Research Article

Determination of Methyl Methanesulfonate, Ethyl Methanesulfonate and Isopropyl Methanesulfonate Impurities in Lopinavir API by GC/MS/MS using Electron Ionization

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ABSTRACT

A high sensitive rapid Gas Chromatography mass spectrometry method was developed and validated for the determination of three carcinogenic Alkyl methanesulfonates viz. methyl methanesulfonate, ethyl methanesulfonate, isopropyl methanesulfonate impurities in Lopinavir API (active pharmaceutical ingredient). Alkyl methanesulfonates have been highlighted as potential genotoxic impurities (PGIs). The optimum separation was achieved between methyl methanesulfonate, ethyl methanesulfonate, isopropyl methanesulfonate on DB–624 capillary column (30mt×0.32mm), 1.8µm, with EI in Selective ion monitoring (SIM) mode under programming temperature was used. Methanol was used as diluents. This method was validated as per International Conference on Harmonization guidelines. The proposed method was specific, linear, accurate, rugged and precise. The calibration curves showed good linearity over the concentration range of 0.7 to 2.1 ppm. The correlation coefficient was >0.999 in each case. Method had very low limit of detection (LOD) as 0.12 ppm for Methyl methanesulfonate, 0.13 ppm for Ethyl methanesulfonate and 0.11 ppm for Isopropyl methanesulfonate and limit of quantification (LOQ) as 0.37 ppm for MMS, 0.38 ppm for EMS, 0.34 ppm for respectively for the analytes. Accuracy was observed within 70%–130% for the analytes. This method can be further extended a good quality control tool for low level quantitation of Alkyl methanesulfonate impurities in other API.

Keywords: Ethyl methanesulfonate, GC/MS/MS, isopropyl methanesulfonate, lopinavir, methyl methanesulfonate

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INTRODUCTION

Lopinavir is an antiretroviral of the protease inhibitor class. Lopinavir inhibiting the HIV viral protease enzyme. This prevents cleavage of the gag-pol polyprotein and, therefore, improper viral assembly results. This subsequently results in non-infectious, immature viral particles [1]. Its chemical name was (2S)-N-[(2S, 4S, 5S)-5-[2-(2, 6-dimethyl phenoxy) acetamido] -4-hydroxy -1,6-diphenyl hexan-2-yl] -3-methyl -2-(2-oxo-1,3 diazinan-1-yl) butanamide with molecular weight 628.810 and its molecular formula was $C_{37}H_{48}N_4O_5$ [2]. Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during formulation, or upon aging of both API and formulated APIs to medicines.

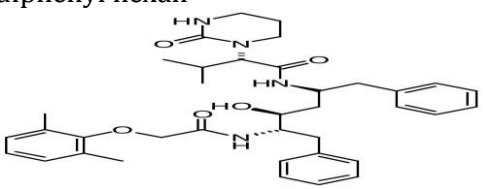

Figure 1: Structure of the Lopinavir

The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. The potential genotoxic impurities (PGIs) are known to induce genetic mutations or chromosomal aberrations and are reported as known carcinogens in rats and mice. The different pharmacopoeias such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly incorporating limits to allowable levels of impurities present in the APIs or formulations. International Conference on Harmonization (ICH) has published guidelines on impurities in new drug substances, product and residual solvents, including the identification and monitoring of impurities found in drug products [3].

Experimental:

MATERIALS AND METHODS

Methyl methanesulfonate, ethyl methanesulfonate and isopropyl methanesulfonate (MMS, EMS, and IPMS respectively) were purchased from Sigma Aldrich, Fluka, Acros Organics. Methanol was procured from Rankem (HPLC grade). Pure sample of Lopinavir was obtained from well reputed research laboratory.

Instrument:

GC-MS analysis was carried out on GCMS-QP 7000 system (Agilent) having GCMS Mass hunter software. AMSs were separated on DB-624 capillary column (Agilent Technologies, 30 m×0.32 mm, i.d.×1.8 µm).

Chromatographic Conditions:

The GC oven temperature program utilized an initial temperature of 110°C and an initial holding time of 15min, and then increased at 25°C/min to 225°C. The final temperature was held for 15min. The injection temperature, GC-MS interface and ion source temperature were 200°C, 270°C and 230°C, respectively. Helium was used as the carrier gas with a flow rate of 2ml/min. All the three alkyl methanesulfonates were identified using the National Institute of Standard Technology (NIST) mass spectral library. Validation was done in Selective Ion Monitoring (SIM) mode.

