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METHOD DEVELOPMENT AND VALIDATION OF GENOTOXIC IMPURITIES METHYL METHANESULFONATE AND METHYL IODIDE IN MONTELUKAST SODIUM DRUG SUBSTANCE BY GC-MS

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ABSTRACT

Montelukast sodium is a leukotriene receptor antagonist (LTRA) and is used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Manufacturing process of montelukast(Fig. 1) sodium involves use of methyl iodide (fig. 2) and methanesulfonyl chloride. Also methanol was used as solvent in the process and it is a well known fact that methanesulfonyl chloride does react with alcohols to form corresponding mesylate i.e. it would form methylmethanesulfonate (fig. 3). As per the IARC monograph methyl methanesulfonateisgenotoxic impurity and being aliphatic halide, methyl iodide is also a genotoxic alerting impurity. Hencethe study wasproposed and completed for the content of methyl iodide and methyl methanesulfonate in montelukast sodium using gas chromatographic technique with mass spectrometer as detector. Based on the daily dose evaluation limit wasdecided as 150µg/g. Validation of the method was proposed and completedbased on International conference on harmonization guidelines.The results obtained after completion of validation were all within the set acceptance criteria. The LOD and LOQ values established for Methyl iodide were2µg/g and6µg/g (i.e. 0.02µg/ml and 0.06µg/ml) respectively. Similarly LOD and LOQ values established for Methyl methanesulfonatewere2µg/g and 6µg/g (i.e. 0.02µg/ml and 0.06µg/ml) respectively. Percentage recovery results and Linearity for both the components were very well within the limits and hence the range of the method was from LOQ to 150% with respect to the evaluation limit. Also three commercial batches of montelukast sodium were analyzed in the validated method. In all the three batches the

content of Methyl Iodide was observed below LOQ while, the content of Methyl methanesulfonatewas not detected.

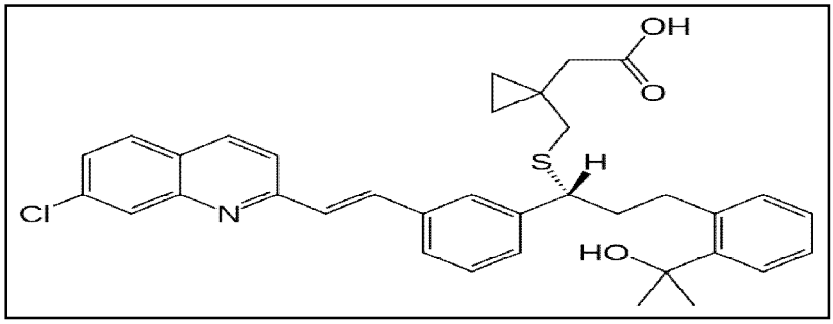

Fig. 1 Montelukast

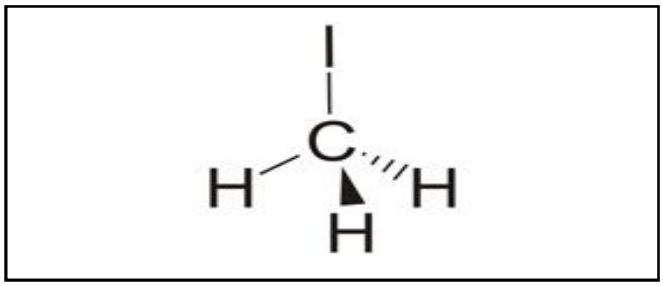

Fig. 2 Methyl iodide

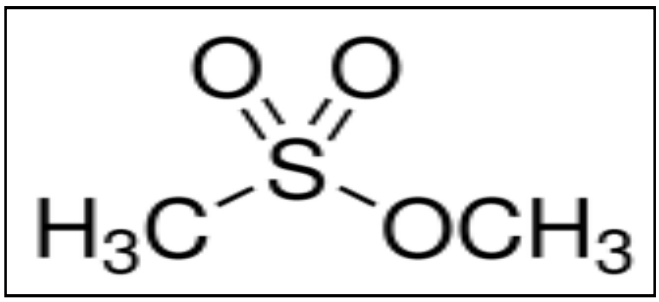

Fig. 3 Methyl methanesulfonate

KEY WORDS : Montelukast Sodium, Gas chromatography, Massspectrometer, Methyl Iodide, Methyl methanesulfonate, Genotoxic Impurity.

