IJPT
Available Online through
www.ijptonline.com

ISSN: 0975-766X
Research Article

AN ION CHROMATOGRAPHIC METHOD FOR ESTIMATION OF METHANE SULPHONIC ACID IN GEMIFLOXACIN MESYLATE

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Received on 16-02-2010 ***Accepted on 10-03-2010***

Abstract

The development and validation of an Ion chromatographic method for estimation of methane sulphonic acid in gemifloxacin mesylate drug substance was performed using acetone and buffer (1:9) as mobile phase and metrosep anion dual 2 as stationary phase at 0.5ml/min as flow rate. The analytes were monitored using suppressed conductivity detectors and linearity was obtained at the concentration range of 35.02 to 65.48 µg/ml and the correlation coefficient was found to be 0.9989. The recovery was found to be in the range of 103.53 to 116.30%w/w.

Key words: Gemifloxacin mesylate, conductivity detection, Ion chromatography, Methane sulphonic acid.

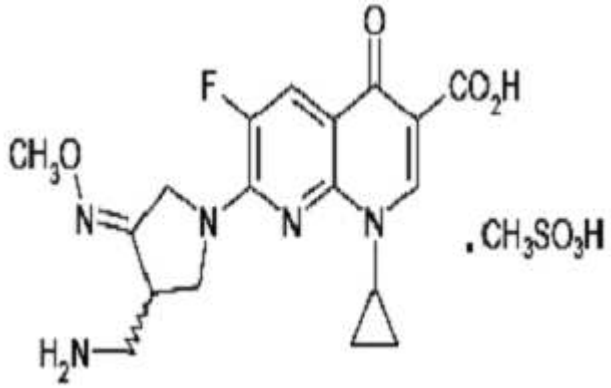
INTRODUCTION:

Rapid analytical methods are required to determine impurities in active pharmaceutical ingredient (API). Impurities like aliphatic and aromatic sulphonic acids are added to the API in order to increase its physical and pharmacological properties, but their limits play a major role as they are toxic in nature.

An ion chromatography applies to any modern method of separation of ions and it permits the determination of both organic and inorganic species in minimum concentration. It has found increasing application in a number of different areas of chemical analysis and particularly for the quantitative determination of anions[1].

Gemifloxacin is flouroquinalone class of antibiotic and available as a mesylate in sesquihydrate form and it is chemically, (R, S)-7-(3-aminomethyl-4-syn-methoxyimino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylicid methanesulfonate, is being developed for the treatment of respiratory and urinary tract infections. The compound has a broad spectrum of activity against Gram-positive and Gram-negativebacteria[2, 3] (Fig- 1). The literature survey revealed that only few analytical method have been reported for the estimation of methane sulphonic acid in different pharmaceutical drug substance and for the estimation of gemifloxacin. They include : ion chromatographic and iso tachophoretic method[4-6] for determination of MSA in different pharmaceutical drug substance and spectrophotometric method for estimation of gemifloxacin[7] . But no method has been developed for estimation of MSA in gemifloxacin mesylate drug substances hence the author has made an attempt to develop a simple ion chromatography method for the estimation of methane sulphonic acid in Gemifloxacin Mesylate drug substance. The developed method was validated as per to ICH guidelines[8, 9].

Fig: 1. Structure of Gemifloxacin Mesylate



Materials and methods:

Instrumentation

A modular Ion Chromatograph consisting of a serial dual piston pump with flow range of 0.05 to 5.00 mL/min with a Conductivity Detector having the conductivity measuring range between 100 µS/cm and 10 mS/cm and Full Scale between 0.05 µS/cm and 10 mS/cm and the data handling system is IC Net Metro data version 2.3. A suppressor module consisting of suppression block was used to reduce the back ground conductivity.

Preparation of mobile phase:

Sodium carbonate and sodium bicarbonate was dissolved and 50ml of water and from the above solution 10ml was transferred to 1000ml and the volume was made up with water. To 100ml of acetone 900ml of above prepared buffer was added and the solution was sonicated and filtered and used as a mobile phase.