Preparation of solutions:

Standard solution:

MMS, EMS, IPMS, and NBMS stock solutions were prepared by dissolving 10mg individually in 100ml of diluent. Methanol was used as diluent. MMS, EMS, IPMS, and NBMS mixture solution 10µg/ml was prepared by diluting the appropriate volume of above stock solution with diluent.

Sample solution:

Weigh and transfer accurately 500mg of sample into 10ml volumetric flask and make upto the mark with diluent. Further diluted the solution to get the concentration of 0.00009mg/mL of MMS, EMS & IPMS.

Results and Discussion:

All the three alkyl methanesulfonates are liquids, hence it was planned to separate them by gas chromatography, identify and confirm them by mass spectrometry. Initially the experiments were carried out by using DB-1 column for the separation of AMSs, but the resolutions were found to be very poor. Then, this column was replaced by DB-5 capillary column and same result was found. Hence, DB-624 column was used and good resolutions were observed. An optimum injection volume of 1µl was chosen. The split ratio was fixed as 1:1 depending on the detector response. An initial column temperature of 110° was found to be optimum. The present method is validated as per ICH guidelines.

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 values for all the AMSs were found to be 0.12 ppm for MMS, 0.13 ppm for EMS and 0.11 ppm for IMS and limit of quantification (LOQ) as 0.37 ppm for MMS, 0.38 ppm for EMS, 0.34 ppm for respectively.

Linearity of the method was checked by plotting calibration curves between the peak areas versus the concentration of AMSs over the range 0.7-2.1 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.

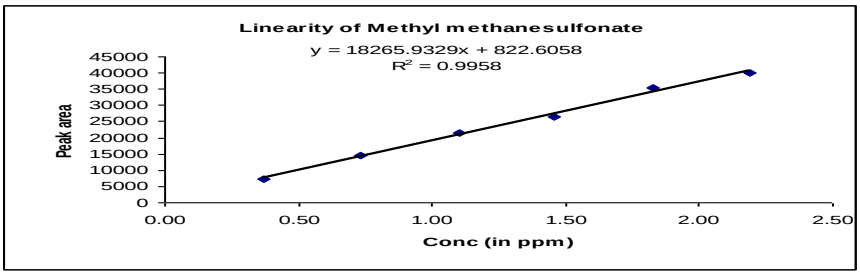

Figure 2: Linearity Graph for Methyl methanesulfonate

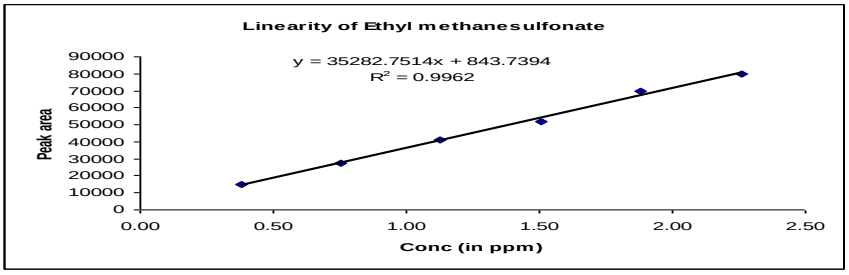

Figure 3: Linearity Graph for Ethyl methanesulfonate

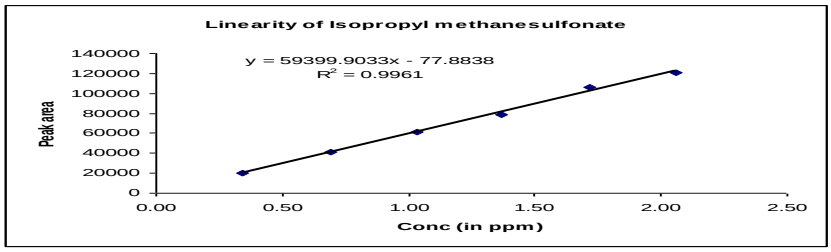

Figure 4: Linearity Graph for Isopropyl methanesulfonate

Table 1: Linear Regression Data for Calibration Curves

Component	Slope	Intercept	Correlation coefficient (R)	R^2
Methyl methanesulfonate	18265.9329	822.6058	0.9979	0.9958
Ethyl methanesulfonate	35282.7514	843.7394	0.9981	0.9962
Isopropyl methanesulfonate	59399.9033	-77.8838	0.9980	0.9961

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 10 ppm mixture solution separately on the same day. For intermediate precision, 10 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 2**).

