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Genotoxic impurity method development and validation by GC-MS for the analysis of methyl methane sulfonate (MMS) in Zidovudine drug substance

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ABSTRACT

Zidovudine is chemically known as 1-(3-Azido-2, 3-dideoxy-beta-D-ribofuranosyl) thymine and is the first approved treatment for HIV and is included in world health organization essential drug list. Zidovudine that is also termed as azidothymidine (AZA), is a nucleoside analog reverse transcriptor inhibitor (NRTI) and is a kind of antiretroviral drug for the treatment of human immunodeficiency virus infection / acquired immunodeficiency syndrome (HIV/AIDS).

Methyl methanesulfonate is a know genotoxic impurity and is potential process impurity of zidovudine. The method development and subsequent validation activity was done for the analysis of methyl methanesulfonate (MMS) in zidovudine drug substance. The development activity was conducted by gas chromatography technique with mass spectrometer as detector. Capillary column used in the method was Rtx-1301, with length 60 meter, internal diameter 0.25 mm, film thickness 0.25 µm with helium as carrier gas. Validation of the method is conducted based on international conference on harmonization (ICH) guidelines. The LOD and LOQ values are found to be 0.04 and 0.12 µg/g (i.e. 0.004 and 0.012 µg/mL) respectively for MMS.

KEYWORDS

Zidovudine drug substance (AZT);
Gas chromatography;
Mass spectrometer;
Methyl methanesulfonate (MMS);
Limit of quantification (LOQ);
Limit of detection (LOD).

INTRODUCTION

MMS is a known carcinogen and genotoxin, it is incorporated by international agency for research on cancer (IARC) in group 2A[1]. During the manufacturing process of zidovudine the formation of MMS is possible because of reaction between Methane sulfonyl chloride and Methanol to form corresponding mesylate.

As per the guidelines by european medicines agency the genotoxins are required to be limited to 1.5 µg/day[2,3]. Raman et al. has worked and explained on strategies for identification, control and determination of genotoxic impurities in drug substances[4].

The many coworkers worked on different genotoxic impurities in various drug substances and products. Li et al. reported the method for analysis of mesylate derivatives of alcohols by GC-FID technique, but as the sensitivity is the major concern, we need to have more sensitive method[5]. Also some co-workers had worked on low level determination of methyl methanesulfonate and ethyl methanesulfonate in imatinib mesylate[6]. Simi-

larly some co-workers had worked on mesylate derivatives of different alcohols using different analytical techniques as LC-MS, GC-MS and GC-FID on different drug substances and products[7,8,9]. Development and further validation of method for determination of MMS in zidovudine with more sensitivity was performed as per international conference on harmonisation[10,11]. Highly sensitive method is successfully developed and validated with LOD 0.004 µg/mL and LOQ 0.012 µg/mL.

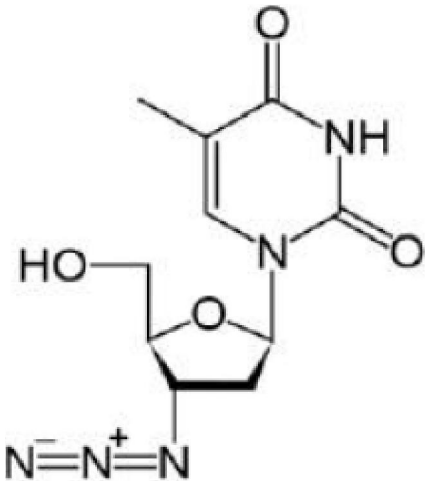

Figure 1 : Structure of Zidovudine

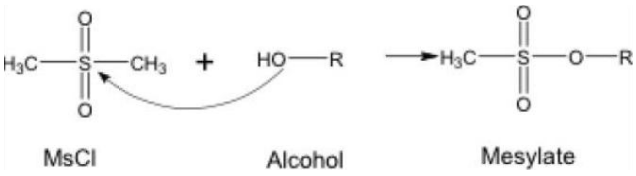

Figure 2 : General reaction of formation of alkyl methanesulfonate

EXPERIMENTAL

Materials

All the reagents used are of analytical grade. Zidovudine drug substance batch for the method development and validation was of commercial grade. Methyl methanesulfonate is procured from sisco research laboratories. Diluent that is used during the analysis for the preparation of standard and sample solution is dichloromethane and is purchased from ranbaxy fine chemicals limited.