Preparation of standard solution:

10µg/ml concentration of standard methane sulphonic acid solution was prepared and filtered through 0.2 µm finer porosity membrane filter. The prepared solution was injected in to chromatograph. The retention time of Methane sulphonic acid peak is about 4.7 minutes.

Determination of methane sulphonic acid in gemifloxacin mesylate:

100mg of sample was weighed and dissolved in 100ml water and from this 5ml was diluted to 100ml and this solution was sonicated and filtered. 10 µL of diluent as blank and the sample solution was injected into the ion chromatograph, using thechromatographic parameters. The chromatograms were recorded and using the peak area of standard and sample content of methane sulphonic acid in gemifloxacin was calculated and the result was given in table: 1

Table: 1. Result of analysis of Pharmaceutical Drug Substance and Recovery studies.

Anion	**Theoretical value (% w/w)**	**Content of methane sulphonic acid (%w/w)[a]**	**Percentage Recovery (%w/w)**
Methane sulphonate ion	17.72-19.91	18.91 ± 0.51	103.53-116.30%w/w.

a. Average of 6 determinations ± standard deviations

VALIDATION:

The linearity response of methane sulphonic acid was determined at 7 concentration levels of sample solution. These solutions were injected in to chromatographic system with chromatographic condition given previously. Using the data obtained correlation coefficient was calculated.

The accuracy of the method was determined by spiking a known quantity of Methane sulphonic acid in the sample at three different levels (50%, 100% and 120% of specification limit) in triplicate in the sample preparation and analyzed as per the proposed method. After making corrections for the Methane sulphonic acid already present in the sample, the percent recovery was calculated.

The developed method was found to be rugged by analyzing a single batch of Gemifloxacin Mesylate drug substance by two different analysts using two different columns on different instruments and different days as per the proposed method.

The robustness of the method was evaluated by deliberately varying the chromatographic conditions viz. the flow rate by $\pm$ 10% and the content of organic modifier in the eluent composition by $\pm$ 2 % absolute.At these varied conditions standard solution and the sample solution spiked with Methane sulphonic acid were analyzed as per the proposed method.

RESULTS AND DISCUSSIONS:

Optimization of mobile phase was carried out by taking different proportions of aqueous and organic phase to obtain rapid simple assay method for methane sulphonic acid with appropriate run time, asymmetric factor and theoretical plates.

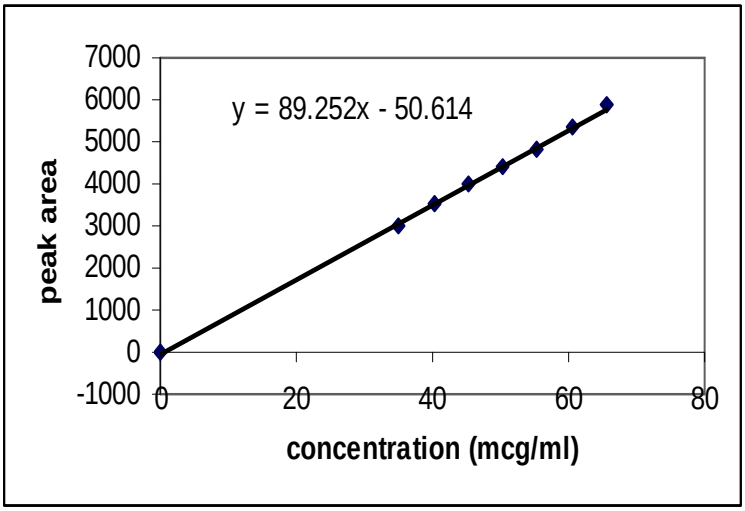
Mobile phase consisting of buffer: acetone in the ratio 9:1 v/v was found to give satisfactory symmetric peak at 4.7 min at a flow rate of 0.5min/ml.

