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Development and Validation of UV Spectroscopic Method for Estimation of Fosfomycin In Fosfomycin for Injection

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ABSTRACT

To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Fosfomycin in Fosfomycin for Injection. The drug is freely soluble in analytical grade water [1]. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics [2]. It showed absorption maxima were determined in analytical grade water. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity [4, 5, 6]. The UV spectroscopic method was developed for estimation of Fosfomycin in injection dosage form and also validated as per ICH guidelines. The drug is freely soluble in analytical grade water, slightly soluble in 95% Methanol and Ethanol. Almost insoluble in anhydrous acetone, ether and chlorinated solvent. So, the analytical grade water is used as a diluent in method. The melting point of Fosfomycin was found to be 94 - 95˚C (uncorrected). It showed absorption maxima 254 nm in analytical grade water. On the basis of absorption spectrum the working concentration was set on 50µg/ml (PPM). The linearity was observed between 30-70 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 101.00 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.41%. A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Fosfomycin in Fosfomycin for Injection dosage form. The method could be considered for the determination of Fosfomycin in quality control laboratories.

Keywords: Fosfomycin, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision

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INTRODUCTION

Fosfomycin is a phosphonic acid having an (R, S)-1, 2-epoxypropyl group attached to phosphorus. It has a role as an antimicrobial agent and an EC 2.5.1.7 (UDP-N-acetylglucosamine 1-carboxyvinyltransferase) inhibitor [1]. It is an epoxide and a member of phosphonic acids. It derives from a phosphonic acid. It is a conjugate acid of a (1R, 2S)-epoxypropylphosphonate (1-)[2]. Fosfomycin is a broad spectrum antibiotic that concentrates in kidney and bladder and is used to treat uncomplicated urinary tract infections. Fosfomycin also reduces nephrotoxicity and ototoxicity of platinum-containing anti-tumor agents [2, 3].

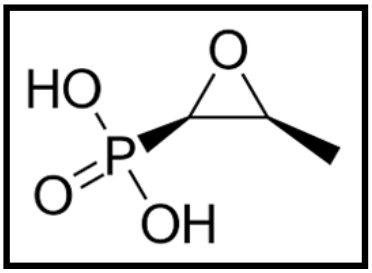

Figure 1: Chemical Structure of Fosfomycin

Fosfomycin is a phosphoenolpyruvate analogue produced by Streptomyces that irreversibly inhibits enolpyruvate transferase, which prevents the formation of N-Acetylmuramic Acid, an essential element of the peptidoglycan cell wall. Fosfomycin tromethamine is rapidly absorbed following oral administration and converted to fosfomycin. Oral bioavailability under fasting conditions is 37%. When given with food, oral bioavailability is reduced to 30% [4].

Literature review reveals that Fosfomycin is a broad-spectrum bactericidal antibiotic with a unique mechanism of action, inhibition of phosphoenolpyruvate transferase, the enzyme involved in the synthesis of peptidoglycan which is found in the cell wall of Gram-negative and Gram-positive bacteria [4, 5, 6].

From literature review it's found According to literature survey, the developed LC-MS [5], the HPLC-UV [6, 7] and methods for estimation of Fosfomycin in bulk drug. The Capillary Electrophoresis method reported by [8], for the Determination of fosfomycin in biological fluids by capillary electrophoresis. Recently [9], Multi-spectroscopic investigation of the binding interaction of fosfomycin with bovine serum albumin was done. But no methods were reported on estimation of Fosfomycin in Fosfomycin for Injection dosage form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Fosfomycin in Fosfomycin for injection dosage forms.

MATERIALS AND METHOD

Instruments:

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.

Materials:

Fosfomycin was obtained as a gift sample. Fosfomycin Injection was procured from local pharmacy. Water used was of analytical grade. Glass double distilled analytical grade water was used throughout the experiment. Freshly prepared solutions were employed.

