ORIGINAL RESEARCH PAPER

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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FOSFOMYCIN TROMETAMOL IN ITS BULK AND PHARMACEUTICAL DOSAGE FORM

KEY WORDS: Fosfomycin Trometamol, Derivatization, Validation, Liquid Chromatography

Binit Patel* Research Scientist-II Senores Pharmaceuticals Private Limited, Ahmedabad, Gujarat, India. *Corresponding Author

Vishal Prajapati Research Associate Cadila Healthcare Limited, Ahmedabad, Gujarat, India.

Shalin Parikh Senior Manager Senores Pharmaceuticals Private Limited, Ahmedabad, Gujarat, India.

ABSTRACT

The goal of the current study is to develop and validate a new chromatographic method for Fosfomycin Trometamol's determination in both pure form and pharmaceutical formulation. In the present study, a pre chromatographic derivatization of Fosfomycin Trometamol was done by forming an ion-pair complex of Heterocyclic nitrogen using the acidic dye methyl orange and phthalate buffer of pH-6. The yellow ion-pair complex was extracted with chloroform and it was further extracted with aqueous solution of 0.01M HCL. The ion-pair complex of Fosfomycin Trometamol and methyl orange obeyed Beer's law in the range of 30 -70 µg/ml with a correlation coefficient (r2) of 0.9946. A Liquid chromatography system equipped with an Agilent ACE C18 column (250 X 4.6mm, 5µm) was used as a stationary phase in this work. The developed method was validated according to ICH guidelines. For determining the medication in bulk and formulation, the devised approach was proven to be accurate, sensitive, and repeatable.

INTRODUCTION

A urinary tract infection is an infection of the kidneys, ureters, bladder, or urethra. Upper tract infections, which affect the Kidneys, can be distinguished from lower tract infections, which affect the Bladder (Cystitis), Urethra (Urethritis), and Prostate (Prostatitis) (Pyelonephritis). [1,3]

Fosfomycin chemically known as 1, 3-dihydroxy-2-(hydroxymethyl) propan-2-aminium hydrogen [(2R, 3S)-3-methyloxiran-2-yl] phosphonate. Some flaws in Fosfomycin metal salt are addressed by Fosfomycin Trometamol, which also offers the benefits of mild effectiveness, high oral absorption concentration, and lengthy action time. It is a milder but still powerful antibacterial medication. The bactericidal activity of Fosfomycin is due to its identical chemical structure to that of phosphoenolpyruvate, which forms an irreversible bond with the enzyme pyruvate-diphosphate uracil-acetylglucosaminyltransferase, which prevents the initial step of the formation of bacterial cell wall mucin. This medicine can be used by pregnant women, children, and elderly patients because it solely affects the bacterial cell wall; human cells that are not its target are unaffected. [4-6]

Numerous analytical method has been available for estimation of Fosfomycin Trometamol in bulk dosage or pharmaceutical dosage form using HPLC and UV spectrophotometry alongside LC-MS method [7-8]. A multi-spectroscopic analysis of Fosfomycin's binding interaction with bovine serum albumin was conducted. [9] The capillary electrophoresis method for detecting Fosfomycin Trometamol in bodily fluids was also found, it was identified during a literature search. [10]

There is currently no method available for derivatization-based HPLC estimation of Fosfomycin Trometamol. According to the most recent ICH guidelines, the developed technique was validated taking Specificity, Linearity, Precision, Recovery, and other validation parameters into account.

MATERIALS AND METHODOLOGY

Chemicals and Reagents

Fosfomycin Trometamol working standards was received as gift samples from local market. Chloroform, Methanol and Acetonitrile were of HPLC grade from Merck. The other reagents like Potassium dihydrogen phosphate, Hydrogen peroxide and Hydrochloric acid were of analytical grade. Water used to prepare buffers and other solutions was from Rankem.

Experimental Conditions

Preparation Of Standard Stock Solution

Weighed and dissolved about 100 mg of Fosfomycin Trometamol working standard in 100 ml of volumetric flask using 10% hydroalocholic solution to form final concentration of 1mg/mL. The same solution was further diluted to make series of working standard solution in range of 30 to 70 µg/mL.

Preparation of Sample Solution

Weighed and dissolved about 3000 mg of Fosfomycin Trometamol in 100 ml of volumetric flask using 10% hydroalocholic solution to form final concentration of 30 mg/mL. The same solution was further diluted to make final solution 50 µg/mL.

