ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 5, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

DEVELOPMENT AND VALIDATION OF A GAS CHROMATOGRAPHY-MASS SPECTROMETRY WITH SELECTED ION MONITORING METHOD FOR THE DETERMINATION OF TRACE LEVELS OF METHANE SULFONYL CHLORIDE AS AN IMPURITY IN ITRACONAZOLE ACTIVE PHARMACEUTICAL INGREDIENT

MANNEM DURGA BABU*, SURENDRABABU K, KISHORE M

Department of Post-graduate Chemistry, SVRM College (Autonomous), Nagaram, Andhra Pradesh, India.
Email: m.durgababu1989@gmail.com

Received: 13 May 2016, Revised and Accepted: 24 May 2016

ABSTRACT

Objective: To develop an accurate, precise, and linear gas chromatographic-mass spectrometry selective ion monitoring (SIM) method for quantitative estimation of methane sulfonyl chloride (MSC) as an impurity in itraconazole (ICR) active pharmaceutical ingredient (API) at ppm level and validated as per International Council of Harmonization (ICH) guidelines.

Methods: This method used in SIM mode mass selective detection was developed and validated for the trace level analysis of an impurity. Chromatographic separation of MSC was achieved in 5.45 minutes and m/z value was 79 on SIM mode, ZB-5 ms 30 m × 0.25 mm × 0.25 µm column, using helium carrier gas with 1.0 ml/min.

Results: The method was linear for MSC in ICR 1.90-7.5 µg/ml, respectively. The coefficient of correlation (r^2) for the MSC was better than 0.999. The limit of detection and limit of quantification (LOQ) obtained were 0.44 and 1.32 µg/ml. The method was fully validated, complying Food and Drug Administration, ICH, and European Medicines Agency guidelines. Furthermore, verified precision, accuracy, LOQ precision, LOQ accuracy, ruggedness, and robustness.

Conclusion: The methods were successfully validated to determination and quantification of MSC in ICR API. Hence, the method holds good for the routine trace analysis of MSC in ICR and various pharmaceutical industries as well as academics.

Keywords: Methane sulfonyl chloride, Itraconazole, Gas chromatography-mass spectrometry, Method development, Method validation.

INTRODUCTION

Numerous analytical methods for the determination of pharmaceuticals and their metabolites in aqueous solutions have been described in the literature. Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-MS (GC-MS) are the most widely used techniques [1]. An MS is typically utilized in one of two-ways: Full scan or selected ion monitoring (SIM). The typical GC-MS instrument is capable of performing both functions either individually or concomitantly depending on the setup of the particular instrument. The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum in SIM certain ion fragments are entered into the instrument method, and only those mass fragments are detected by the MS. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g., three fragments) during each scan [2]. SIM mode the MS is "targeting a limited mass range," the number of scans across the peak has increased resulting in better peak shape. This is an easy solution for getting better quantitation for early eluting peaks. Inspect the ions obtained for the peak in full scan mode and use at least one of the ions in SIM to obtain a better scan rate [3].

Methane sulfonyl chloride (MSC) (Fig. 1) is an organosulfur compound with the formula CH_3SO_2Cl. It is a colorless liquid that dissolves in polar organic solvents but is reactive toward water, alcohols, and many amines. During the manufacturing process of itraconazole, formation of MSC is possible due to residual methanol available in the manufacturing process and may also be formed due to thermal interaction in the presence of methanol.

MSC is a potential genotoxic impurity in itraconazole (ICR) drug substance as it was the part of the synthesis process. As per the International Conference on Harmonization Guidelines from European Medical Agency, the genotoxins were to be limited to 1.5 µg/day [4,5]. MSC is having chloro as a functional group with aliphatic chain as per the guideline it is a genotoxic alerting compound. Sensitive method for the analysis of ICR as genotoxic impurity was not available. While developing method at such a low-level, interferences due to drug substance as well as other process impurities and degradation products were the major problems in achieving specificity. Hence, based on published general strategies for genotoxic impurities and the threshold of toxicological concern, MSC was evaluated in ICR drug substance.