GC-MS operating conditions

The zidovudine sample was analyzed with previously validated method for the content of MMS. The analysis was performed with shimadzu GCMS QP-2010 with quadrupole mass analyzer and software control used is GCMS solution version 2.61. Analysis was performed on column Rtx-1301, with length 60 meter, internal diameter 0.25 mm, film thickness 0.25 µm. Injection volume of 1 µl was used with split of 1:5 for injection. GC oven programme used is as initial temperature 40°C and initial holding time of 5 min, then the temperature is raised to 250°C at the rate 30°C/minute and the final temperature hold is 5 minutes. Injector temperature is maintained at 170°C. Ion source temperature and interface temperatures are 200°C and 220°C respectively. Helium is used as the carrier gas with the flow rate as 1.2 mL/min. Ionization energy used for the optimum ionization is 70 eV.

Full scan of the components in the analysis is conducted at 10-500 amu and a spectrum is used for the identification. GCMS solution software version 2.61 is used for the mass spectral analysis. Compounds were identified using the reference spectra in the library of national institute of standard technology.

Solvent cut time is kept at 0.0 min to 7.0 min and acquisition time is kept at 7.5 min to 10.0 min. Analysis is conducted in selective ion monitoring mode (SIM mode).

Preparation of solutions for analysis

Methyl methanesulfonate solution was prepared diluting 20 mg to 100 mL using diluent Dichloromethane. Further 1.0 mL of the above solution is diluted to 100 mL with diluent Dichloromethane (2.0µg/mL). Again 2.5 mL of the resulting solution is diluted to 50 mL (0.1µg/mL). Similarly sample solution was prepared by diluting the 500 mg of Zidovudine sample to 5.0 mL with diluent Dichloromethane.

For the LOD-LOQ prediction solutions with concentration between 0.01µg/mL and 0.08µg/mL were prepared. Also the Linearity solutions were prepared between the concentrations ranges from LOQ and 0.25 µg/mL. Dichloromethane is injected as blank during the analysis and validation activity.

RESULTS AND DISCUSSION

Method development

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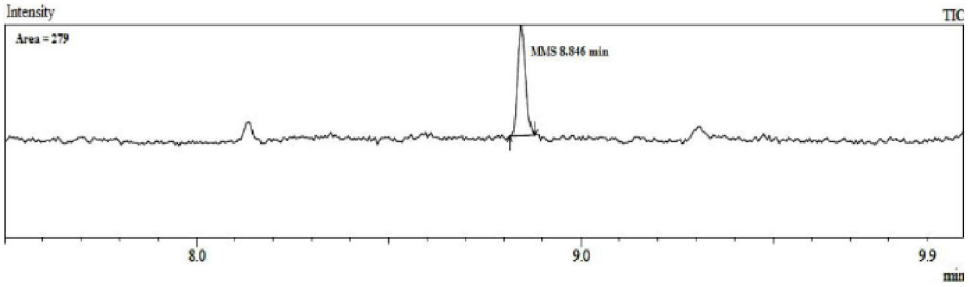

