ANALYZING THREE GENOTOXIC IMPURITIES OF ATORVASTATIN CALCIUM EMPLOYING GC-MS SINGLE QUAD DETECTOR WITH ELECTRON IMPACT TECHNOLOGY

Mohinish Sahai^{1,✉}, Nayakanti Devanna² and Rahul Kumar Rajput³

¹Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur -515002, (Andhra Pradesh) India.

²Director, Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University, Anantapur -515002, (Andhra Pradesh) India.

³Department of Research and Development, Mankind Research Centre, Manesar, Gurugram-122051, (Haryana) India.

✉Corresponding Author: mohinish_sahai@rediffmail.com

ABSTRACT

This paper described the first report on GC-MS methodology development and validation for the simultaneous determination of potential genotoxic materials, ethyl methanesulfonate (ELMS), methyl methanesulfonate (MLMS), and isopropyl methanesulfonate (ILMS), in Atorvastatin calcium (ATC). The quantification limits for this methodology ranged from 0.0115 to 0.0767 ppm (ELMS), 0.0111 to 0.0738 ppm (ILMS) and 0.0129 to 0.0858 ppm (MLMS). This methodology has presented excellent eminence parameters for case, linearity (squared correlation coefficient value > 0.990), precision for method (0.6-1.1%, n=6), ruggedness (1.1-2.1%, n=12), system suitability (5.1-6.1%, n = 6) in relations of relative standard deviation percentage. The recovery values attained in low, medium and high levels of spiked ATC samples were between 87.8% and 119.1%. The GC-MS methodology demonstrates its versatility in evaluating ELMS, ILMS and MLMS concurrently in ATC 's daily quality assurance evaluations.

Keywords: Impurities, Genotoxic, Atorvastatin Calcium, Gas Chromatography, Mass Spectrometer.

RASĀYAN J. Chem., Vol. 14, No.2, 2021

INTRODUCTION

Alkyl mesylate esters of alcohols with small chains are proved as agents having reactivity with DNA, genotoxicity and possibly carcinogenicity.¹⁻⁴ The practice of using alcohols like ethyl, methyl and isopropyl at any stage of synthesis of pharmaceutical active ingredient or during purification of the pharmaceutical active ingredient, leads to the formation of genotoxic ethyl methanesulfonate (ELMS), methyl methanesulfonate (MLMS), and isopropyl methanesulfonate (ILMS), respectively.⁵ Sometimes, also, these ELMS, MLMS and ILMS are used as starting preparatory materials for pharmaceutical active ingredient synthesis. A toxicological risk threshold value of 1.5 µg a day intake of genotoxic impurity is tolerated as is currently the case.⁶⁻⁸

Atorvastatin calcium (ATC) is a lipid-reducing medication that reduces anomalous levels of cholesterol and other lipids, and ultimately reduces the danger of cardiovascular disorder.^{9,10} Since ATC is given at a maximum allowable dose of 80 mg per day. A permitted intake of genotoxic impurities (ELMS, MLMS and ILMS) in ATC is 18.75 ppm per day. The same quantity value was used as specification limit concentration for ELMS, MLMS and ILMS.

Monitoring and controlling the quality feature of the pharmaceutically active ingredient and formulation products for impurities is just as worthy as signifying efficacy.¹¹⁻¹⁴ Quantification of ELMS and MLMS simultaneously in emtricitabine by LC/MS/MS,¹⁵ methane sulfonic acid by HPLC-UV,¹⁶ and in imatinib mesylate by GC/MS^{17,18} was documented. Quantification of ELMS, ILMS and MLMS simultaneously in

dapoxetine hydrochloride by GC-ECD,¹⁹ in rasagiline mesylate by GC,²⁰ in lopinavir by GC/MS/MS,²¹ and in doxazosin mesylate by GC/MS²² were also documented. This paper describes the first report on GC-MS methodology development and validation for the simultaneously ELMS, ILMS and MLMS contaminants determination in ATC.