Optimized Prederivatization Process

Fosfomycin Trometamol, because to its high polarity and lack of chromophores was difficult to retain and detect using traditional reserved-phase HPLC-UV techniques. Thus, it needs the process of derivatization, which forms yellow colored ion pair complex where drug react with methyl orange dye in presence of pH 6.0 phthalate buffer using chloroform as extracting solvent. Furthermore, chloroform containing organic layer was allowed to interact with 0.01M hydrochloric acid solution to break complex followed by generation of light pink colored dye, which can be used for detection on HPLC coupled with UV detector. This solution composed of equal portion of drug and methyl orange reagent derived by applying Job's method of continuous variations and same has been depicted in **Figure 1**.

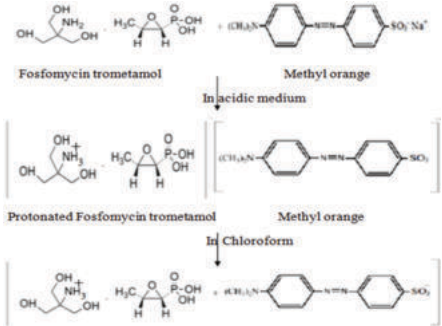

Figure: 1 Mechanism of Fosfomycin Trometamol –Methyl Orange ion-pair complex formation

Optimization of ion-pair complex was achieved through hit and run basis, considering evaluation of various parameter such as pH, type of buffer and organic solvent, volume of the dye, and shaking time. Finally, phthalate buffer pH 6.0 followed by chloroform as extraction solvent with 2 min shaking minute and 5.0 mL of 0.1% methyl orange solution was considered as most suitable conditions for ion-pair complex extraction.

Optimized Process For Extraction Of Complex

From the working standard solution, 30, 40, 50, 60, 70 µL were transferred to a series of 100 mL separating funnels containing 5 mL of phthalate buffer solution (pH 6) and 2 mL of 0.1 % w/v methyl orange solution. After 2 minutes of vigorous shaking and setting aside, the funnels were extracted with 5 mL of chloroform. The contents were shaken well and kept aside for separation. The chloroform layer was then separated by passing through anhydrous sodium sulphate anhydrous and the extraction was again repeated using a fresh 5ml aliquot of chloroform. The yellow colored organic phases were combined. Each concentration was studied in triplicate.

These solutions were further extracted with 25 mL aqueous solution of Hydrochloric acid (0.01M) in triplicate. This combined aqueous layer was evaporated on water bath at 100°C till it reduced to approximately 2 to 3 mL. At last, 1.0 mL of aqueous layer was transferred in 10mL volumetric flask and volume was made upto the mark with HPLC grade water.

Optimization Of Chromatographic Conditions

The chromatographic condition was optimized on trial and error basis. Numerous trials were taken with different range of buffer in composition with suitable solvent to achieve proper elution of Fosfomycin Trometamol. On a conclusion basis, mobile phase in combination of Acetonitrile: Potassium dihydrogen phosphate buffer 10mM (pH3.0, adjusted with dilute hydrochloric acid solution) with ratio of 60:40, 1.0 mL/minute flow rate with ACE C18 column (250 X 4.6mm, 5µm)was chosen for better elution pattern. The injection volume was 20µL and 507 nm wavelength was selected for symmetry of analyte peak.

Validation Of Analytical Method

The ICH criteria were followed for the validation of the analytical method, and the limit for that method was taken into account for the experiment. To rule out any interference from a blank or impurity at the retention period of the analyte peak, the specificity of the analytical procedure was established. The linearity of Fosfomycin Trometamol was plotted by series of dilution in concentration range of 30 to 70 µg/mL. The regression correlation was achieved using plot of peak area against concentration to establish linear relationship. Precision was also carried out on six sample preparation at target concentration and outcome of it should be lie within the limit of 2.0%. Alongside, recovery was performed at 80%, 100% and 120% level by spiking previously analyzed samples of the Fosfomycin Trometamol test (20µg/mL, i.e.100%) in triplicate preparation. Limit of Detection (LOD) and Limit of Quantification (LOQ) has been established through calibration curve of linearity. In addition, stability indicating activity for above method was also carried out, acid, alkali, thermal and peroxide degradation in different experimental conditions. The acid and alkali degradation was performed using 0.5M HCl and 0.5M NaOH solution respectively. Whereas, peroxide degradation undertaken using 3% Hydrogen Peroxide as oxidizing agent.

RESULT AND DISCUSSION

To achieve proper symmetry of analyte peak, Fosfomycin Trometamol, various trials were conducted using combination of different mobile phase ratio at varied flow rate with different USP column type, like L1 and L7. Finally, 10mM phosphate buffer pH 3.0 in combination with Acetonitrile in ratio of 60:40 (v/v) with ACE C18 column (250 X 4.6mm, 5µm) at 1.0 mL per minute flow shows better separation of analyte peak. The injection volume was adjusted to 20µL and analyte peak was detected at 507 nm. The analyte peak elutes within the time frame of 6 minutes.