ICR (Fig. 2) is a classical member of the triazole class and is an important drug in our arsenal to treat fungal infections because it exhibits broad-spectrum antifungal activity [4-7]. ICR, (+-)-ics-4[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy] phenyl]-1-piperazinyl] phenyl]-2,4-dihydro-2(1-methylpropyl)-3H-1,2,4-triazol-3-one, is an orally active triazole antifungal agent which demonstrates broad-spectrum activity against a number of fungal species including dermatophytes. It has been demonstrated that GC-MS method offers several advantages over high-performance LC (HPLC) method including better sensitivity, specificity, and higher throughput. This paper presents a highly specific and sensitive GC-MS method for the MSC in ICR active pharmaceutical ingredient (API) as per International Council of Harmonization guidelines [11]. This approach eliminated the time-consuming liquid-liquid extraction used in HPLC-ultraviolet method, increased the detection limit, and greatly reduced sample processing and instrument acquisition time. Thus, the paper reports an economical, simple, and accurate GC-MS method for MSC in ICR.

METHODS

Apparatus

GC-MS analysis was carried out on GC-MS-QP 2010 plus system (Shimadzu) having GC-MS Solutions software, an analytical balance (XS 205 from Mettler Toledo) and autopipette (100 µL - 1000 µL from Eppendorf) were used. The GC-MS experimental conditions for MSC content in ICR as shown in Table 1.

Chemicals and reagents

MSC was purchased from Sigma-Aldrich, Fluka, Acros Organics. Dichloromethane was procured from Rankem (HPLC grade). Pure sample of ICR was obtained from Local Research Laboratory.

Preparation of standard solution

Diluent

Dichloromethane is an organic compound with the formula CH_2Cl_2. This colorless, volatile liquid with a moderately sweet aroma is widely used as a solvent. Although it is not miscible with water, it is miscible with many organic solvents. Dichloromethane was selected as the standard and sample diluent because of its ability to dissolve a wide variety of substance.

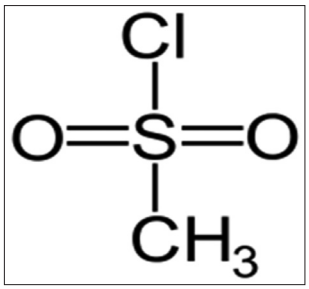

Fig. 1: Chemical structure of methane sulfonyl chloride

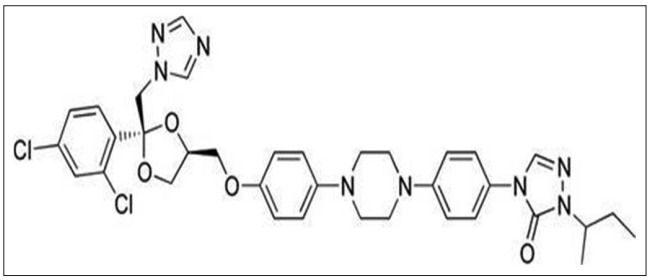

Fig. 2: Chemical structure of itraconazole

Table 1: GC-MS experimental conditions

Column	ZB-5MS, 30 m×0.25 mm ID×0.25 µm
Injector temperature	150°C
Carrier gas	Helium
Carrier gas flow	1.0 mL/min
Split ratio	5:00
Oven program	Initial temperature 40°C hold time 4.0 minutes raise 20.0°C/min up to 200°C hold time 13.00 minutes
Total run time	25.0 minutes
Injection volume	1.0 µl
Ionization source	EI
Electron energy	70 Ev
Source temperature	280°C
Interface temperature	260°C
m/z fragment	79
Solvent cut time	3.0 minutes
Detector voltage	0.92 KV
Start time	3.01 minutes
End time	8.0 minutes

GC-MS: Gas chromatographic-mass spectrometer

Preparation of standard stock solution

Weighed accurately 50 mg of MSC in 50 ml of volumetric flask dissolve and make up diluents (1000 µg/ml). Transfer 1.0 ml of above solution into a 100 ml volumetric flask make up with diluents (10 µg/ml).

