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Short communication

Development and validation of GC–MS method for the determination of methyl methanesulfonate and ethyl methanesulfonate in imatinib mesylate

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Abstract

A gas chromatography–mass spectrometry (GC–MS) method has been developed for the identification and determination of two carcinogenic and genotoxic mesylate esters viz. methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) in imatinib mesylate (INM). The method was optimized based on the peak shapes and resolution of MMS and EMS. The method was validated as per International Conference of Harmonization (ICH) guidelines in terms of limits of detection (LOD), limit of quantitation (LOQ), linearity, precision, accuracy, specificity and robustness. The LOD and LOQ values were found to be 0.3 and 1.0 μg/ml, respectively. The method is linear within the range of 1–15 μg/ml for both the compounds. These mesylate esters were not found in three different batches of pure and pharmaceutical formulations of INM.

Keywords: Development; Validation; Mesylate esters; Imatinib mesylate

1. Introduction

The potential health hazards of two mesylate esters viz. methyl methanesulfonate (MMS) [1] and ethyl methanesulfonate (EMS) [2] in pharmaceuticals have attracted the attention of regulatory authorities because these are known carcinogens and genotoxins [3]. Their presence in the pharmaceuticals is due to the formation of by-products resulting from the reaction between methanesulfonic acid (MSA) which is used as a counter ion in drug molecule and methanol or ethanol used as a solvent in the manufacturing process. Genotoxins are limited to a daily dose of 1.5 μg/day as per International Conference on Harmonisation (ICH) guidelines from the European Medicines Agency [4,5].

Imatinib mesylate, chemically known as 4-[(4-methyl-1-piperazinyl)methyl]-*N*-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]benzamide methanesulfonate (INM) [6], is approved by the FDA for the treatment of a rare form of cancer called gastrointestinal stromal tumor. It blocks a different abnormal enzyme found on the tumor cells, there by curing the disease. It is also used for the treatment of newly diagnosed adult patients with philadelphia chromosome positive chronic myeloid leukemia (CML) in blast crisis, accelerated phase or in chronic phase after failure of interferon-alpha therapy, and pediatric patients with Ph+ chronic phase CML whose disease has recurred after stem cell transplant or who are resistant to interferon-alpha therapy. Since, INM is generally used to cure cancer, the presence of carcinogenic and genotoxic impurities like MMS and EMS in it (Scheme 1), may affect adversely. Hence, in order to meet the regulatory requirements, it is essential to develop a sensitive analytical method that can identify and determine MMS and EMS in INM.

Ramjitt et al. [7] reported a capillary GC–MS method for the determination of MMS and EMS in pharmaceuticals. In this method, acetonitrile was used as solvent for the dissolution. Since, INM is insoluble in acetonitrile, this method is not suitable for the analysis of INM. In addition, this method was not validated. Lee et al. [8] also reported a GC–MS method

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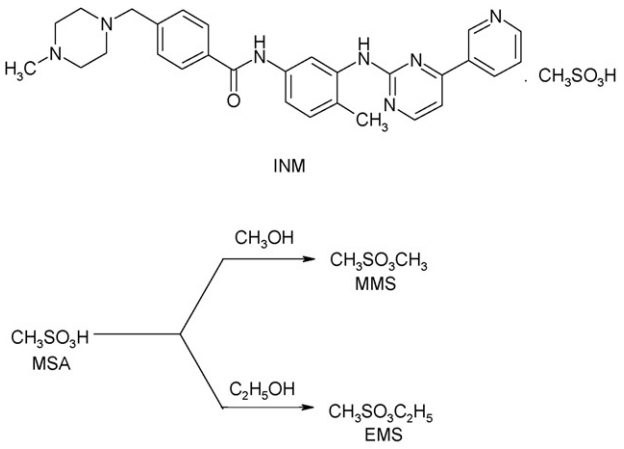

Scheme 1. Formation of MMS and EMS from INM.

which involves the derivatization of mesylate esters with aqueous sodium thiocyante and the analysis of resulting products with an LOQ value of 1 μg/g. However, this method suffers from the resulting derivatized products viz. alkyl thiocyanates and isothiocyantes which are volatile and irritant in nature. Li [9], developed a GC method with FID detector for the quantification of these two esters in pharmaceuticals. Since, the LOQ value was found to be 5 μg/g, it was suggested to go for GC–MS method to quantify these two esters below this level. Since, no method was reported so far for the identification and determination of MMS and EMS in INM, an attempt was made to overcome the shortcomings of the existing methods and succeeded in developing a highly sensitive, undervatized GC–MS method by using a *n*-hexane, the solvent in which INM is freely soluble with 1 μg/ml limit of quantitation of MMS and EMS in 10 mg/ml of INM.

2. Experimental

2.1. Materials

All reagents and solvents were of analytical grade. MMS, EMS and *n*-hexane were purchased from Sigma-Aldrich Chemicals Pvt. Ltd. New Delhi, India. Pure and pharmaceutical formulations of imatinib mesylate were obtained from R&D and formulation divisions of Hetero Drugs Ltd., Hyderabad India.

