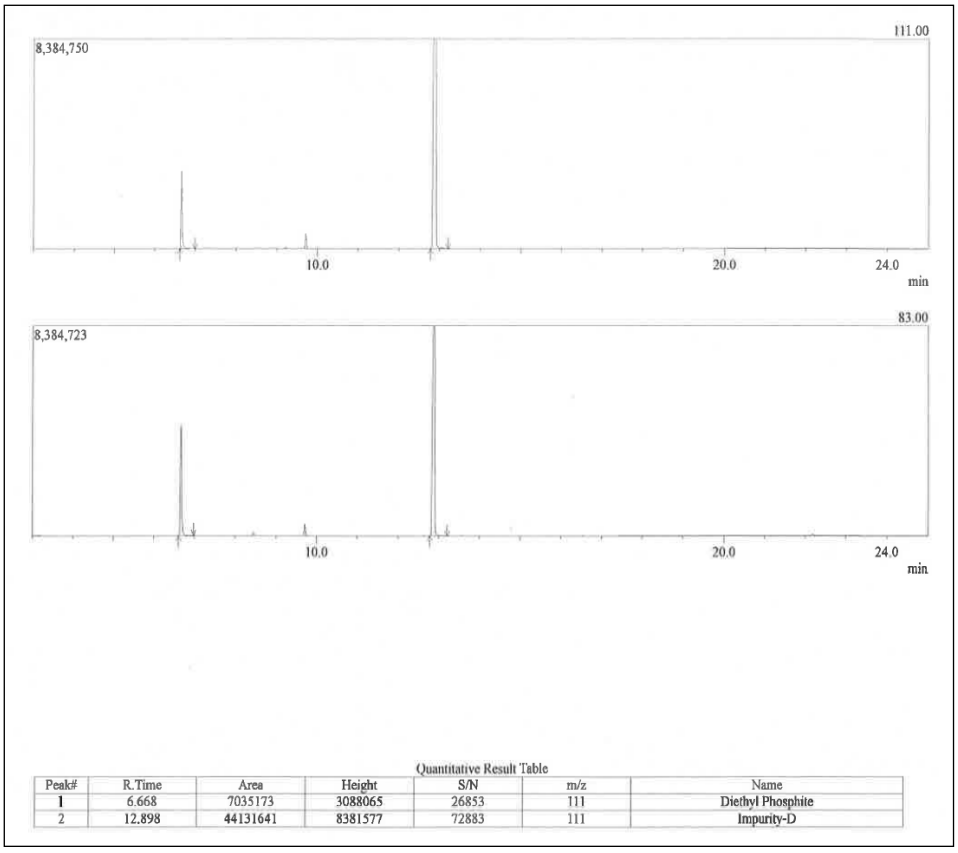
Fig. 3. Specificity: Standard preparation



Quantitative Result Table

Peak#	R.Time	Area	Height	S/N	m/z	Name
1	6.724	4229	1487	11	111	Diethyl Phosphite
2	-	---	---	-	111	Impurity-D

Fig.4. Specificity: Unspiked Test preparation



Quantitative Result Table

Peak#	R.Time	Area	Height	S/N	m/z	Name
1	6.668	7035173	3088065	26853	111	Diethyl Phosphite
2	12.898	44131641	8381577	72883	111	Impurity-D

Fig. 5. Spiked Impurities in Foscarnet sodium Typical chromatogram for Selectivity

Table 6. LOD and LOQ data in Diethyl phosphate

Name of Impurity	Conc. w.r.t test (in ppm)		s/n ratio	
	LOQ level	LOD level	LOQ level	LOD level
Diethyl phosphite impurity	0.025	0.008	14	6

Table 7. Linearity data for the Diethyl phosphite impurity (LOQ to 150% Concentration)

Sr. No.	C conc. w.r.t. standard Conc. in%	Concentration (In ppm w.r.t. test conc.)	concentration (in ppm)	Average area (n = 3)
1	LOQ	0.038	0.025	5218
2	50	0.060	0.040	13400
3	70	0.084	0.056	18360
4	80	0.096	0.064	21650
5	100	0.120	0.080	26800
6	120	0.144	0.096	32260
7	140	0.168	0.112	37520
8	150	0.180	0.120	40266
Slope				355522.1673
Intercept				-1918.830648
Correlation coefficient				0.99730

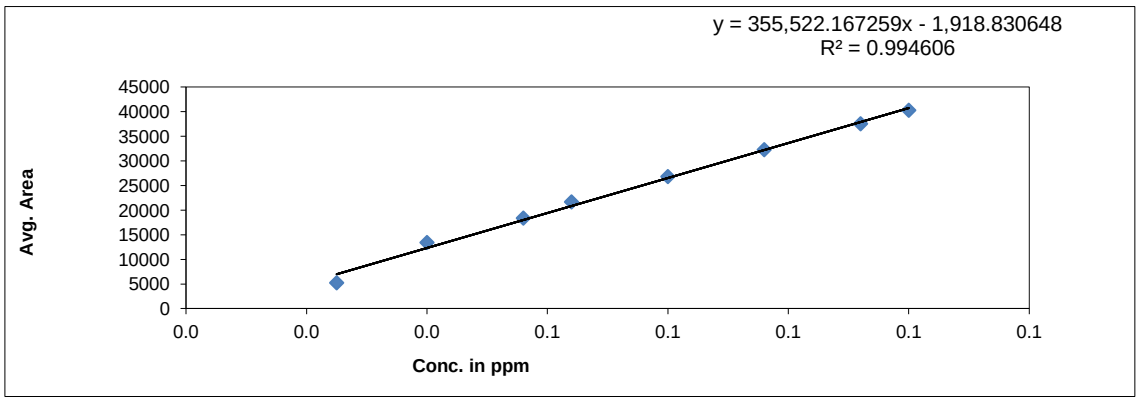


Fig. 6. Linearity graph for the Diethyl phosphite impurity content from LOQ to 150% concentration range