Preparation of standard solution (1.87 µg/ml)

Transfer 3.74 ml from standard stock solution into a 20 ml volumetric flask and make up to the mark with the same diluents to get a standard solution (standard solution was prepared with respect to sample concentration).

Preparation of sample solution

Weighed accurately 10.0 g of the ICR API into 20 ml of volumetric flask, add 10 ml of diluents mix well then makeup with the same diluents (final concentration is 500 mg/ml of API).

RESULTS AND DISCUSSION

Method optimization

The various genotoxic impurities are present in API is the foremost prerequisite for successful method development in GC-MS. The successful method development should result in a fast, simple, and time efficient method that is capable of being utilized in a manufacturing setting. Following were the stepwise strategies for the method development in our case.

Column selection

The primary goal of column selection was to resolve a genotoxic impurity which is formed during the synthesis and manufacturing of ICR API. Several columns were initially investigated to finalize a single method for the separation and quantitation of solvent. Wall-coated capillary columns of various brands with a variety of phases and dimensions have been investigated, e.g. column A is ZB-624 (30 m length, 0.32 mm i.d. with a stationary phase of 6% cyanopropyl phenyl and 94% dimethyl polysiloxane film of 1.8 µm) and column B is ZB-5 MS (30 m length, 0.25 mm i.d. with a stationary phase of 5% cyanopropyl phenyl and 95% dimethyl polysiloxane film of 0.25 µm). In the above two columns, the response was found to be comparatively lower, and peak shapes were found to be satisfactory in column B. Finally, column B is proved to be the best column that could fulfill all the needs of the GC-MS method, i.e., higher sensitivity and shorter runtime.

MS analysis

As per the analysis conducted by GC-MS and the retention times of MSC was in the range 5.0-6.0 minutes, respectively. As per the MS of MSC, the fragments were observed at m/z 79. The spectrum of MSC the analytes match to the reference spectrum of NIST. The MS and reference MS of MSC shown in Fig. 3.

Method validation

The method validation was done by evaluating specificity, repeatability, linearity and range, accuracy, limit of detection (LOD) and limit of quantitation (LOQ), LOQ - repeatability, LOQ-accuracy, ruggedness, and robustness.

Specificity

The ICR API sample was spiked with MSC, and sample was chromatographed to examine interference, if any, of the residual solvent peaks with each other. The retention time for standard MSC 5.45 minutes, respectively. The chromatograms of blank, standard MSC, and ICR API were as shown in Fig. 4.

Repeatability

The MSC was prepared at 1.87 ppm absolute with respect to sample concentration and injected in six replicates. The relative standard deviation (RSD) (n=6) values obtained for the area of MSC is 40,452.

