Biological Forum – An International Journal **5**(1): 68-72 (2013)

ISSN No. (Print) : 0975-1130
ISSN No. (Online) : 2249-3239

Determination of MMS, EMS and IPMS content in Imatinib Mesylate by Gas Chromatography

Ravi R. Yadav and Manoj D. Rokade
Department of Chemistry,
Jagdishprasad Jhabarmal Tibrewala University,
Vidyanagari, Jhunjhunu Churu, Rajasthan, 333001, India

(Received 05 February, 2013, Accepted 30 March, 2013)

ABSTRACT: High sensitive rapid gas chromatography method has been developed for the determination of three carcinogenic and Genotoxic mesylate esters *viz.* Methyl methane sulphonate (MMS), Ethyl methane sulphonate (EMS) and isopropyl methane sulphonate (IPMS). The optimum separation was achieved between methyl methanesulfonate, ethyl methanesulfonate and isopropyl methanesulfonate on a DB-5 (60 m×0.32 mm×5.0 µm) capillary column under programming temperature. Carbon tetra chloride was used as diluent. This method was validated as per International Conference on Harmonization guidelines (Q2R1). The limit of quantitation of methyl methanesulfonate, ethyl methanesulfonate and isopropyl methanesulfonate is 2.5 ppm with respect to 1g/ml of Imatinib mesylate.

Keywords: Methyl Methane Sulphonate, Ethyl Methyl Sulphonate, Isopropyl Methane Sulphonate, Imatinib Mesylate, Gas chromatography

INTRODUCTION

Alkyl mesylate esters of short chain alcohols are reactive, genotoxic and possibly carcinogenic alkylating agents. Regulatory authorities made it mandatory to study and submit the impurity profile for the active pharmaceutical ingredients (APIs) [1-9].Imatinib Mesylate Chemically known as 4-[(4-Methyl-1-piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl-2pyrimidinyl] amino]phenyl] benzamide methane sulfonate is approved by the Food and Drug Administration (FDA) for the treatment of a rare form of cancer called gastrointestinal stromal tumor. It blocks a different abnormal enzymes found on the tumor cells, thereby curing the disease. It also used for the treatment of newly diagonosed adult patients with Philadelphia chromosome positive chronic myeloid leukemia (CML) is blast crisis, accelerated phase or in chronic phase after failure of interferon-alpha therapy and pediatric patients with Ph^+ chronic phase whose disease has recurred after stem cell transplant or who are resistant to interferon alpha therapy. Since Imatinib is generally used for curing cancer, the presence of carcinogenic and genotoxic impurities like MMS, EMS and IMS in it, 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, EMS and IPMS in Imatinib mesylate.

As per ICH M7 guideline for control genotoxic impurities in pharmaceuticals the limit will be correspondingly depend on the intake level for the life time. (The calculation for less than lifetime acceptable intake is predicated on the principle of Haber's rule, a fundamental concept in toxicology where concentration (C) x time (T) = constant (K)). Therefore, the carcinogenic effect is based on both dose and duration of exposure. The recommended daily dose of Imatinib mesylate is 800 mg/day for a span of 1-6 months. So as per ICH M7 the limit for genotoxic impurities in Imatinib mesylate should be 120µg. In the current method the limit level was set to 50µg.[11]

MATERIAL AND METHODS

Methyl Methane Sulfonate, Ethyl Methane Sulfonate and Isopropyl (MMS, EMS and IPMS respectively) were purchased from SRL, Spectrochem and across. Carbon tetra chloride were procured from SD Fine Ltd., Gift sample of Imatinib mesylate was obtained Triveni Interchem Pvt Ltd from Vapi, India.

MMS, EMS and IPMS stock solutions were prepared by dissolving 100 mg individually in 100 ml of diluent. Carbon tetra chloride was used as diluent. MMS, EMS and IPMS mixture solution 10 ppm was prepared by diluting the appropriate volume of above stock solution with diluent.

Gas chromatography analysis was carried out on Perkin Elmer system (Clarus 500) with head space sampler (Turbo matrix 40) having total chrome software. MMS, EMS and IPMS were separated on DB-5 capillary column (Agilent Technologies, USA, 60 m×0.32 mm i.d.×5.0 µm film). One ml (Head Space) of 10 ppm mixture solution with 1:5 split inlets was selected for injection. The GC oven temperature program utilized an initial temperature of 90° and an initial holding time of 1 min, and then increased at 5°/min to 180°. The final temperature was held for 5 min.

The injector and detector temperature for GC were 220°C and 240°C respectively. The attenuation was -4 and range was 1. Nitrogen was used as the carrier gas with a flow rate of 1.9 ml/min. The parameter set for head space sampler was.