EXPERIMENTAL

Instrumentation Employed and Conditions for Quantification

The analysis and detection of ELMS, ILMS and MLMS were performed on a gas chromatography system (Shimadzu, GC-2010 Plus) fixed with a mass spectrometer (Shimadzu, GCMS TQ8050). The gas chromatographic separation of ELMS, ILMS and MLMS was completed on a DB-5 column with 30 m length, 0.32 mm interior diameter and 0.25-micron film thickness and flow rate of 1.5 ml/min. The functional temperature schedule was started at 40°C initially, hold for 5 min, then risen at 12°C per min to 105°C, hold for 0 min and then again risen at 25°C per min to 310°C, hold for 12 min. Injector temperature was regulated at 285 °C. The quantity of sample injected was 2 µL and was performed in split form 1:2. Ion source and interface temperatures were regulated at 230 °C and 310 °C, respectively. The identification of ELMS, ILMS and MLMS did use a mass spectrometer system in chosen ion mode of monitoring (ions – 80 m/z for MLMS, 79 m/z for ELMS and 123 m/z for MLMS). The data acquirement and processing were examined using Real-Time GCMS solution software (Shimadzu).

Chemicals

Sun Bio (Bangalore, India) gifted the reference samples of ELMS, ILMS, MLMS and ATC with the potency of 99.9%, 100.0%, 93.4% and 99.5% respectively. Methanol from Merck, India was employed as diluent.

ELMS, ILMS and MLMS Solutions

The stock ELMS, ILMS and MLMS solution was prepared in methanol diluent at the quantity of 235 ppm. The working ELMS, ILMS and MLMS solution was also prepared in methanol diluent from stock solution at a specification limit quantity of 18.75 ppm. Six calibration solutions of ELMS, ILMS and MLMS at various levels (ELMS – 0.0115 to 0.0767 ppm, ILMS - 0.0111 to 0.0738 ppm and MLMS - 0.0129 to 0.0858 ppm) were made in methanol diluent from serial dilution of stock solution.

ATC Sample Solution

ATC was dissolved in methanol diluent at 2000 ppm ATC concentration.

Quantification of ELMS, ILMS and MLMS in ATC Sample

Conditioned the column at 310°C for approximately 30 min. Allowed the gas chromatograph to get equilibrated at 40°C till a sturdy baseline was obtained (at least approximately for 30 min). After the system has been equilibrated, injected methanol diluent blank solution (n=1), and working ELMS, ILMS and MLMS solution (6=6) and ATC sample solution (n=1) into the gas chromatograph system. Using conditions described in the section “Instrumentation employed and conditions for quantification”, determined the peak areas of ELMS, ILMS and MLMS. The ELMS, ILMS and MLMS content (ppm) of the ATC sample solution was determined utilizing the below-given formula:

$$\text{Impurity (ppm)} = [(A_{IT} - A_{BL}) / (A_{STD} - A_{BL})] \times (W_{tSTD} / \text{Dil}_{STD}) \times (\text{Dil}_{TEST} / W_{tTEST}) \times P \times 10000$$

In above equation, A_{BL} = Response area of impurity in methanol diluent blank chromatogram; A_{IT} = Response area of ELMS, ILMS and MLMS in test solution chromatogram; A_{STD} = Average response area of impurity in working ELMS, ILMS and MLMS solution; W_{tSTD} = Weight quantity of impurity in working solution; W_{tTEST} = Weight quantity of ATC in sample solution; Dil_{STD} = Dilution factor of working solution; Dil_{TEST} = Dilution factor of ATC sample solution; P = Potency of impurities.

RESULTS AND DISCUSSION

Method Optimization

Experimental trial studies were carried out to optimize the condition requirements of gas chromatography, so that better resolution was accomplished between ELMS, ILMS and MLMS. Initial investigations were pursued employing various columns with distinct chemistries including DB-624, DB-5 (film thickness of

0.25 micron, length of 30 m and internal diameter of 0.32 mm). Parameters of GC-MS including the program of oven temperature and flow rate have been configured. The influence of constant pressure and constant mode of flow also was judged. Trials on DB-5 column with ultimate optimized oven program and flow rate (see section “Instrumentation employed and conditions for quantification”) were found to be better compared to the evaluated trials. The identification of ELMS, ILMS and MLMS was done in chosen ion mode of monitoring (ions – 79 m/z for ELMS, 80 m/z for MLMS and 123 m/z for MLMS).