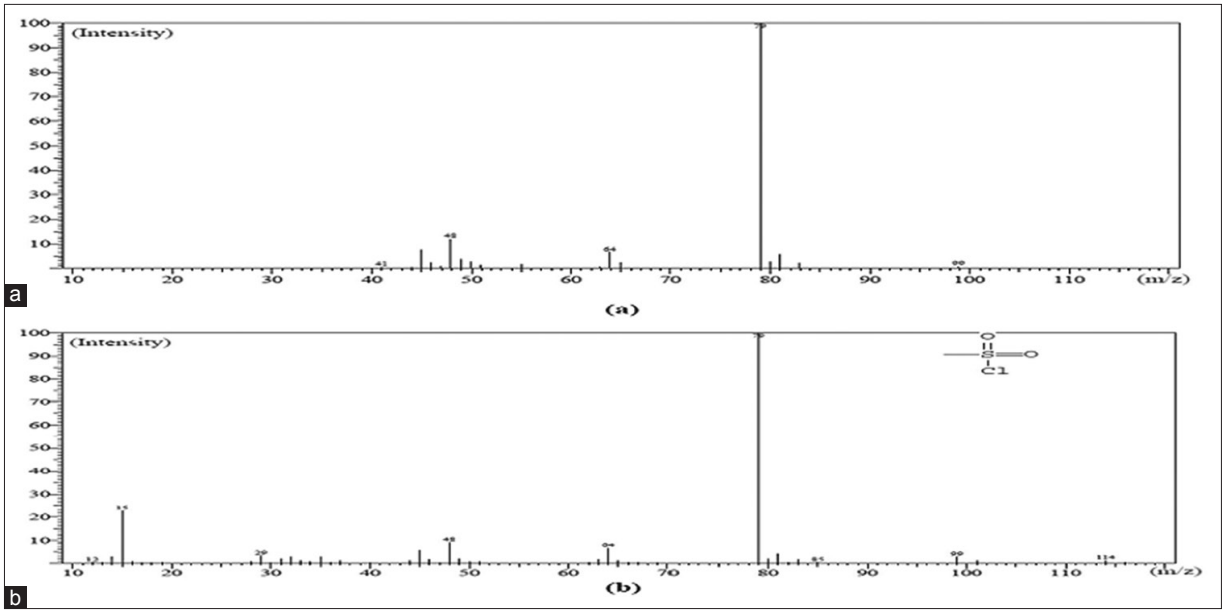

Fig. 3: (a) Mass spectrum (MS) of methane sulfonyl chloride (MSC) and (b) reference MS of MSC in NIST

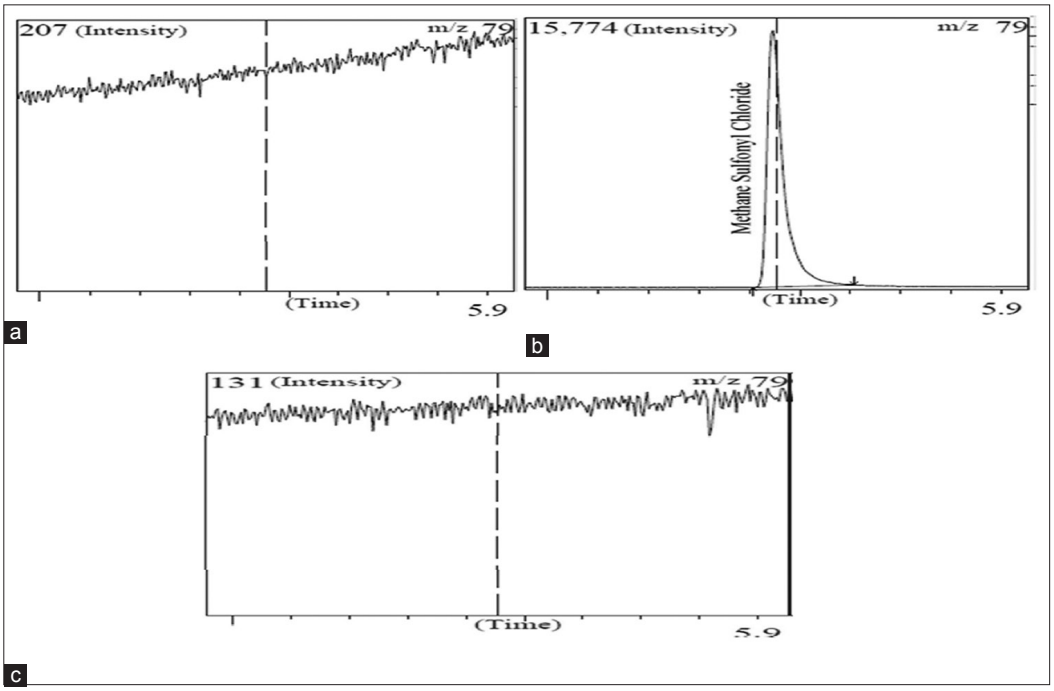

Fig. 4: Typical chromatograms of (a) Blank (dichloromethane), (b) methane sulfonyl chloride, (c) itraconazole active pharmaceutical ingredient

The % RSD for MSC peak area response of standard six injections should be not more than 15% as per United States Pharmacopoeia [12]. The data of repeatability were as shown in Table 2.