1.INTRODUCTION

Drug substances are in general manufactured by multiple manufacturing steps. While providing rationale for impurities, it is always necessary to account for presence of raw materials, impurities from process, intermediate stages and degradation products. Special emphasis is expected while dealing with genotoxic impurities in drug compounds and it is very much necessary to apply more stringent strategies due to the potential adverse effects to the patients because of these impurities.In the manufacturing process of montelukast sodium, methyl iodide and methyl methanesulfonatewerethe potential genotoxicimpurities. Methyl

iodide was used as a reagent and methyl methanesulfonate was the process impurity. As per the IARC monograph Methyl methanesulfonate is genotoxic impurity [1]. While Methyl iodide, which is also termed as iodomethane, was also found to be causing for the DNA damage [2,3 , 4].Insuch case based on the data of daily dosage, it was required to control these both the impurities as per their TTC(Toxicological threshold concern). Genotoxinsare always required to be limited to a daily dose of 1.5µg/day as per International conference on harmonization(ICH) guidelines from the europeanmedicinesagency[5, 6].

In literature, work on the analysis of mesylate derivative of methanol and ethanol in Imatinibmesylate drug substance as well as drug product was published[7]. Various co-workers had worked on the mesylate derivatives using GC-MS techniques [8, 9] in different drug products and substances.Similarly work was published on the analysis of mesylates using gas chromatographic technique with Flame Ionization Detector [10, 11].LC-MS method for low level determination of methyl methanesulfonate and ethyl methane sulfonate in emtricitabinewas also available in literature[12].Further to the method development, validation activity was proposed and completed based on the International conference on harmonisation guidelines on validation [13,14]. Method was proved to be specific, sensitive to the required limits, linear and accurate. LOD and LOQ values for both the analytes namely methyl iodide and methyl methanesulfonate were found to be 0.02µg/ml and 0.06µg/ml respectively.

2. EXPERIMENTAL

2.1 MATERIALS

All the reagents used during development, validation and analysis activity were of the analytical grade. Three commercial scale batches of montelukast sodium were used during the complete assessment for validation and analysis activity. Chloroform was used as diluent for preparation of standard solutions and sample solutions. Chloroform was procured from rankem. Methyl iodide was obtained from LobaChemie and methyl methanesulfonate was obtained from Sisco Research laboratories.

2.2 GC-MS Operating Conditions

Method development and validation activity was performed on ShimadzuGCMS QP-2010 with quadrupole mass analyzer and software control used was GCMS solution version 2.61. Column used for the analysis was Rtx-1301, 60 meter length; internal diameter 0.25mm, film thickness 0.25µm. Injection volume of 1µL was used with split of 1:25 for injection. GC column oven programmewas initial temperature 50°C and initial holdtime of 7 min, then the

temperature was raised to 230°C at the rate 30°C/minute and the final temperature hold was kept for5 minutes. Injector port temperature was maintained at 170°C. Ion source temperature and Interface temperature wereprogrammedat200°C and 220°C respectively. Helium was used as the carrier gas with constant linear velocity 28.1 cm/sec. Ionization energy used for the optimum ionization is 70 eV.Solvent cut time was 0.0 to 1.0 min. Further to the programming MS acquisition was kept on between 2.0 to 5.0 minutes for methyl iodide and 7.0 to 11.0 minutes for methyl methanesulfonate. For better sensitivity and specificity SIM mode is used for the analysis. Based on mass spectra of both the components m/z values screened for SIM mode are 142 and 79 for methyl iodide and methyl methanesulfonaterespectively.

2.3 Preparationof Solutions for Analysis

Chloroform was used as blank and as also used as diluent for the preparation of standards as well as sample solutions.Methyl iodide and Methyl methanesulfonatestandard solution was prepared by diluting the weighed quantity of 37.5 mg of each in to 50ml volumetric flask. Further 1.0ml of the resulting solution was diluted to 50ml with diluent (15.0µg/ml). This resulting solution was used as standard stock for the preparation of final standard solution as well as for the preparation of other standard solutions required during linearity, Limit of quantification and Limit of detection activities.