Oven (^{0}C)	95
Needle (^{0}C)	105
Transfer (^{0}C)	110
Thermostat time (min)	20
Injection (ml)	0.2
Withdrawing time(min)	0.2
Pressurizing time (min)	3.0
GC cycle time(min)	According to GC run time
Shaker	Enable
Vial vent	Enable
Operating mode	Constant
Injection mode	Volume
Column pressure	38

The elution order observed was MMS, EMS and IPMS (Fig 2).

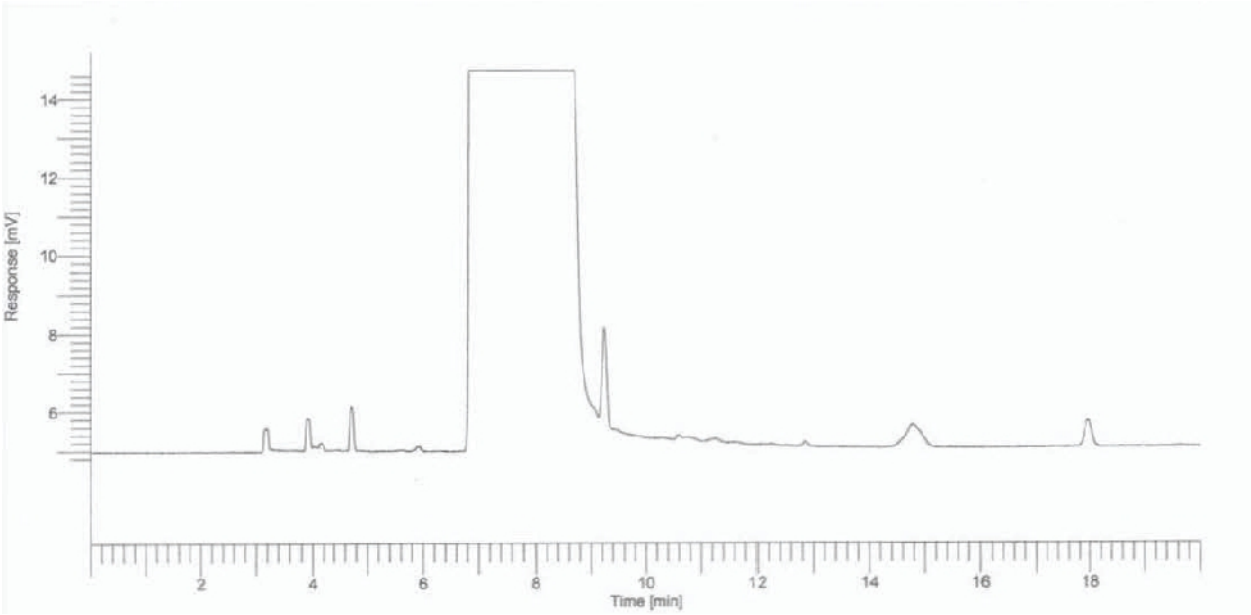

Fig. 1. Gas chromatogram of Blank solution.

MMS, EMS and IPMS are liquids, hence it was planned to separate them by gas chromatography. Imatinib mesylate is insoluble in neutral/alkaline aqueous solution. Initially the experiments were carried out by using DB-624 column (Mid polar column) for the separation of MMS, EMS and IPMS, but the resolutions and peak shape were found to be very poor. Then, this column was replaced by DB-1 column and same result was found. Hence, DB-5 column (5%-phenyl-95%-dimethylpolysiloxane) was used and good resolutions were observed. An optimum injection volume of 1ml (Head space) was chosen. The split ratio was fixed as 1:5 depending on the detector response. An initial column temperature of 90° was found to be optimum.