Validation

System Suitability

The system suitability was judged by analyzing the methanol diluent blank and six times working ELMS, ILMS and MLMS solution. The relative standard deviation for ELMS (5.3%), ILMS (6.1%) and MLMS (5.1%) response peak areas from six injections were fewer than 15%, suggesting suitability for the system to evaluate ELMS, ILMS and MLMS simultaneously.

Specificity/Selectivity

The specificity/selectivity was judged by analyzing the methanol diluent blank, working ELMS, ILMS and MLMS solution, ATC control sample solution and ATC spiked with ELMS, ILMS and MLMS. The quantities of ELMS, ILMS and MLMS in working and spiked ATC solutions were 18.75 ppm. The archetypal chromatograms of methanol diluent blank, working ELMS, ILMS and MLMS solution and ATC spiked with ELMS, ILMS and MLMS were put on view in Fig. -1. As seen by Fig. -1, there's no noticeable interference at retention periods of ELMS, ILMS, and MLMS in chromatograms of methanol diluent blank and ATC control sample solution.

Fig. -1: Chromatograms of [a] Methanol Diluent Blank [b] ATC Control Sample Solution [c] Working ELMS, ILMS and MLMS Solution [d] ATC Spiked with ELMS, ILMS and MLMS

Limits of Detection (Ld) and Quantification (Lq)

Ld confirmation was done by visual response detection at 0.5%, 5.0%, 10.0%, 15.0%, and 25.0% quantity of MLMS, ELMS and ILMS, and concentration that was reliably detected visually was assessed as Ld. The Lq and Ld were assessed to be precise by injecting six replicate injections. The Ld values were 0.004 ppm (concentration of 2.0 ppm for test sample) for MLMS and ELMS, and 0.004 ppm (concentration of 2.1 ppm for test sample) for ILMS. The Lq values were 0.013 ppm (concentration of 8.1 ppm for test sample) for MLMS, 0.012 ppm (concentration of 6.8 ppm for test sample) for ELMS, and 0.011 ppm (concentration of 7.7 ppm for test sample) for ILMS. The Ld and Lq values were confirmed by analyzing six times the ELMS, ILMS and MLMS solutions prepared at a concentration of Ld and Lq values. The relative standard deviation for ELMS (4.5% at Lq and 8.6 at Ld), ILMS (4.9% at Lq and 8.9 at Ld) and MLMS (7.1% at Lq and 8.2 at Ld) area response was fewer than 33%, suggesting adequate Ld values and fewer than 15% suitability suggesting adequate Lq values for analysis.

Linearity

Linearity was judged by analyzing five concentrations ranges from LOQ(Lq) to 200% of specification limit quantity (18.75 ppm) of ELMS (0.0115 to 0.0767 ppm), ILMS (0.0111 to 0.0738 ppm) and MLMS (0.0129 to 0.0858 ppm). The linearity graphs of ELMS, ILMS and MLMS were mapped between response areas and the respective concentrations. Squared correlation coefficient (correlation coefficient, slope, residual sum of the square, and Y-intercept) values for ELMS, ILMS and MLMS were calculated (Table-1). The squared correlation coefficient values for ELMS, ILMS and MLMS were more than 0.990, suggesting good linearity from LOQ to 200% of specification limit quantity.

Table-1: Values Confirming Linearity of ELMS, ILMS and MLMS

Parameter	ELMS	ILMS	MLMS
Linearity range	0.0115 to 0.0767 ppm	0.0111 to 0.0738 ppm	0.0129 to 0.0858 ppm
Correlation coefficient	0.999	0.999	0.999
Squared Correlation coefficient	0.998	0.998	0.998
Slope	2848990.48	1971999.09	1850934.46
Residual sum of square	36812672.15	20529243.89	21566826.07
Y-Intercept	6573.05	13164.76	10959.52

Precision

The system precision was judged by analyzing the methanol diluent blank one time and six times working ELMS, ILMS and MLMS solution. The relative standard deviation for ELMS (5.3%), ILMS (6.1%) and MLMS (5.1%) response peak areas from six injections were fewer than 15%, suggesting precision for the system to evaluate ELMS, ILMS and MLMS simultaneously.