Standard stock solution was again diluted ten times i.e. 5.0 ml of the standard solution was diluted to 50ml to get the solution with concentration 1.5µg/mlof each, methyl iodide and methyl methanesulfonate.Similarly solutions with concentration 0.02µg/mland 2.25µg/ml were prepared applying appropriate dilutions for LOD-LOQ prediction, Accuracy and Linearity study.

Montelukast sample solutions were prepared by weighing and diluting 50.0 mg of sample to 5ml with Chloroformsonicated the resulting solution for 5 minutes and supernatant solution was used for the analysis.Montelukast sodium samples were analyzed on previously validated method for the content of methyl Iodide and methyl methanesulfonate.

3. RESULTS AND DISCUSSION

3.1 Method Development

Boiling point of methyl methanesulfonate is 202-203 ºC and that of methyl iodide is 42-43 ºC. So GC-MS was selected as the analytical tool. To start with the method development one

of the major factor in consideration was diluent. Different diluents were used such as methanol, Isopropyl alcohol, Tetrahydrofuran, hexane but due to interference at the retention time of analytes and solubility related problems, all these solvents were rejected. Finally Chloroform was decided as a diluent. Further different intermediate polarity GC columns were considered for the analysis, as analytesofinterest were polar so with intermediate polarity columns peak shapes would be acceptable. Hence during this method development, columns like DB-5, DB-624 and Rtx-624 with different dimensions were tried. Finally based on peak performance and minimum baseline interference, Rtx-1301 column with length 60 meter, internal diameter 0.25mm and 0.25µm film thickness was finalized.

Based on the retention time obtained from standard injection, solvent cut time and MS acquisition time was decided. Retention time of methyl iodide was between 4.0 to 5.0 minutes and that of methyl methanesulfonatewas between 8.5-9.5 min. Hence solvent cut time was kept at 0.0 to 1.0 minutes and MS acquisition time kept on at 2.0 to 5.0 minutes and 7.0 to 11.0 minutes.

Full scan of the components in the analysis was conducted at 10-500 amu and a spectrum was used for the identification. GCMS solution software version 2.61 was used for the mass spectral analysis. Compounds were identified using the reference spectra and m/z values for the SIM mode were finalized as 142 for Methyl iodide and 79 for Methyl methanesulfonate.

Table 1 Percentage Accuracy Results of The Method For Methyl Methanesulfonate And Methyl Iodide

Montelukast Na spiked, Methyl Iodide and Methyl methanesulfonate	**Recovery of Methyl Iodide, % (mean ±R.S.D.)**			**Recovery of Methyl methanesulfonate, % (mean ±R.S.D.)**		
	0.756µg/ml level	1.512µg/ml level	2.269µg/ml level	0.754µg/ml level	1.509µg/ml level	2.263µg/ml level
sample preparation-1	97.9±0.4	97.4±0.6	97.5±0.1	100.0±0.5	97.6±0.2	100.2±1.0
sample preparation-2	97.4±0.1	98.4±0.4	97.4±0.2	99.3±0.2	98.0±0.2	98.6±0.6
sample preparation-3	97.2±0.3	98.2±0.2	97.9±0.3	99.2±0.3	97.9±0.1	99.2±0.3

3.2 METHOD VALIDATION

3.2.1 Specificity

Method was developed for the content of methyl iodide and methyl methanesulfonate in montelukast sodium. Further method was validated based on the international conference on harmonisation validation guidelines. Before starting any parameter of validation, six

injections of standard solution were injected as a part of system suitability and percentage relative standard deviation was monitored against the limit 15.0 percent. While performing the specificity study interference of other solvents which were used in the process of montelukast sodium was checked. No interference was observed at the retention time of analytes.

3.2.2 Limit of Detection and Limit Of Quantification

Further LOD and LOQ values were established using calibration curve method. Standard solutions ranging from 0.02 to 0.6µg/ml for both the analytes were injected into the system for performing LOD and LOQ prediction study. Based on the concentrations obtained from slope and Intercept of the Prediction activity, LOD and LOQ precision activity performed. LOD and LOQ values for Methyl iodide were 0.02µg/ml and 0.06µg/ml respectively. LOD and LOQ values for Methyl methanesulfonate were 0.02µg/ml and 0.06µg/ml respectively.