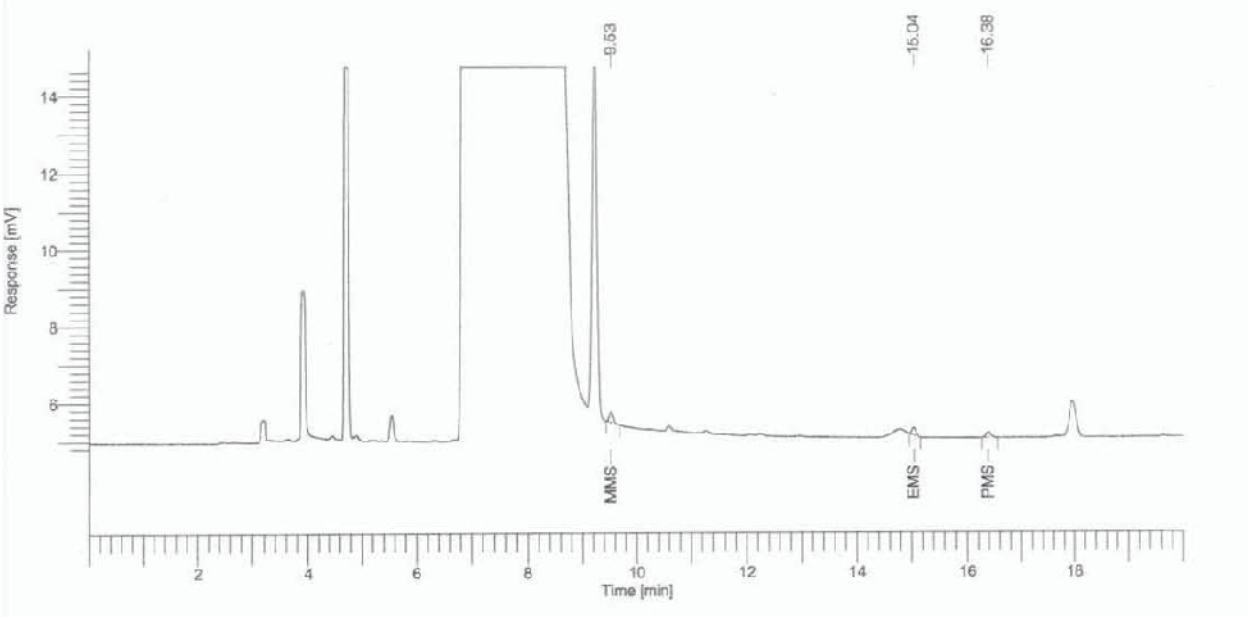

Fig. 2. Gas chromatogram for MMS, EMS and IPMS.

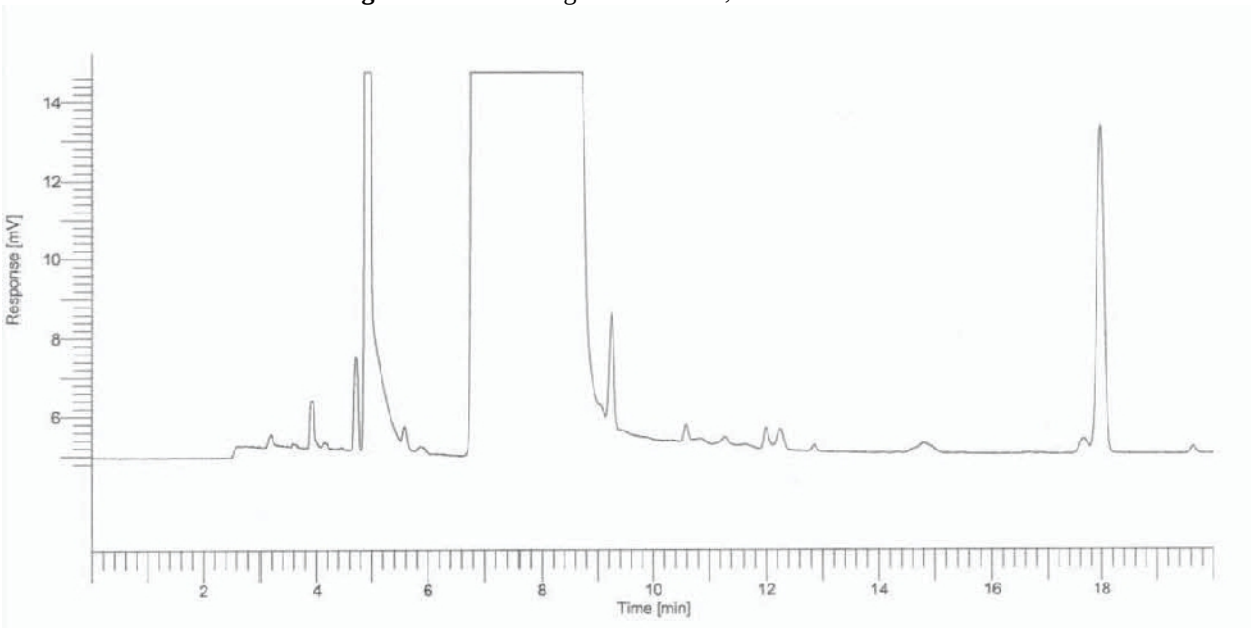

Fig. 3. Gas chromatogram for Imatinib mesylate.

RESULTS AND DISCUSSION

The present method is validated as per ICH guidelines Q2 (R1)[10]. MMS, EMS and IPMS mixture solution (10 ppm) was injected and the limit of detection (LOD) and the limit of quantification (LOQ) values were determined at the lowest concentrations at which signal-to-noise ratio is 3 and 10, respectively. LOD and LOQ values for MMS, EMS and IPMS were found to be 1 and 2.5 ppm respectively. Linearity of the method was checked by plotting calibration curves between the peak areas versus the concentration of MMS, EMS and IPMS over the range 2.5-75 ppm. The slope, intercept and correlation coefficient values were derived from liner least-square regression treatment. The correlation coefficient values reported in (Table 1) indicate the best linearity of the method.