Figure 3 : GC-MS chromatogram of LOD level

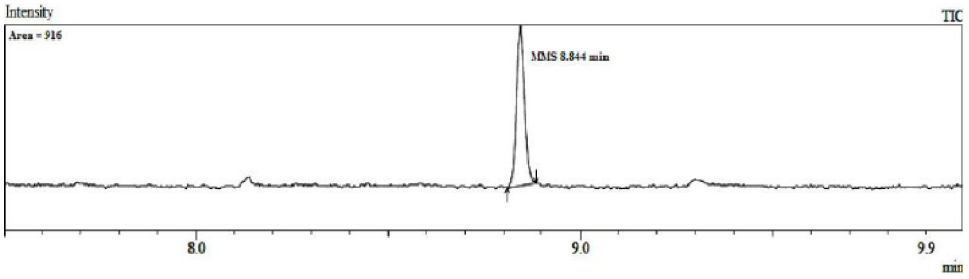

Figure 4 : GC-MS chromatogram of LOQ level

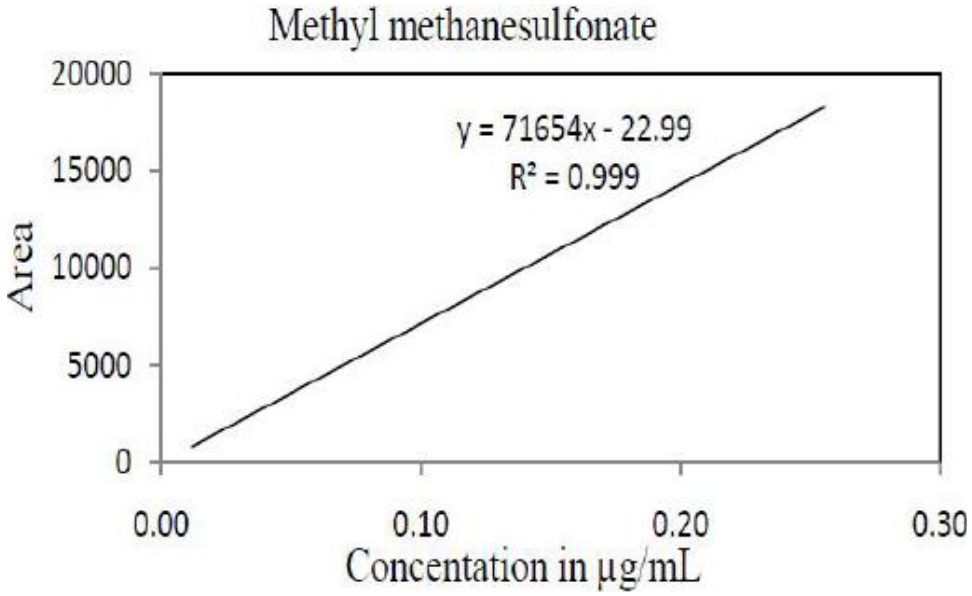

Figure 5 : Linearity graph of Methane methanesulfonate

Different columns with different dimensions were used during the method development activity, like DB-5, Rtx-624 and DB-624 for the analysis of MMS. In this peak shape and the resulting response was not as satisfactory as for the validating the method. Then we have opted Rtx-1301 with lower internal diameter and lower film thickness that resulted in good peak shape and response. As well as the column flow used during is also lower (1.2 mL/min). During the SIM mode in MS we have selected target ion as 79 m/Z for MMS. The Retention time is in the range 8.0 to 9.0 minutes. In this method a solvent cut time is 7.0 minutes and kept gradient temp programming starting from 40 °C with holding time 5.0 minutes, then further raised to 250 °C at the rate of 30 °C/minutes and final hold time is 5.0 minutes. Method is optimized and validated.