3.2.3 Linearity

Linearity study was planned and completed in the range from LOQ to 150% of the evaluation limit. Calibration curves were plotted between the peak areas against the concentration of respective analyte and the linearity equation observed was methyl iodide (fig. 4) (y = 8684.x – 11.28) and methyl methanesulfonate (fig. 5) (y = 13895.x + 97.61). Also the correlation coefficient values observed were 0.99964 and 0.99985 for methyl iodide and methyl methanesulfonate respectively.

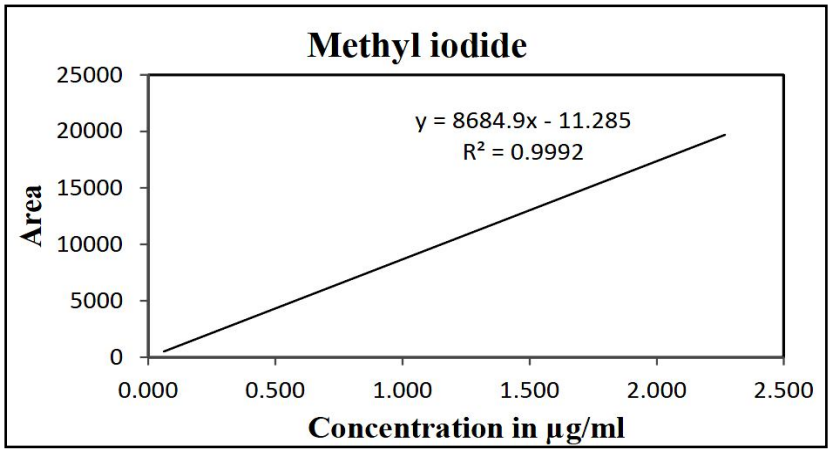

Fig. 4 Linearity graph of Methyl iodide

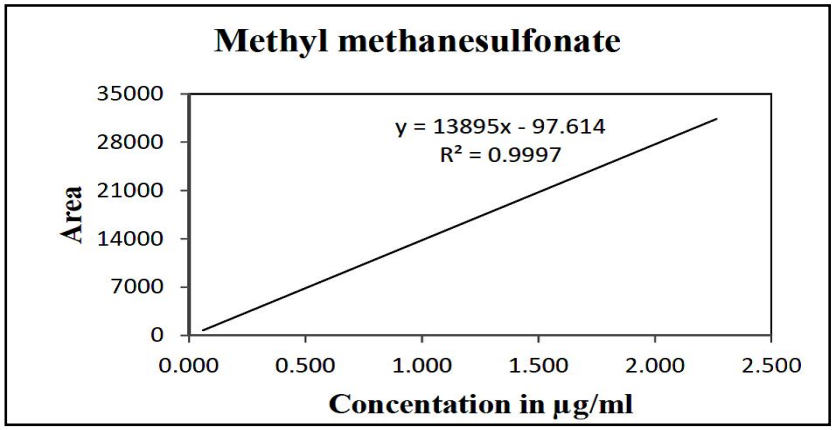

Fig. 5 Linearity graph of Methyl methanesulfonate

3.2.4 Precision and Accuracy

As per the precision activity performed the percentage Relative standard deviation for system precision was observed to be 1.0 and 0.7 respectively for methyl iodide and methyl methanesulfonate. The low values of RSD confirm to the precision of the method. Recovery study was performed at the level 50%, 100% and 150% of the evaluation limit i.e. 0.75, 1.50 and 2.25µg/ml. The percentage recovery values and comparison are as shown in the Table 1. For recovery calculation, results were reported based on comparison between blank (fig. 6), sample (fig. 7), standard (fig. 8) and spiked sample (fig.9).