The precision of the method was evaluated in terms of repeatability and intermediate precision. The repeatability is determined by calculating the relative standard deviation (% RSD) of six replicate determinations by injecting freshly prepared 50 ppm mixture solution separately on the same day. For intermediate precision, 50 ppm mixture solution was injected on six different days. The low % RSD values via peak areas confirm the good precision of the developed method (Table 1). MMS, EMS and IPMS were not detected when three pure R&D samples of Imatinib mesylate (1 g/ml) were analyzed in the present method. Hence, the accuracy of the method was determined by spiking MMS, EMS and IPMS mixture at three concentration levels (25, 50 and 75 ppm) to 1 g of Imatinib Mesylate and making the volume to 5 ml with diluent. Each determination was carried out for three times. The recovery data presented in (Table 1) indicates the accuracy of the method. The blank and standard chromatograms are shown in fig 1 and fig 2. In the varied gas chromatographic conditions of ±5° on the carrier gas flow, ±5° on the initial oven temperature, ±1°/ min on the ramp rate, the retention times and peak areas of MMS, EMS and IPMS were found to be same indicating the robustness of the method.

Table 1: **Analytical Data**.

Parameter	**MMS**	**EMS**	**IPMS**
LOD (ppm)	1	1	1
LOQ (ppm)	2.5	2.5	2.5
Linearity Range (ppm)	2.5-75	2.5-75	2.5-75
Correlation Coefficient	0.999	0.998	0.999
% Y Intercept	-2.8	+1.9	+2.0
Intermediate Precision(%RSD)	1.2	2.1	0.9
% Recovery			
25 ppm	94.0-98.5 %	102.1-105.0 %	98.0-97.7 %
50 ppm	99.0-93.3 %	100.1-102.0 %	99.5-98.3 %
75 ppm	99.8-100.6 %	99.8-100.2 %	98.9-100.3 %

The aim of this study is to develop a GC method that can quantify MMS, EMS and IPMS in Imatinib Mesylate. The developed GC method was optimized based on the resolutions of MMS, EMS and IPMS peaks and validated as per ICH guidelines. The method well suits for the intended purpose.

REFERENCES

McGovern, T. & Jacobson-Kram, D. (2006). Regulation of genotoxic and carcinogenic, Impurities in drug substances and products. Trends in Analytical Chemistry, Vol. **25**, pp 790-795.

Dearfield, K. L., Cimino, M. C., McCarroll, N. E., Mauer, I. & Valcovic, L. R. (2002).Genotoxicity risk assessment: a proposed classification strategy. *Mutation Research -Genetic Toxicology and Environmental Mutagenesis*, Vol. **521**, pp. 121–135.

Robinson, D.I. (2010). Control of genotoxic impurities in active pharmaceutical ingredients: A review and perspective. *Organic Process Research and Development*, Vol. **14**, pp 946-959.

Ashby, J. (1990). Determination of the genotoxic status of a chemical. *Mutation Research*, Vol. **248,** pp 221-231.

Elder, D. P. & Snodin, D. J. (2009). Drug substances presented as sulfonic acid salts:Overview of utility, safety and regulation. *Journal of Pharmacy and Pharmacology*,Vol. **61**, pp 269-278.

Snodin, D.J. (2006). Residues of genotoxic alkyl mesylates in mesylate salt drug substances: real or imaginary problems? *Regulatory Toxicology and Pharmacology*, Vol. **45**, pp 79-90.

Teasdale, A., Eyley, S. C., Delaney, E., Jacq, K., Taylor-Worth, K., Lipczynski, A., Reif, V., Elder, D.P., Facchine, K..L., Golec, S., Oestrich, R.S., Sandra, P. & David, F. (2009).

Mechanism and processing parameters affecting the formation of Methyl methane sulfonate from methanol and methanesulfonic acid: an illustrative example for sulfonate ester impurity formation. *Organic process Research and development*, Vol. **13**, pp. 429-433.

Elder, D.P. & Snodin, D.J. (2009). Drug substances presented as sulfonic acid salts: Overview of utility, safety and regulation. *Journal of Pharmacy and Pharmacology*, Vol. **61**, pp 269-278.

www.ICH.org, Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use, S2 (R1).

www.ICH.org, Validation of analytical procedure, Text and methodology Q2 (R1).

ICH guideline M7 on assessment and control of DNA reactive (Mutagenic) impurities in pharmaceutical carcinogenic risk, EMA/CHMP/ICH/83812/2013.