Table 8. Diethyl phosphite impurity result of the method and intermediate precision

Spiked sample solutions	% of Diethyl phosphite impurity (in ppm)	
	Method Precision	Intermediate Precision
Preparation 1.	0.1265	0.1283
Preparation 2.	0.1244	0.1179
Preparation 3.	0.1246	0.1352
Preparation 4.	0.1344	0.1213
Preparation 5.	0.1182	0.1232
Preparation 6.	0.1288	0.1256
Mean	**0.1262**	**0.1253**
SD	0.01	0.01
RSD	**7.92**	**7.98**
Overall Mean (n=12)	0.1257	
Overall SD **(n=12)**	0.01	
Overall% RSD(n=12)	**7.96**	

Table 9. The percent accuracy data of Diethyl Phosphite

Tests	LOQ Level	50% Level	100% Level	150% Level
Preparation -1	103.4	105.5	105.3	100.7
Preparation -2	116.7	115.5	113.6	105.7
Preparation -3	111.7	102.2	101.1	107.9
Mean	110.6	107.7	106.6	104.8
SD	6.76	6.94	6.36	3.70
% RSD	6.11	6.44	5.96	3.53

Table 10. RSD of Robustness study for the Diethyl phosphite impurity

parameters	Diethyl phosphite impurity			
	Column oven temperature		Column Pressure	
	75 °C	**65 °C**	**3.3 psi**	**2.7 psi**
Retention time	6.604	6.732	6.622	6.724
% RSD (n=6) replicates of standard preparation	**3.35**	**2.05**	**2.86**	**2.80**
Overall mean in ppm#	0.1255	0.1258	0.1262	0.1259
Overall% RSD#	**0.00**	**0.00**	**0.00**	**7.94**

 # n=8 (n=6 sample preparation of method precision and (n=2) preparation of Robustness)

Table 7 shows the correlation coefficient, slope, concentrations, and intercept of linearity data, and Figure 6 shows the linearity graph. Least squares linear regression analysis was used to examine the peak area versus concentration data. The Diethyl phosphite impurity has a correlation coefficient of 0.9973, which is higher than 0.99.

4.4 Precision

As stated in the technique of analysis, system precision was achieved by injecting five replicates of the standard preparation. For replicate injections, the observed percent RSD is 3.00. For method precision, six distinct samples were prepared and analysed; for intermediate precision, six separate samples were prepared and analysed on various days, systems, and columns. The observed percent RSD in method precision and intermediate precision is 7.92 and 7.98, respectively. The overall percent RSD is 7.96, which is less than 15.0%, for twelve test preparations (six from procedure precision and six from intermediate precision). Table 8 provides the outcomes of method precision and intermediate precision.

4.5 Accuracy

Spiking test preparation with an impurity at LOQ level, 50% level, 100 and 150 percent of specification limit concentrations was used to establish method accuracy. Table 9 shows the percent accuracy data for the Diethyl phosphite impurity. The percent accuracy observed at the LOQ level and 50% level, 100 and 150 percent is between 100.7 and 116.7 percent, which is within acceptable limits. (An accuracy of 70 to 130 percent is recommended).

4.6 Robustness

The method's robustness was tested by altering the pressure by±10% psi. The pressure is changed from3.0 psi to 2.7 psi and 3.3 psi. In the actual procedure, the column oven temperature is varied by± 5 °C from 70 °C to 65 °C and 75 °C. Table 7 displays the observed standard deviation, and percent RSD. The retention times in all of the studies above differed by ±0.2 minutes from the original retention times. For robustness studies, the percent RSD ranges from 2.05 to 3.35%. Changes in method parameters (pressure and column oven temperature) had no significant impact on system suitability criteria ie. percent RSD, according to Table 10. The values obtained are considerably within the acceptable range.

4.7 Solution Stability

The solution stability of the test preparation was tested at 25°C on a day-by-day basis for up to three days. Up to 3 days, the cumulative percent RSD values of the Diethyl phosphite impurity are substantially below acceptable limits. This

implies that when stored at 25°C temperature, Analytical test preparations are stable for 3 days.

5. CONCLUSION

The GC-MS method for Diethyl phosphite impurity content determination of Foscarnet sodium is very precise, selective, accurate, and stable, and follows ICH criteriaQ2(R1) also has been accurately developed and validated. The selectivity of method demonstrates that the Diethyl phosphite impurity peak is fully resolved from both known and unknown impurities. With LOQ - 150% level w.r.t. specification concentration, the method is linear, and the observed Correlation coefficient is 0.997 and Diethyl phosphite impurity was recovered between 100.7 and 116.7%.. System suitability, such as percent RSD, has no substantial impact on robustness. The observed outcomes were deemed to be within acceptable criteria. For all of the technical parameters that have been examined, the validated method has shown satisfactory results. As a result, the current method is specific, linear, selective, precise, robust, and stable, and can be used well in analysis.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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