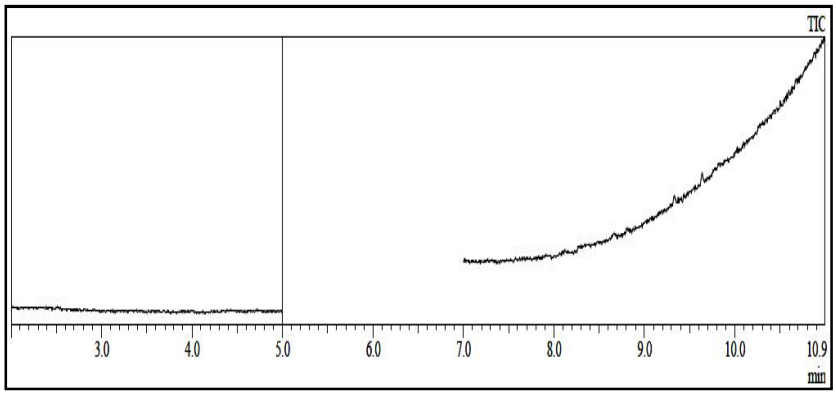

Fig. 6 Chromatogram of Blank solution

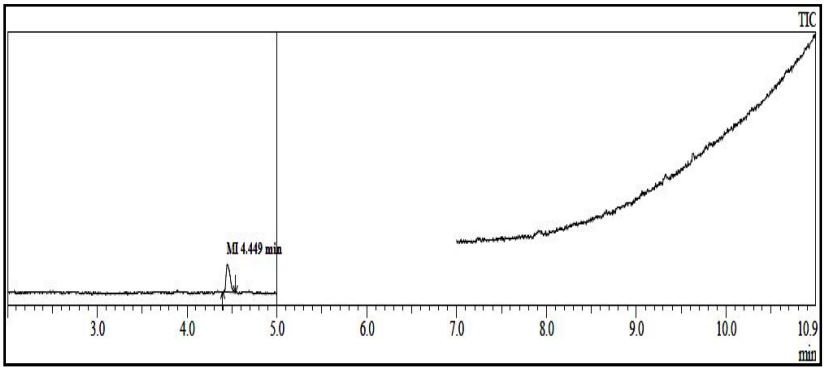

Fig. 7 Chromatogram of Sample solution

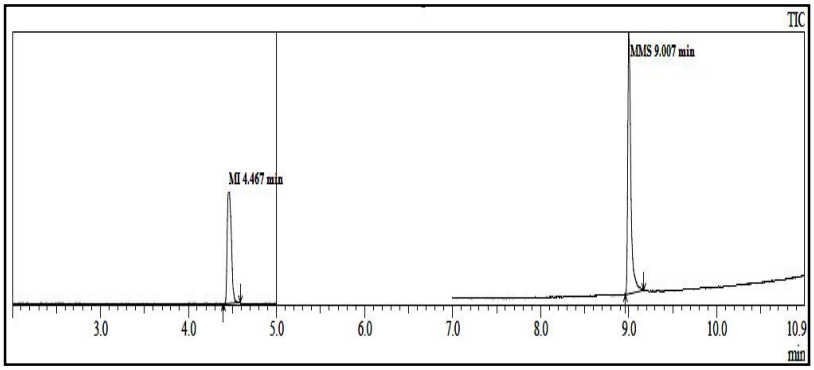

Fig. 8 Chromatogram of Standard solution at 1.5µg/ml concentration SIM mode

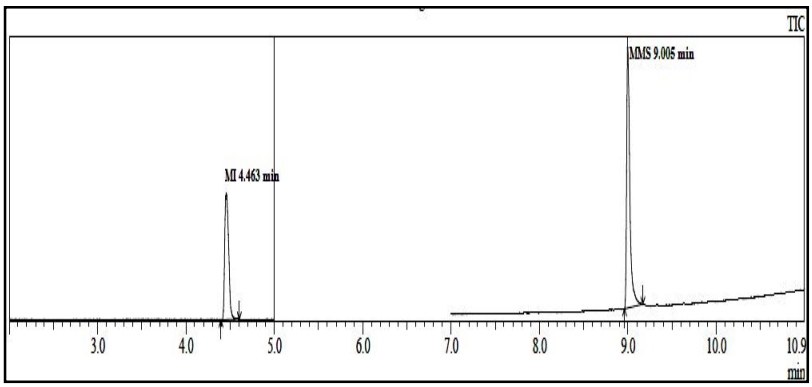

Fig. 9 Chromatogram of Spiked sample solution at 1.5µg/ml concentration

3.3 Mass Spectral Analysis

As per the analysis conducted by GC-MS and the retention times of methyl Iodide and methyl methanesulfonate were in the range 2.0 to 5.0 minutes and 7.0 to 11.0 minutes respectively. The Mass spectrum of methyl iodide is as shown in fig.10 and that of methyl methanesulfonate is as shown in fig. 11. As per the mass spectrum of methyl iodide, the fragments were observed at 142 and 127. As shown in the spectrum of Methyl methanesulfonate, major fragments were observed at 110, 109, 80, 79, and 65. The spectrum of both the analytes match to the reference spectrum of NIST.