Table 2: Data for Method Precision

Preparation No.	MMS	EMS	IPMS
1	1.8	1.9	1.6
2	1.8	2.0	1.7
3	1.8	2.0	1.7
4	1.8	2.0	1.6
5	1.7	1.9	1.6
6	1.8	2.0	1.7
Average ppm	1.8	2.0	1.7
% RSD	2.9	0.9	1.2

AMSs were not detected when three pure R and D samples of Lopinavir were analyzed in the present method. Hence, the accuracy of the method was determined by spiking AMSs mixture at three concentration levels (0.7, 1 and 2.1 ppm) to 500 mg of Lopinavir and making the volume to 10 ml with diluent. Each determination was carried out for three times. The recovery data presented in (**Table 3**) indicates the accuracy of the method.

Table 3: Data for Accuracy of MMS, EMS, IPMS

% Accuracy	% Recovery		
	MMS	EMS	IPMS
40% Level	100.0	100.0	95.7
	112.3	105.3	97.1
	95.9	102.7	92.8
60% Level	94.5	95.6	92.2
	97.3	95.6	91.3
	107.3	98.2	92.2
100% Level	100.5	102.7	95.3
	100.5	104.8	97.7
	97.8	103.7	96.5
120% Level	93.6	96.9	91.3
	95.0	96.2	90.3
	96.3	96.2	90.3

Table 4: Ruggedness Data of MMS, EMS, IPMS

Sample	MMS		EMS		IPMS	
	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
Sample-1	1.8	1.8	1.9	1.8	1.6	1.7
Sample-2	1.8	1.9	2.0	1.8	1.7	1.7
Sample-3	1.8	1.8	1.9	1.8	1.7	1.7
Sample-4	1.8	1.8	2.0	1.8	1.6	1.7
Sample-5	1.7	1.8	1.9	1.8	1.6	1.7
Sample-6	1.8	1.8	2.0	1.8	1.7	1.8
Average ppm	1.8	1.8	1.9	1.8	1.7	1.7
% RSD	2.9	2.4	0.8	0.7	1.2	1.4

The mass spectra of EMS, IPMS, and MMS, shows Parent peaks in (**fig. 3**) at m/z109 ($C_3H_8O_3S$), 123 ($C_4H_{10}O_3S$) and 80 ($C_2H_6O_3S$), respectively corresponding to their molecular weights. In the varied gas chromatographic conditions of ±1° on the carrier gas flow, ±2° on the initial oven temperature, the retention times and peak areas of AMSs were found to be same indicating the robustness of the method.

CONCLUSION

The aim of this study is to develop a GC-MS method that can quantify three alkyl methanesulfonates in Lopinavir. The developed GC-MS method was optimized based on the resolutions of AMSs peaks and validated as per ICH guidelines. The method well suits for the intended purpose.

Table 5: Data for Robustness

Parameter condition	Retention time		
	MMS	EMS	IPMS
Actual	5.2	7.4	8.7
Flow : 1.9 mL/min	5.3	7.6	8.9
Flow : 2.1 mL/min	5.1	7.3	8.5
Column oven temperature- 108°C	5.5	7.9	9.2
Column oven temperature 112°C	4.9	7.0	8.2