2.2. Optimized GC–MS conditions

Analysis was carried out on a GC system coupled with quadrupole mass spectrometer (GCMS-QP2010, Shimadzu Corporation, Japan). The compounds were separated on DB-1 capillary column (Agilent Technologies, USA, 30 m × 0.25 mm i.d. × 0.25 μm film). Two microliters volume with 1:200 split inlet was selected for injection. The GC oven temperature program utilized an initial temperature of 80 °C and an initial holding time of 1 min, then increased at 20 °C/min to 200 °C. The final temperature was held for 1 min. The injection temperature, GC–MS interface and ion source temperatures were 140, 250 and 250 °C, respectively. Helium was used as the carrier gas with a flow rate of 3 ml/min. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the *m/z* scan range of 35–130 amu. GCMS solution software (Version 2.50) was used for gas chromatographic as well as mass spectral analysis. Compounds were identified using the National Institute of Standard Technology mass spectral library.

2.3. Sample preparation

MMS solution was prepared by diluting 7.7 μl (10 mg) solution to 10 ml of *n*-hexane. EMS solution was prepared by diluting 8.5 μl (10 mg) solution to 10 ml of *n*-hexane. MMS and EMS mixture solution was prepared by diluting 7.7 and 8.5 μl solutions to 10 ml of *n*-hexane. One milliliter each of the solutions were further diluted separately to 100 ml with the same solvent to get 10 μg/ml solutions. This solution was further diluted to get the solutions in the linearity range of 1–15 μg/ml. The MMS and EMS solutions (1, 10, 15 μg/ml) spiked to 10 mg/ml of INM was prepared by adding appropriate volumes of MMS and EMS mixture solutions to 100 mg of pure INM solid (or tablet powder equivalent to 100 mg of INM) in 10 ml volumetric flask and making the volume up to the mark with *n*-hexane (blank).

3. Results and discussion

3.1. Method development

MMS and EMS are liquids at ambient temperature with a boiling point around 200 °C, hence it was planned to separate these compounds by gas chromatography, identify and confirm them by mass spectrometry. Three solvents viz. acetonitrile, methylene chloride and *n*-hexane were tried for dissolving MMS, EMS and INM. INM is insoluble in acetonitrile. *n*-Hexane was used as solvent because of low noise and the absence of interfering peaks on the chromatogram at the retention times of MMS and EMS when compared to methylene chloride. The experiments were carried out initially by using DB-5 column (5% phenyl–95% dimethylpolysiolxane) for the separation of MMS and EMS, but the peak shapes are not good. Then, this column was replaced by DB-1 column (100% dimethylpolysiolxane) and sharp peaks were observed. The effect of injection volume on separation and determination was investigated by injecting 10 μg/ml mixture solution of MMS and EMS in the range of 1–5 μl and the results indicated that the peak widths of the MMS and EMS are independent of injection volume. Hence, an optimum injection volume of 2 μl was chosen. The split ratio was fixed as 1:200 depending on the detector response. The effect of initial column temperature on the separation of the mesylate esters was investigated. An initial column temperature of 80 °C was chosen, which allowed baseline separation of MMS and EMS from each other.

3.2. Method validation

The present method is validated as per International Conference on Harmonization (ICH) guidelines for analytical method validation [10,11]. Standard solutions of MMS and EMS (10 μg/ml) were injected individually and the limits of detection

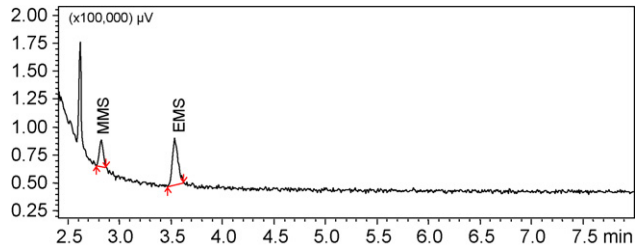

Fig. 1. GC–MS total ion chromatogram of 10 mg/ml of INM spiked with 1 μg/ml each of MMS and EMS.

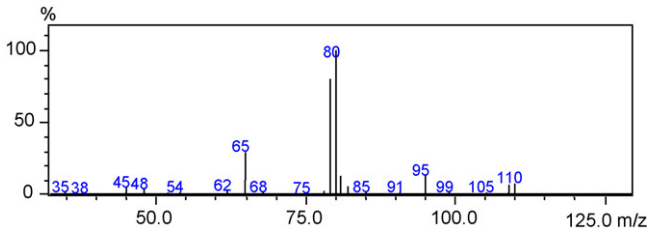

Fig. 2. Mass spectrum of MMS.