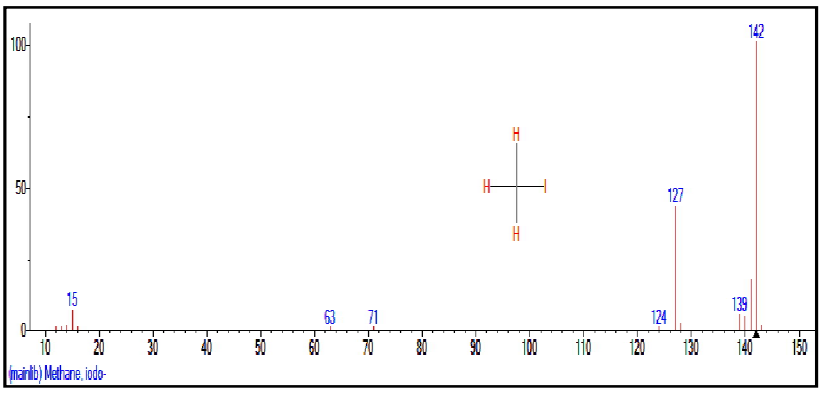

Fig. 10 Mass spectrum of Methyl iodide

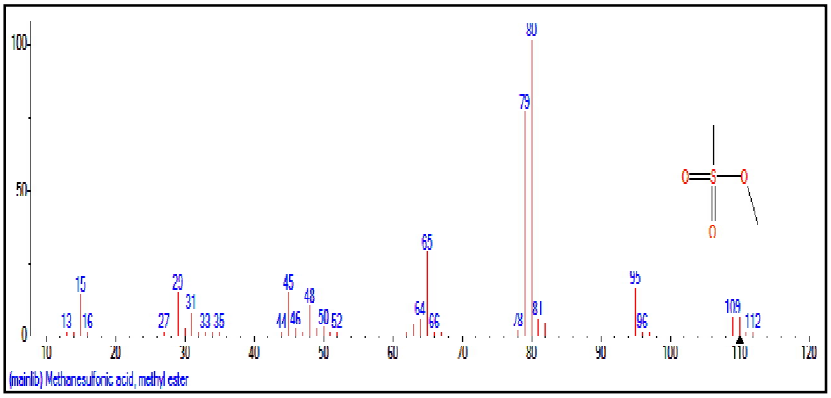

Fig. 11 Mass spectrum of Methyl methanesulfonate

4. CONCLUSION

As per the developed and validated method for the content of methyl iodide and methyl methanesulfonate in montelukast sodium, observations obtained were well within the acceptance criteria. Based on the LOD and LOQ study conducted the values observed are 0.02 and 0.06µg/ml respectively for both the analytes namely methyl iodide and methyl methanesulfonate. Values of LOD and LOQ indicate the sensitivity of the method which was well below the evaluation limit. In linearity study the correlation coefficient values observed were more than 0.999, which confirms the linearity of the method for both the analytes. Finally precision and accuracy values were observed to be well within the set acceptance criteria. Further to the validation study, three batches of montelukast sodium were analyzed for the content of methyl iodide and methyl methanesulfonate and the results showed that the methyl iodide content was below LOQ while methyl methanesulfonate was absent in all the three batches.

Hence, after studying the data obtained from the validation activity and analysis of three batches it is established that the method is suitable for regular analysis purposes.

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ABBREVIATIONS

IARC: International association for research on cancer

TTC: Threshold of toxicological concern

ICH: International conference on harmonization

GC: Gas chromatography

MS: Mass spectrometer

LC: Liquid chromatography

LOD: Limit of detection

LOQ: Limit of quantification

SIM: Selected ion monitoring

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