Linearity

The linearity of the method was determined by making injections of standard MSC at five concentration levels from 50% to 200%. Three replicates were performed at each level. The calibration curves were obtained with the average of peak area ratios of three replicates. The correlation coefficient (r^2) value for MSC was found to be higher than 0.999, and the calibration curves were linear within the range. These results revealed an excellent linearity. The linearity values for the MSC as shown in Table 3 and linearity graph is shown in Fig. 5.

Table 2: Repeatability data for MSC

Number of injections	MSC area
Injection 1	38,014
Injection 2	40,212
Injection 3	43,256
Injection 4	40,125
Injection 5	39,851
Injection 6	41,256
Average area	40,452
Standard deviation	1731
Percentage of RSD	4.28

RSD: Relative standard deviation, MSC: Methane sulfonyl chloride

Accuracy (% recovery)

Weighed accurately 10.0 g of the ICR API into three different 20 ml of volumetric flasks and spiked with 1.9, 3.75, and 5.6 µg/ml standard solutions of MSC, add 10 ml of diluents mix well then make up with the same diluents. Inject three levels in triplicate. From accuracy data, the % recovery of MSC was found within the limits (100±15%). Results indicate that the method has an acceptable level of accuracy. The results are presented in Table 4.

LOD and LOQ

The LOD and LOQ were calculated by instrumental and statistical methods. For the instrumental method, LOD is determined as the lowest amount to detect, and LOQ is the lowest amount to quantify by the detector. The LOD and LOQ of MSC in ICR API were determined based on linearity. Prepare the standard MSC solution at LOD (0.44 µg/ml) and LOQ (1.32 µg/ml) concentrations. The area of MSC at LOD concentration is 3985 and LOQ concentration 13134. The linearity also passed at LOQ concentration. The data of LOD and LOQ were as shown in Table 5.

Repeatability at LOQ concentration

Prepare the standard MSC solution at LOQ concentration (1.32 ppm) and injected in six replicates. The RSD (n=6) values obtained for the area of MSC is 13134. The acceptance criteria of % RSD for MSC are more than 15%. The LOQ repeatability data and chromatograms of LOD and LOQ were as shown in Table 6 and Fig. 6.

Accuracy at LOQ concentration

Weighed accurately 10.0 g of the ICR API into three different 20 ml of volumetric flasks and spiked with LOQ level (1.32 µg/ml) standard solution of MSC, add 10 ml of diluents mix well then make up with the same diluents and inject in triplicate. From accuracy data at LOQ level, the % recovery of MSC was found within the limits (100±15%). The data of LOQ-accuracy were as shown in Table 7.

Ruggedness

Ruggedness of the method was evaluated by performing the sample analysis in six replicates using different analyst on different days. The % RSD values of <15.0% for MSC content indicate that the method adopted is rugged. The data of ruggedness were shown in Table 8

Robustness

This study was performed by making small but deliberate variations in the method parameters. The effect of variations in flow rate of carrier gas and column oven temperature was studied. Under all the variations, system suitability requirement is found to be within the acceptance criteria and hence the proposed method is robust. The RSD of area counts for MSC peak obtained from six replicate injections of standard solution should be not more than 15.0%. The data of robustness were shown in Table 9.

CONCLUSION

A simple high throughput GC-MS method has been developed and fully validated for the determination of MSC in ICR API. This method is specific, sensitive, and reproducible and has been successfully to

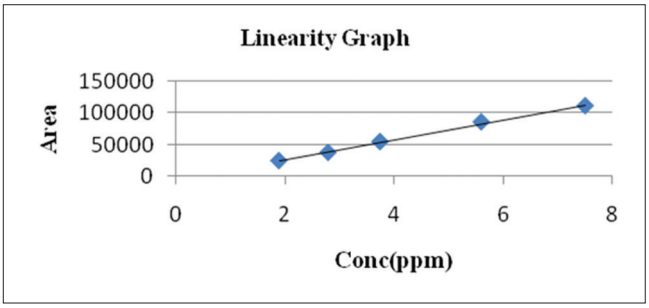

Fig. 5: Linearity graph of methane sulfonyl chloride

Table 3: Linearity data for MSC

S. No.	Concentration level (%)	Run-I area	Run-II area	Run-III area	Average area
1	50	24,122	23,999	24,911	24,344
2	75	37,528	37,241	37,002	37,257
3	100	54,851	54,113	53,989	54,318
4	150	85,521	85,426	85,422	85,456
5	200	110,826	110,852	111,001	110,893
6	Correlation coefficient (r^2)	0.999			