METHOD DEVELOPMENT AND VALIDATION

Determination of λ max (100 PPM) [12, 13, 14]

20 mg weighed amount of Fosfomycin was dissolved into 100 ml of volumetric flask with analytical grade water. Pipette out 5 ml and added in 10 ml of volumetric flask dissolved and diluted up to the mark with analytical grade water. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

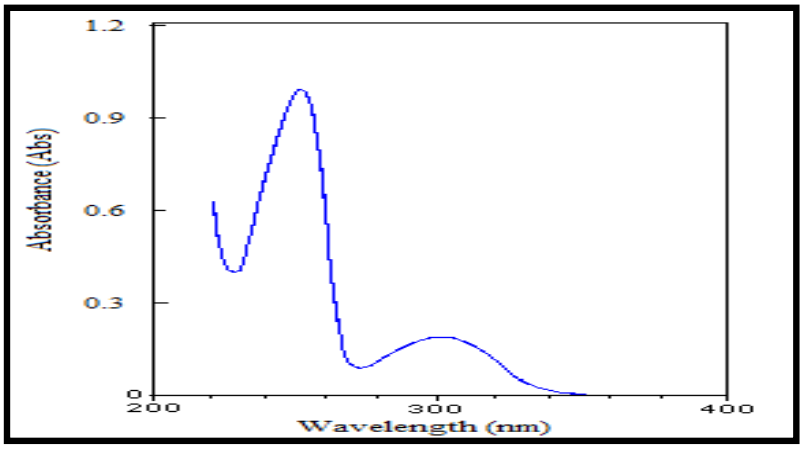

Figure 2: Calibration Curve

Preparation of working concentration [12, 13, 14]

Preparation of Standard stock solution:

Standard stock was prepared by dissolving 20 mg of Fosfomycin in 100 ml of analytical grade water to get concentration of 200 µg/ml (PPM).

Preparation of Standard solution:

Pipette out 2.5 ml from standard stock solution and diluted up to 10 ml with analytical grade water to get concentration of 50 µg/ml (PPM).

Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade water. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution:

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution:

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Procedure for sample preparations [15,16,17]

For analysis of commercial formulations; 20 mg of Fosfomycin for injection powder is weighed accurately from vial and transferred into the 100 ml of volumetric flask, added 60 ml analytical grade water, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade water. Filtered the solution through whatmann filter paper if required. Pipette out 2.5 ml of the above solution and diluted up to 10 ml with analytical grade water. The absorbance was measured at 254 nm. The absorbance was recorded:

Table 1: Absorbance of Dosage Form

Alniche Pharmaceutical Limited (4 gm per vial)		
Sr. no.	**Sample**	**Absorbance (Reading)**
1	Blank Solution	0.0001
2	Standard Solution	0.6958
3	Sample Solution	0.6915

Table 2: Dosage Form Specifications

Sr. No.	Company/Product	M.D.	E.D.	Assay (%)
1	Alniche Pharma LTD/ FosFona™ Powder for solution for Infusion(4mg/vial)	08/2018	07/2021	99.38

Method of validation [16,18,19]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity:

The linearity of the proposed assay was studied in the concentration range 30 - 70 PPM at 254nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies

Sr. no.	Sample Concentration	Absorbance
1	30 PPM	0.4121
2	40 PPM	0.5663
3	50 PPM	0.6945

4	60 PPM	0.8318
5	70 PPM	0.9668
Correlation coefficient		**0.999**

Accuracy:

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade water and measuring the absorbance at 254nm.

Table 4: Accuracy Studies

Spectrophotometric Method			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.79	98.75
100	1	1.01	101.00
120	1.2	1.19	99.17

Precision:

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies

Sr. No.	**Sample Solution**	**Absorbance**
1	Sample Solution 1	0.6918
2	Sample Solution 2	0.6985
3	Sample Solution 3	0.6945
4	Sample Solution 4	0.6921
5	Sample Solution 5	0.6965
Mean		**0.6947**
SD		**0.0029**
% RSD		**0.4128**

Ruggedness:

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies

Sr. No.	**Analyst**	**Results**	**Mean**	**% Assay**	**% RSD**
1	Analyst 1	0.6895	0.6905	99.23	0.0997
		0.6915			
2	Analyst 2	0.6923	0.6914	99.37	
		0.6905			

RESULTS AND DISCUSSION

Solubility of Fosfomycin

Solubility test was passed as per criteria.