Method validation

Validation of the developed method for the analysis of MMS in Zidovudine is performed based on International Conference on Harmonization Guidelines for Analytical method validation. The standard solution of MMS was injected as a part of system suitability and

TABLE 1

Zidovudine drug substance spiked with Methyl methanesulfonate	Recovery of Methyl methane sulfonate, % (mean ±difference)		
	0.125 µg/mL level	0.250 µg/mL level	0.375 µg/mL level
sample preparation-1	102.6±3.7	100.4±0.5	99.8±0.4
sample preparation-2	97.3±1.6	100.4±0.5	100.6±0.4
sample preparation-3	96.8±2.1	102.0±1.1	100.2±0.1

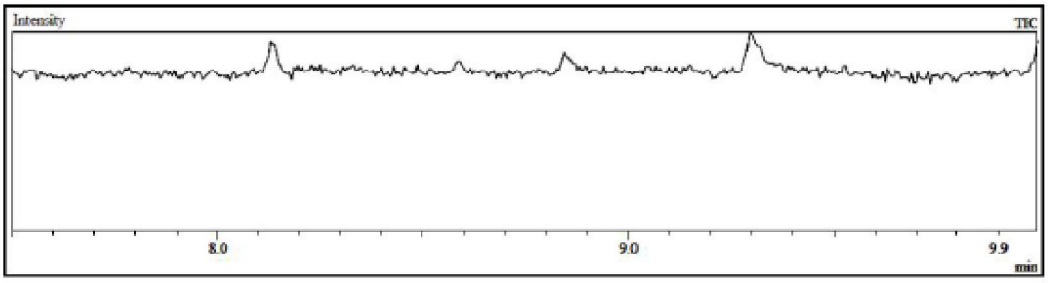

Figure 6 : GC-MS chromatogram of blank

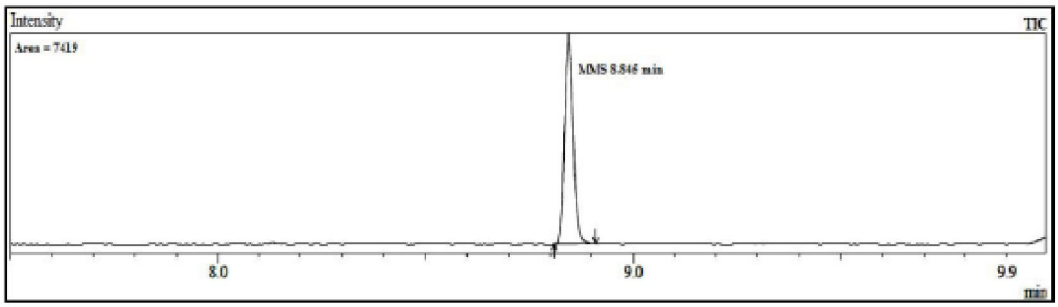

Figure 7 : GC-MS chromatogram of 0.1 µg/mL standard of methyl methanesulfonate

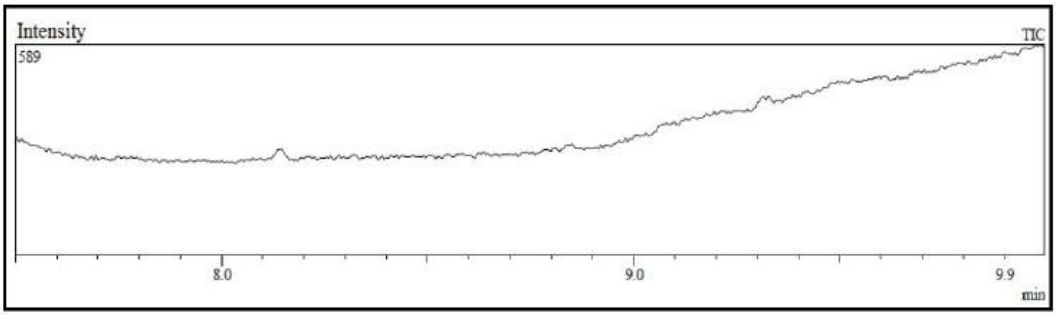

Figure 8 : GC-MS chromatogram of sample

further different concentrations ranging from 0.01 to 0.25µg/mL were injected into the GC-MS for performing different study parameters.