MSC: Methane sulfonyl chloride

Table 4: Accuracy data for MSC

S. No.	Sample+50% area	Sample+100% area	Sample+150% area
1	21,230	42,452	61,638
2	21,290	42,136	61,851
3	21,546	42,546	61,251
Average area	21,355	42,378	61,580
Percentage recovery	105.58	104.76	101.49
Standard average area	40,452		

MSC: Methane sulfonyl chloride

Table 5: Linearity data for MSC at LOQ concentration

S. No.	Concentration (%)	Area
1	50 (1.9 µg/ml)	24,344
2	75 (2.8 µg/ml)	37,257
3	100 (3.75 µg/ml)	54,318
4	150 (5.6 µg/ml)	85,456
5	200 (7.5 µg/ml)	110,893
Correlation coefficient (r^2)	0.999	
Slope	15,742	
STEYX	2078	
LOD	0.44 µg/ml	
LOQ	1.32 µg/ml	

LOQ: Limit of quantification, LOD: Limit of detection, MSC: Methane sulfonyl chloride

Table 6: Repeatability data for MSC at LOQ concentration

Number of injections	MSC area
Injection 1	12,024
Injection 2	13,884
Injection 3	12,076
Injection 4	15,470
Injection 5	11,292
Injection 6	14,058
Average area	13,134
Standard deviation	1589
Percentage of RSD	12.10

RSD: Relative standard deviation, LOQ: Limit of quantification, MSC: Methane sulfonyl chloride

Table 7: LOQ accuracy data for MSC

S. No.	Sample+LOQ level area
1	14,064
2	13,697
3	14,686
Average area	14,149
Percentage recovery	13,134
Standard average area	107.73%

LOQ: Limit of quantification, MSC: Methane sulfonyl chloride

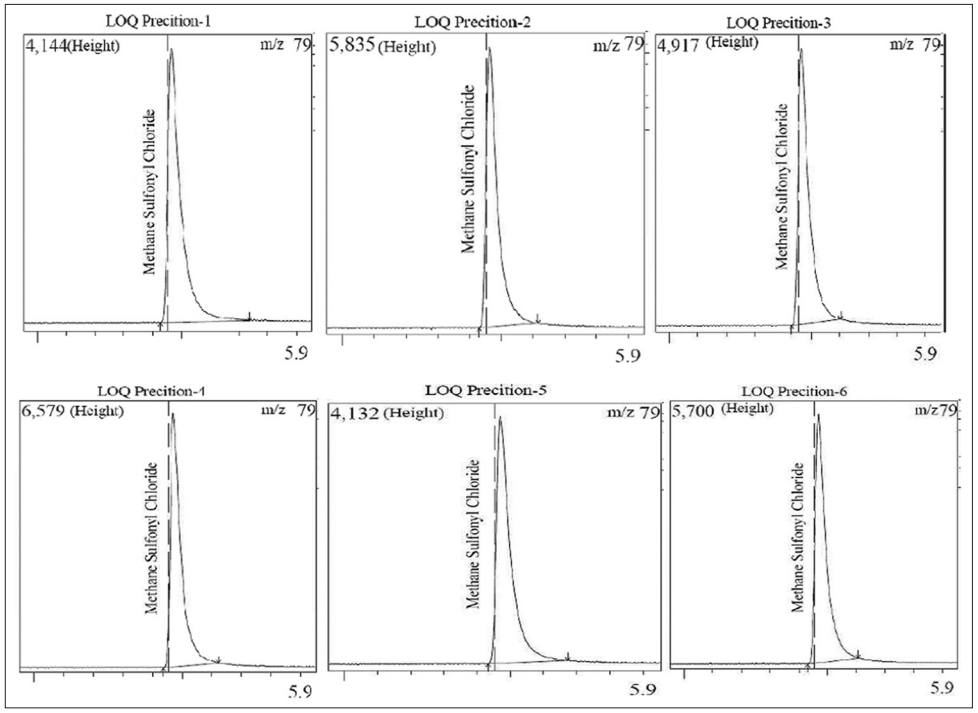

Fig. 6: Typical % relative standard deviation chromatograms at limit of quantification concentration