Table 7: Results for solubility studies

Sr. No.	Chemical	Result
1	Analytical grade water	Soluble
2	Methanol	Slightly soluble
3	Acetone	Practically insoluble

Melting point of Fosfomycin

The melting point of Fosfomycin was found to be 94-95˚C (uncorrected).

Results for linearity for assay method of Fosfomycin

The linearity of method was determined at concentration level ranging from 30 to 70 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.999.**

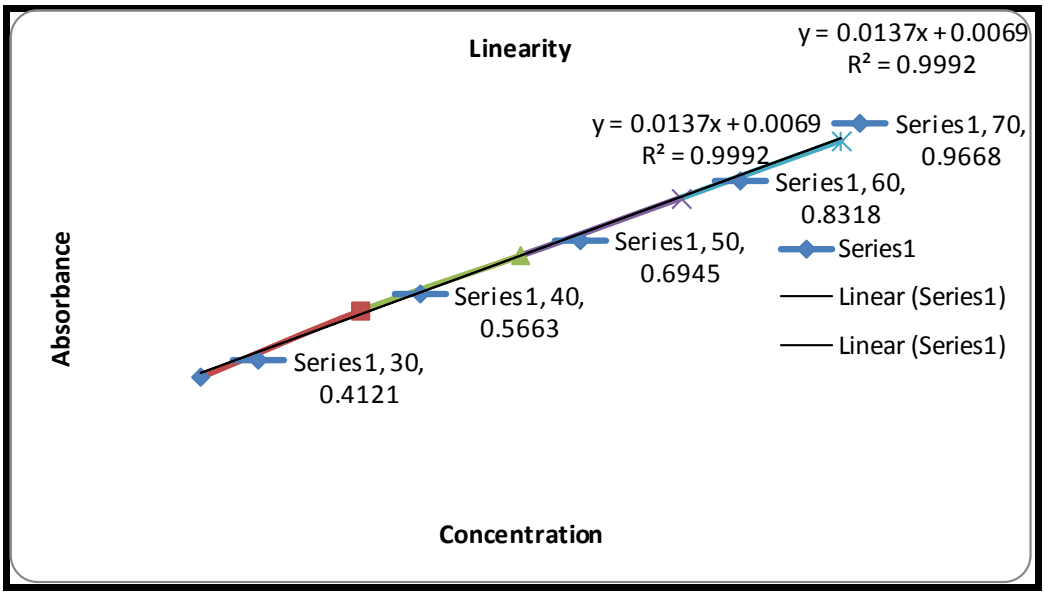

Figure 3: Fosfomycin Standard Curve

Results for accuracy for assay method of Fosfomycin

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98 - 102 %.

Results for precision for assay method of Fosfomycin

The % RSD for different sample of precision was found to be 0.4128 and it is within acceptance criteria represented in Table - 5.

Results for ruggedness for assay method of Fosfomycin

The %RSD for different sample of ruggedness was found to be 0.0997 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Fosfomycin in injection form has been developed. From the spectrum of Fosfomycin, it was found that the maximum absorbance was 254 nm in analytical grade water. A good linear relationship was observed in the concentration range of 30-70 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Fosfomycin in solid dosage forms. Hence, the method could be considered for the determination of Fosfomycin in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. DNA - Deoxyribonucleic acid
6. HIV - Human Immunodeficiency Virus
7. ICH - International Council for Harmonization
8. RSD - Relative Standard Deviation
9. SD - Standard Deviation
10. Qty - Quantity
11. C - Celsius
12. M.D. - Manufacturing Date
13. E.D. - Expiry Date

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