The analytical method was found specific, as there was no interference of diluent at the retention of Fosfomycin Trometamol. The linearity was plotted using peak area against concentration with the range from 30 to 70 µg/mL and correlation coefficient was found 0.9946, which is in-line with the guideline shows in **Figure 2**.

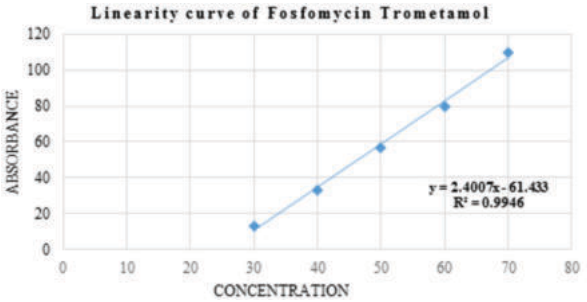

Figure: 2 Linearity curve of Fosfomycin Trometamol

The method precision was carried out on six replicate of preparation at target concentration 50µg/mL and result of it was found with the limit of 2% RSD. The intermediate precision was performed on different day using different lot of chromatography column and sample and comparative result of both precision, intermediate precision was found satisfactory in line with % difference not more than 3.0 and same has been depicted in **Table 1.** Accuracy was determined in terms of recovery study and the recoveries were done at three levels i.e. 80%, 100% and 120%. % recovery data obtained by the proposed method are shown in the **Table 1.** Limit of Detection (LOD) and Limit of Quantitation (LOQ) were established based on calibration curve, which was amounted 1.37 µg/mL and 4.16 µg/mL respectively. Robustness was carried out by against normal chromatography conditions includes changes such as Phosphate buffer pH 3: ACN (40:60) to (50:50) and (30:70), wavelength 507 nm, 506 nm, 508 nm and flow rate from 1 mL/min to 0.8 mL/min and 1.2 mL/min. At this small but deliberate changes the method parameters remains unaffected and produces the same response as that of normal condition. The % RSD was calculated for all changes and the values of % RSD was found to be less than 2%. Thus developed method was found to be robust. The applicability of method was carried out on marketed formulation and the mean percentage assay of the same was found 98.5%, which is within the limit of 98.0% to 102.0%.

Table 1: Result of Precision and Recovery of Fosfomycin Trometamol

Fosfomycin Trometamol								
Precision			Recovery					
Sr. No.	Precision	Inter mediate Precision	Test (µg/mL)	Std (µg/mL)	Total amount of Drug (µg/mL)	Mean Peak Area ± SD	Total amount of Drug found (µg/mL) (n=3)	Mean % recovery ± SD
1	56.12	57.12	20	16	36	23.21±0.31	35.86	99.61±0.31
2	56.65	56.10		20	40	31.53±0.46	39.25	98.12±0.46
3	56.83	55.75		24	44	41.10±0.12	43.15	98.06±0.12
4	57.02	57.21	--	--	--	--	--	--
5	55.82	56.95	--	--	--	--	--	--

6	57.36	55.10	--	--	--	--	--	--
Avg	56.63	56.37	--	--	--	--	--	98.50
SD	0.57	0.86	--	--	--	--	--	0.302
%RSD	1.01	1.52	--	--	--	--	--	1.405

At last, the stability indicating activity was also evaluated in different conditions such as, Acidic, Alkali, Peroxide and Thermal conditions. The percentage degradation in terms of final amount found was shown as 82.06%, 79.39%, 94.66% and 50.00% respectively for Acidic, Alkali, Peroxide and Thermal conditions. Above results concludes that the Fosfomycin Trometamol was thermolabile in nature and sensitive to thermal degradation.

CONCLUSION

The objective of the current study was to develop and validate an analytical method for the estimation of Fosfomycin Trometamol in its bulk and pharmaceutical dosage form. Before choosing the one that turned out to be best appropriate for this purpose, several circumstances were investigated. The relatively shorter run time of 6 minutes for product fasten the process for estimation. The correlation of coefficient was found 0.9946 and recovery at different concentration was found 99.61%, 98.12% and 98.06% respectively. As the proposed method proved to be adequate, reliable, and feasible, all the parameters evaluated in this study meets the criteria. This method could be considered as scientific evidence for future official pharmacopeial methods and for studies in the pharmaceutical industry where Fosfomycin Trometamol estimation is required.

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