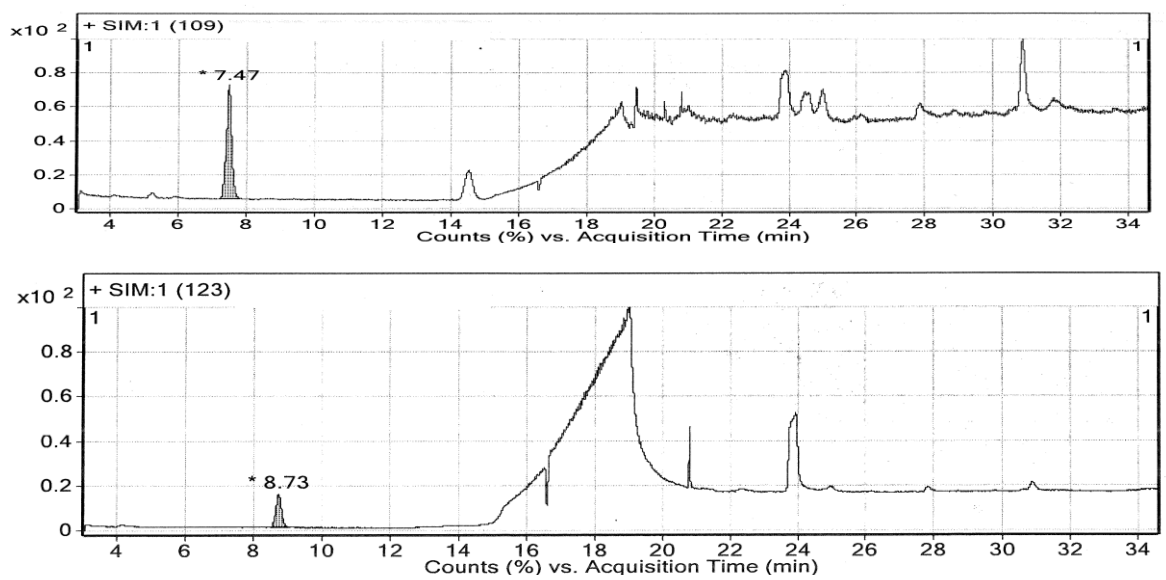

Figure 5: Chromatograms of Optimized method for MMS, EMS & IPMS

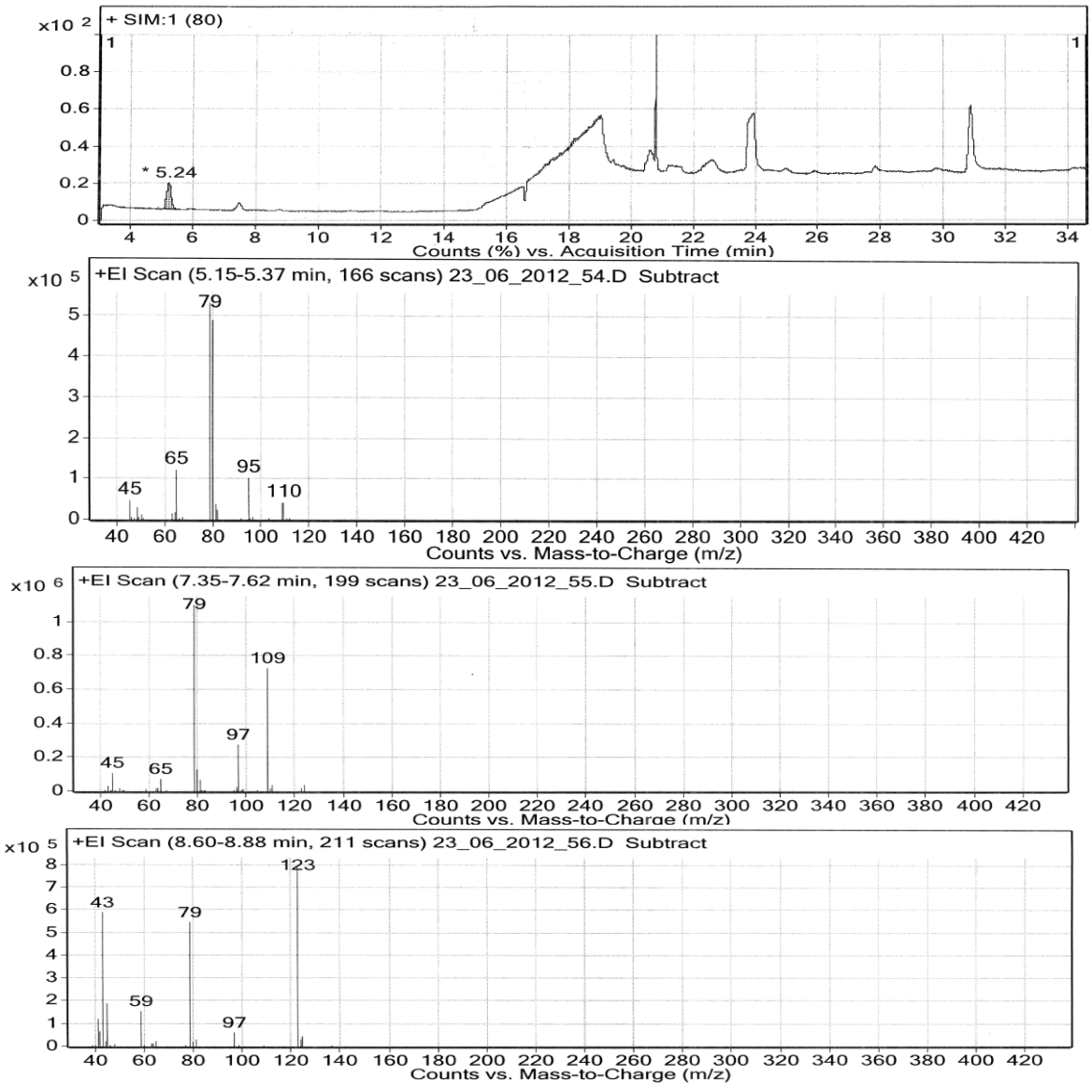

Figure 6: Mass Spectra of MMS, EMS, and IPMS

REFERENCES

1. www.drugbank.ca/drugs/DB01601
2. http://en.wikipedia.org/wiki/Lopinavir
3. http://www.ijperonline.com/july_sep_2010/301-310.pdf
4. P.R.Kakadiya, B.P.Reddy, V.Singh, S.Ganguly, T.G.ChandraSekhar. Low level determinations of methyl methanesulfonate and ethyl methanesulfonate impurities in Lopinavir and Ritonavir Active pharmaceutical ingredients by LC/MS/MS using electrospray ionization. Journal of Pharmaceutical and Biomedical Analysis (2011);55 (2):379–384.
5. Chitturi Sr, Sreenivas Rao Ch, Raghava Reddy A, V.Himabindu, Ramesh D. Impurity Profile Study Of Lopinavir And Validation Of HPLC Method For The Determination Of Related Substances In Lopinavir Drug Substance. J Pharm Biomed Anal (2008);48(5):1430-40.
6. A. Suneetha, S.Kathirvel, G.Ramachandrika. A Validated RP HPLC Method For Simultaneous Estimation Of Lopinavir And Ritonavir In Combined Dosage Form. International Journal of Pharmacy and Pharmaceutical Sciences (2011);3(1):49-51.
7. Yoshiko Usami etal. Simultaneous Determination Of Lopinavir, Ritonavir And Efavirenz By HPLC Method. Chemical and Pharmaceutical Bulletin (2003);51(6):715-718.