The method precision was judged by analyzing six times the ATC solution (2000 ppm) spiked with ELMS, ILMS and MLMS at specification limit quantity. The mean quantity for ELMS, ILMS and MLMS assessed are 18.8 ppm, 18.6 ppm and 18.6 ppm, respectively. Relative standard deviation values for ELMS, ILMS and MLMS were 1.1%, 0.9% and 0.6%. The relative standard deviation from six quantifications was fewer than 20%, suggesting reliability and repeatability in evaluating ELMS, ILMS and MLMS simultaneously. The intermediate precision/ruggedness was judged by analyzing six times the ATC solution (2000 ppm) spiked with ELMS, ILMS and MLMS at specification limit quantity by the different analyst (n=2) on a different day (n=2). The cumulative relative standard deviation values for 12 quantifications of ELMS, ILMS and MLMS were assessed (Table-2) and were found fewer than 20%, suggesting intermediate precision/ruggedness in evaluating ELMS, ILMS and MLMS simultaneously.

Table-2: Confirming Intermediate Precision/Ruggedness for ELMS, ILMS and MLMS

Parameter	Impurity Quantity Determined (ppm) for Test Sample					
	MLMS		ELMS		ILMS	
	Analyst 1 and Day 1	Analyst 2 and Day 2	Analyst 1 and Day 1	Analyst 2 and Day 2	Analyst 1 and Day 1	Analyst 2 and Day 2
Mean*	18.6	17.9	18.8	18.5	18.6	17.6
% RSD	0.6	1.3	1.1	1.4	0.9	1.6
Overall mean	18.3		18.7		18.1	
Overall% RSD	2.1		1.6		3.0	

* Mean of six quantifications

Accuracy

The accuracy was judged by analyzing three times the ATC solution (2000 ppm) spiked with ELMS, ILMS and MLMS at LOQ (ELMS – 4.98 ppm, ILMS – 5.16 ppm and MLMS – 5.08 ppm), 100% (ELMS – 18.61 ppm, ILMS – 20.64 ppm and MLMS – 20.32 ppm) and 150% (ELMS – 29.85 ppm, ILMS – 30.96 ppm and MLMS – 30.49 ppm) of specification limit quantity. The mean percent quantity ELMS, ILMS and MLMS recovered at three studied levels were assessed (Table-3). The mean percent quantities of ELMS, ILMS and MLMS recovered from three quantifications were within 70 to 130%, suggesting accurateness in evaluating ELMS, ILMS and MLMS simultaneously.

Table-3: Confirming Accuracy for ELMS, ILMS and MLMS

Solvent	Mean percent recovered*			
	LOQ Level	100% level	150% level	Overall Mean
MLMS	116.9	90.0	91.8	99.6
ELMS	119.1	99.2	94.4	104.2
ILMS	116.7	87.8	88.6	97.7

* Mean of three recoveries

CONCLUSION

In the investigation, GC-MS methodology development and validation for the simultaneous determination of ELMS, ILMS and MLMS contaminants in ATC were done. Based on the gathered results through validation, it was concluded that GC-MS methodology for determining ELMS, ILMS and MLMS simultaneously in ATC is specific, precise, sensitive, linear and accurate. Therefore, this GC-MS methodology can be employed for quality control of studied impurities in ATC.

ACKNOWLEDGEMENT

The Authors thank Mr. Pavan of Sun Bio limited for providing gift samples and impurities of atorvastatin calcium for the research project and providing the necessary support and infrastructure to conduct the experimentation.