Specificity

Specificity study is performed by injecting the solvents used during the manufacturing process of Zidovudine. Based on the study it was observed that there no interference at the retention time of the analyte components.

Limit of detection and limit of quantification

As per the International conference on Harmonization guideline for the analytical method validation, calibration curve method is used for the determination for LOD and LOQ values. The LOD and LOQ values obtained were 0.004 and 0.012 µg/mL respectively for MMS. Calibration curve was plotted between the peak areas against the concentration of MMS (y= 71654. x -22.99).

Linearity

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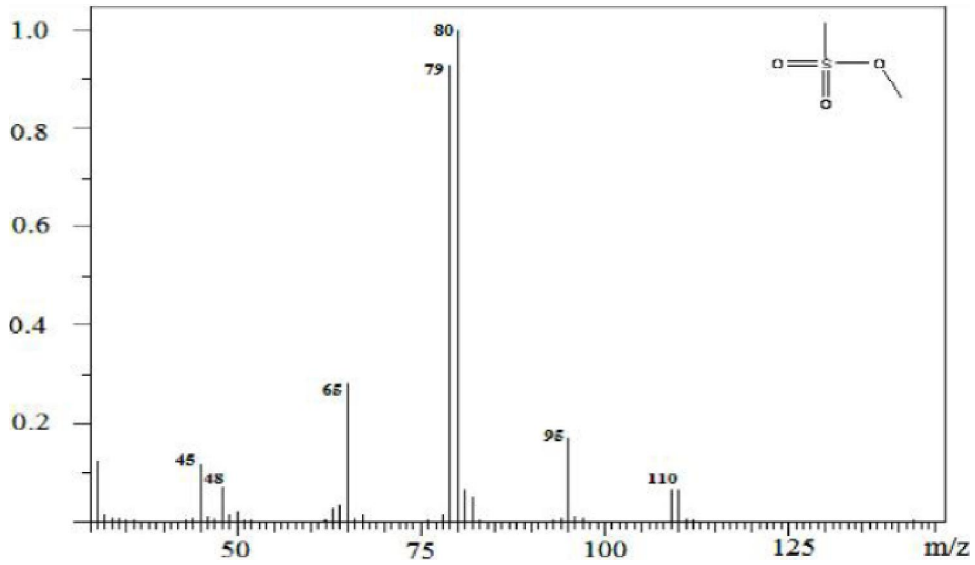

Figure 9 : Mass spectrum of methyl methanesulfonate

Linearity is performed in the range 0.01 and 0.25 µg/mL and the correlation coefficient was observed to be 0.9997.

Precision and accuracy

The system precision is performed by injecting the standard solution containing 0.10 µg/mL of MMS in six replicate. RSD for the six replicates was found to be 2.2 for MMS and that is well within the acceptance criteria. The low %RSD values of the peak areas of analyte confirms to the precision of the developed method. Accuracy was performed by spiking the samples of Zidovudine at 0.125, 0.250 and 0.375 µg/mL concentrations. The average percentage of recovery was observed well within the acceptance criteria as provided in TABLE 1.

Based in the validation activity conducted, it proves that the method is suitable for use in routine analysis.

Mass spectral analysis

Analysis conducted by GC-MS and the retention time of MMS is in the range 8.0 to 9.0 minutes as shown in the Figure 1. The Mass spectrum of Methyl methanesulfonate is as shown in Figure 2. As shown in the spectrum of MMS, the parent peak is observed at 110 that confirm the molecular formula $C_2H_6O_3S$. Major fragments are observed at 110, 109, 80, 79, and 65. The spectrum observed matches exactly to the reference spectrum.

CONCLUSION

The developed method for the analysis of MMS in Zidovudine drug substance using gas chromatographic technique and mass spectrometer as detector, hence it is specific and very highly sensitive as it can quantify MMS up to 0.012 µg/mL and detect up to 0.004 µg/mL. Based on the validation conducted, it confirms that the method may be used successfully for the analysis purpose.

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