Table 8: Result of ruggedness

Day-1 (% RSD)			Day-2 (% RSD)			Analyst-1 (% RSD)	Analyst-2 (% RSD)
Analyst-1	**Analyst-2**	**Analyst-1 and Analyst-2**	**Analyst-1**	**Analyst-2**	**Analyst-1 and Analyst-2**	**Day-1 and 2**	**Day-1 and 2**
6.09	5.01	5.32	4.84	4.91	4.69	5.44	4.77

RSD: Relative standard deviation

Table 9: Result of robustness

Flow variation			
Parameter	0.5 ml/min (flow minus)	1.0 ml/min (control)	1.5 ml/min (flow plus)
Percentage of RSD	5.69	5.28	5.58
Column oven temperature			
Parameter	195°C (temperature minus)	200°C (control)	205°C (temperature plus)
Percentage of RSD	4.83	4.71	4.39

RSD: Relative standard deviation

monitor and control impurity level. The residue MSC was determined in ppm levels also. The method well suits for the intended purpose.

ACNOWLEDGMENT

The author expresses sincere thanks to the Principal and Head of the Chemistry Department, SVRM College, for excellent guidance and encouragement.

REFERENCES

1. Verenitch SS, Lowe CJ, Mazumder A. Determination of acidic drugs and caffeine in municipal wastewaters and receiving waters by gas chromatography-ion trap tandem mass spectrometry. J Chromatogr A 2006;1116(1-2):193-203.
2. Available from: https://www.en.wikipedia.org/wiki/Gas_chromatography%E2%80%93 mass_spectrometry.
3. Available from: http://www.sge.com/uploads/55/6a/556ae4e55c042c95832191d30557f017/TA-0061-C.pdf.
4. The European Agency for the Evaluation of Medicinal Products, ICH Topic S1B, Note for Guidance on Carcinogenicity: Testing for Carcinogenicity of Pharmaceuticals; 1998.
5. European Medicines Agency, Evaluation of Medicines for Human Use, Guideline on the Limits of Genotoxic Impurities; 2007.
6. Dileep Kumar M. Analysis of Genotoxic Impurities in API's in Compliance with ICH Guidelines using Gas Chromatography Mass Spectrometry. Waltham, United States: Centre of Excellence for Analytical Sciences Perkin Elmer (India.) Pvt., Ltd.
7. Zhao L, Quimby B. Analysis of Drugs by using the Ultra Inert Inlet. Liners with Wool. Wilmington, DE, USA: Agilent Technologies, Inc.
8. Bari SB, Kadam BR, Jaiswal YS, Shirkhedkar AA. Impurity profile: Significance in active pharmaceutical ingredient. Eurasian J Anal Chem 2007;2(1):33-53.
9. Veenaeesh P, Manikumar G, Manjusha. P, Padmasri A. Determination of methyl methane sulfonate, ethyl methane sulfonate and isopropyl methane sulfonate impurities in Lopinavir API by GC/MS/MS using electron ionization. Int J Pharm Res Rev 2014; 3(2):11-6.
10. Kashif UL, Kumar N. Validation of LC-MS/MS method for the simultaneous estimation of Itraconazole and its metabolite hydroxyl Itraconazole in plasma. Asian J Pharm Clin Res 2014;7 Suppl 1:131-6.
11. Geneva .The Impact of Implementation of ICH Guidelines in Non ICH Countries. English Distribution General 2001; 1-29.
12. United States Pharmacopoeia (USP). 2006. p. 1577-8.