The calibration curve for methane sulphonic acid was obtained by plotting the peak area vs. concentration. It was found linear in the range of 35.02 to 65.48 µg/ml. peak area and concentration were subjected to least square regression analysis to calculate calibration equation and correlation coefficient. The data of the calibration equation was given in table: 2 and the calibration curve shown in fig: 2.

Table: 2. Regression analysis of the calibration curve.

Parameters	**Methane sulphonic acid.**
Linearity range	35.02-65.48 µg/ml
Slope (m)	89.252
Intercept	- 50.614
Correlation coefficient	0.9989
Regression equation	Y = 89.252 x – 50.614

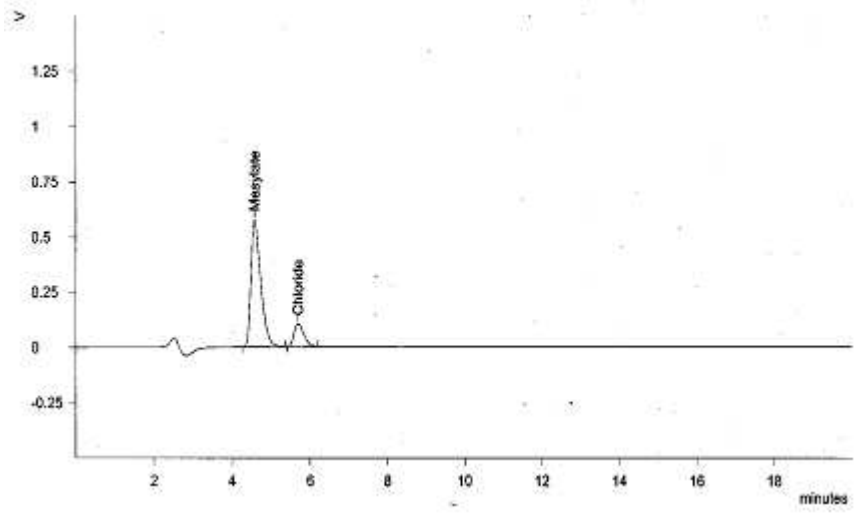
Fig: 2. Calibration curve of methane sulphonate ion



The accuracy of the developed method was determined by standard addition method and the recovery was found to be in the range of 103.53 to 116.30%w/w(table-1).Precision and the robustness studies were carried out and reported in terms of %RSD (table:3).

The specificity of the method was evaluated by injecting the blank and the standard solution prepared as per the proposed method to check for interference, if any, at the retention time of Methane sulphonic acid from the blank. There was no peak eluting at the retention time of Methane sulphonic acid from the blank. The specificity of the method was further evaluated by analyzing sample, chloride ion and by spiking Methane sulphonic acid and chloride ion with the sample solution. It was observed that there was no interference at the retention time of Methane sulphonic acid. The chromatogram was shown in fig: 3.

Fig: 3. Chromatogram showing the spiking of chloride ion ( ) and standard methane sulphonic acid in sample solution ( ).



The developed method was rugged and the solution was stable in the room temperature for at least 6 hour as the %RSD was obtained with in the limit(table:3). The system suitability was determined by injecting standard solution, the asymmetric factor and theoretical plates are calculated and reported in table: 3.

Table: 3. Summary of validation parameter for the assay of methane sulphonic acid

Parameters	**Methane Sulphonate Ion.**
Precision in %RSD a. System precision b. Method precision	 0.79 0.85
Robustness in %RSD a. + 10% change in flow rate b. – 10% change in flow rate c. +2% change in composition of organic modifier. d. -2% change in composition of organic modifier.	 1.60 1.28 0.70 1.94
Solution stability (cumulative %RSD)	Not more than 5%
Ruggedness (% RSD)	0.76
Theoretical plates	1583.60
Asymmetric factor	1.51

RSD- relative standard deviation

CONCLUSION:

The developed method was validated and it was found to rapid, simple, sensitive and accurate. Statistical analysis proved that the method was repeatable and selective for the analysis of methane sulphonic acid.

ACKNOWLEDGEMENT:

The authors are thankful to Orchid chemical and pharmaceutical Ltd.

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