8. Madhukar A. Jagadeeshwar K*, Naresh K., Armstrong Vinod Raj, B. Jayapaul. Simple and sensitive analytical method development and validation of lopinavir bulk drug by RP-HPLC. Der Pharma Chemica (2011);3(6):494-499.
9. R.Vijaya Lakshmi etal. Development and validation of a RP-HPLC method for determination of lopinavir in bulk and pharmaceutical dosage form. International Journal Of Research In Pharmacy And Chemistry (2011);2(2):413-417.
10. Chandrasekhar K, Seshachelam K, Hari Babu B. A novel validated LC method for quantitation of lopinavir in bulk drug and pharmaceutical formulation in the presence of its potential impurities and degradation products. Biomed Chromatogr (2007);21(7):716-23.
11. Li W. Trace Analysis Of Residual Methyl Methanesulfonate, Ethyl Methanesulfonate And Isopropyl Methanesulfonate In Pharmaceuticals By Capillary Gas Chromatography With Flame Ionization Detection. J Chromatogr A (2000);1046 (2):297-301.
12. Colon I etal. Determination of methyl and ethyl esters of methanesulfonic, benzenesulfonic and p-toluenesulfonic acids in active pharmaceutical ingredients by solid-phase microextraction (SPME) coupled to GC/SIM-MS. J Pharm Biomed Anal (2005);39(3-4): 477-85.
13. Jagadeeshwar K etal. Simple and sensitive analytical method development and validation of lopinavir bulk drug by RP-HPLC. Der Pharma Chemica (2011);3 (6): 494-499.
14. Gohlke, R.S. Time-of-Flight Mass Spectrometry and Gas-Liquid Partition Chromatography. Analytical Chemistry (1959);31(4): 535.