REFERENCES

1. D. J. Snodin, *Regulatory Toxicology and Pharmacology*, **45**, 79(2006), DOI:10.1016/j.yrtph.2006.02.003
2. S. L. Coffing, M.O. Kenyon, J.I. Ackerman, T.J. Shutsky, K.L. Dobo, *Environmental and Molecular Mutagenesis*, **56**, 322 (2015), DOI:10.1002/em.21910
3. Methyl Methanesulfonate, International Agency for Research on Cancer (IARC) - Summaries and Evaluations, Vol. 71, p.1059 (1991).
4. E. Gocke, H. Bürgin, L. Müller, T. Pfister, T. *Toxicology Letters*, **190**, 254(2009), DOI:10.1016/j.toxlet.2009.03.016
5. K.L. Dobo, N. Greene, M.O. Cyr, S. Caron, W.W. Ku, *Regulatory Toxicology and Pharmacology*, **44**, 282(2006), DOI:10.1016/j.yrtph.2006.01.004
6. Guidelines on the Limits of Genotoxic Impurities. Committee for Medicinal Products for Human Use [CHMP]. European Medicines Agency [EMA], London (2006).
7. Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches; US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Silver Spring, MD, USA (2008).
8. R. Kroes, J. Kleiner, A. Renwick, *Toxicological Sciences*, **86**, 226(2006), DOI:10.1093/toxsci/kfi169
9. J.T. Davies, S.R. Delfino, C.E. Feinberg, M.F. Johnson, V.L. Nappi, J.T. Olinger, A.P. Schwab, H.I. Swanson, *Lipid Insights*, **9**, 13(2016), DOI:10.4137/LPI.S37450
10. S. Ramkumar, A. Raghunath, S. Raghunath, *Acta Cardiologica Sinica*, **32**, 631(2016), DOI:10.6515/acs20160611a
11. L. Müller, R.J. Mauthe, C.M. Riley, *Regulatory Toxicology and Pharmacology*, **44**, 198(2006), DOI:10.1016/j.yrtph.2005.12.001
12. V. Sathiyarayanan, H. Venkatasubramanian, D. Easwaramoorthy, *Rasayan Journal of Chemistry*, **12**, 2141(2019), DOI:10.31788/RJC.2019.1245419
13. L. P. Magar, B. H. Zaware, C. J. Laheru, D. Singh, S.J. Takate, M.K.Gupta, *Rasayan Journal of Chemistry*, **13**, 940(2020), DOI:10.31788/RJC.2020.1325632
14. S. Mullangi, K. Ravindhranath, *Rasayan Journal of Chemistry*, **13(3)**, 1662(2020), DOI:10.31788/RJC.2020.1335819
15. P. R. Kakadiya, T.G. Chandrashekhar, S. Ganguly, D.K. Singh, V. Singh, *Analytical Chemistry Insights*, **6**, 21(2011), DOI:10.4137/ACI.S6471
16. J. Zhou, J. Xu, X. Zheng, W. Liu, F. Zheng, *Journal of Separation Science*, **40**, 3414(2017), DOI:10.1002/jssc.201700543

17. K. Ramakrishna, N.V. Raman, K.M. Rao, A.V. Prasad, K.S. Reddy, *Journal of Pharmaceutical and Biomedical Analysis*, **46**, 780(2008), DOI:10.1016/j.jpba.2007.11.013
18. C. Zhang, L. Huang, Z. Wu, C. Chang, Z. Yang, *Journal of Separation Science*, **39**, 3558(2016), DOI:10.1002/jssc.201600389
19. Z. Mengmeng, P. Hongjuan, C. Jia, L. Xiaohui, *Chinese Journal of Pharmaceuticals*, **46**, 55(2015).
20. S. Hussain, T. Shaikh, A. Gosar, *International Journal of Innovative Pharmaceutical Sciences and Research*, **3**, 1471(2015).
21. P. Veenaeesh, G. Manikumar, P. Manjusha, A. Padmasri, *International Journal of Pharma Research and Review*, **3**, 11(2014).
22. C. Sitaram, R.B. Rupakula, B.N. Reddy, C.S. Sastry, *Indian Journal of Pharmaceutical Sciences*, **73**, 107(2011), DOI:10.4103/0250-474X.89769

[RJC-6208/2020]