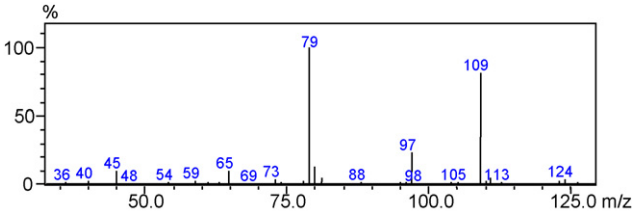

Fig. 3. Mass spectrum of EMS.

and quantitation values for both the mesylate esters were found to be 0.3 and 1 μg/ml, respectively. Calibration curves were drawn between the peak areas versus the concentration of MMS ($y = 28{,}897x - 5332$) and EMS ($y = 67{,}427x - 12{,}890$) separately in the range of 1–15 μg/ml. The correlation coefficient values of MMS (0.9998) and EMS (0.9996) indicate the best linearity of the method. The precision of the method was evaluated by calculating the relative standard deviation (%R.S.D.) of six replicate determinations by injecting freshly prepared 1 μg/ml solutions of MMS and EMS separately on the same day and the %R.S.D. values were found to be 3.51 and 3.58, respectively. For intermediate precision, 1 μg/ml solutions of MMS and EMS were injected separately on six different days and the %R.S.D. values were found to be 3.74, and 3.94, respectively. The low %R.S.D. values via peak areas confirm the good precision of the developed method. The accuracy of the method was determined by spiking the adequate volumes of MMS and EMS mixture (Fig. 1) at three concentration levels (1, 10 and 15 μg/ml) to 100 mg of three different pure INM samples separately and making the volume with 10 ml with *n*-hexane. The recovery data presented in Table 1 indicates the accuracy of the method. The specificity of method was tested through recovery studies of MMS and EMS (Table 1) which were performed as above with three different batches of INM tablets. Since, no extra amount of MMS and EMS than added was found in pure and pharmaceutical formulations of INM, it was concluded that they do not contain MMS and EMS. This also indicates the specificity of the method because the excipients do not interfere in the method. In the varied gas chromatographic conditions of ±10% on the carrier gas flow, ±5 °C on the initial oven temperature, ±1 °C/min on the ramp rate, the retention times and peak areas were found to be same indicating the robustness of the method.

3.3. Mass spectral analysis

The retention times of 2.82 and 3.53 min were observed for MMS and EMS, respectively, from the GC–MS experiments. The mass spectra of MMS (Fig. 2) and EMS (Fig. 3) derived from the total ion chromatogram, confirm the presence of these mesylate esters. The mass spectrum of MMS shows a parent peak at *m/z* 110 corresponding to the molecular formula $C_2H_6O_3S$. This contains peaks corresponding to major fragments at *m/z* values of 110, 109, 95, 80, 79 and 65. Similarly, the mass spectrum of EMS shows a parent peak at *m/z* 124 corresponding to the molecular formula $C_3H_8O_3S$. This contains peaks corresponding to major fragments at *m/z* values of 124, 123, 109, 97, 79 and 65. The mass fragments of MMS and EMS exactly matched with those of the reported ones [7].

4. Conclusion

The developed GC–MS method is specific for MMS and EMS because mass detector is used for confirming their presence in INM. This method is superior over the existing methods because, it is undervatized, highly sensitive method that can quantify MMS and EMS up to 1 μg/ml in 10 mg/ml INM solution, uses *n*-hexane as solvent in which INM is

Table 1
Evaluation of accuracy and specificity of the proposed method

INM spiked with MMS and EMS	Recovery of MMS, % (mean ± %R.S.D.)[a]			Recovery of EMS, % (mean ± %R.S.D.)[a]		
	1 μg/ml level	10 μg/ml level	15 μg/ml level	1 μg/ml level	10 μg/ml level	15 μg/ml level
Pure sample-1	99.6 ± 1.25	99.2 ± 1.28	98.9 ± 1.13	98.1 ± 1.19	98.7 ± 1.26	98.2 ± 1.15
Pure sample-2	98.3 ± 1.19	99.2 ± 1.09	99.2 ± 1.11	98.3 ± 1.24	99.1 ± 1.22	99.5 ± 1.21
Pure sample-3	99.6 ± 1.10	99.1 ± 1.18	99.4 ± 1.12	98.1 ± 1.11	98.5 ± 1.16	98.1 ± 1.22
Tablets-1	98.2 ± 1.24	98.7 ± 1.36	99.3 ± 1.39	98.5 ± 1.38	98.5 ± 1.38	99.2 ± 1.33
Tablets-2	98.6 ± 1.25	98.1 ± 1.13	98.4 ± 1.20	99.6 ± 1.33	98.4 ± 1.39	98.4 ± 1.31
Tablets-3	99.3 ± 1.27	99.2 ± 1.34	98.6 ± 1.21	98.8 ± 1.31	99.1 ± 1.31	98.7 ± 1.28

[a] Mean value of three determinations.

freely soluble and validated as per ICH guidelines. Hence, the present method can be successfully used in quality control laboratories for the identification and determination of unexpected mesylate esters formed during the synthesis of imatinib mesylate.

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