Quantification of Potential Genotoxic Impurities in Amlodipine Besylate Using an Agilent GC/Q-TOF System

Application Note

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Abstract

This Application Note describes a GC/Q-TOF method for the determination of potential genotoxic impurities methylbenzene sulphonate (MBS) and ethylbenzene sulphonate (EBS) in amlodipine drug product. An Agilent 7890A GC coupled with an Agilent 7200A Series Q-TOF was used for the analysis. The unique design features of the Agilent Q-TOF enhances mass accuracy and mass resolution, which helps to confirm the identity of trace impurities with high confidence. An Agilent J&W DB-5ms column with helium as carrier gas was used to develop a 25-minute method to separate both analytes. The method was validated to evaluate the reproducibility analysis. Further MS/MS of EI spectra peaks was processed using Agilent MassHunter Molecular Structure Correlator (MSC) Software to elucidate the impurity structure by assigning substructures to fragments. The Agilent Q-TOF provided excellent sensitivity for the identification and quantitation of these impurities without chemical derivatization.

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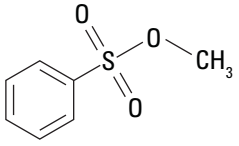
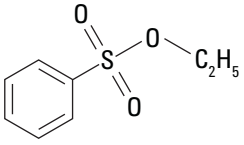
Introduction

Impurities in drug substances or products typically belong to the category of starting materials, intermediates, or by-products. Some of these known impurities are potential mutagens or carcinogens. It may be difficult or nearly impossible to eliminate such impurities completely from the drug synthesis. In general, pharmaceutical guidelines require that impurities > 0.05 % should be specified and identified. Similarly, there is regulatory guidance for the allowed limit of potential genotoxic impurities. Analytical methods should be developed with suitable selectivity and sensitivity for the confident detection and quantitation of trace level potential genotoxic impurities.

Amlodipine besylate belongs to a class of dihydropyridine-type calcium channel blockers. The presence of alcohol either in any stage of synthesis, or the crystallization stage of the salt may cause the formation of sulphonic acid esters, which are considered to be potential genotoxic agents[1,2]. Here we describe a sensitive and selective GC/Q-TOF method for the trace level detection of methyl benzene sulfonate (MBS) and ethyl benzene sulfonate (EBS). The structure of MBS and EBS are given in Figure 1.



MBS
Molecular Formula $C_7H_8O_3S$
Mono isotopic Mass 172.0194 Da



EBS
Molecular Formula $C_8H_{10}O_3S$
Mono isotopic Mass 186.0351 Da

Figure 1. Molecular structure and formula for MBS and EBS.

Experimental Details

Reagents and chemicals

The standards of MBS and EBS were purchased from Aldrich (Bangalore, India), and amlodipine tablets were purchased from a local drug store. LC/MS grade acetonitrile and methanol used for the analysis was purchased from Sigma-Aldrich (Bangalore, India).

Standard and sample preparation

Individual stock solutions of MBS and EBS were prepared by dissolving the appropriate amount of each in acetonitrile to a concentration of 2,000 µg/mL. A standard mixture containing MBS and EBS at a concentration of 10 µg/mL was prepared by taking aliquots of each standard stock solution and diluting with acetonitrile. Calibration standards were prepared by serial dilution of 10 µg/mL standard mixture of analytes to yield the final individual concentrations of 5, 10, 20, 50, 100, 200, 500, 1,000, 2,000, and 5,000 ng/mL with acetonitrile. Calibration curves were obtained by plotting the peak area of each impurity against the corresponding concentrations.

Amlodipine tablet (drug content: 5 mg) was crushed into power and heated at 70 °C for 24 hours. To this degraded drug product, 5 mL of solvent mixture of 50:50; acetonitrile:methanol (v/v) was added and sonicated for 10 minutes. The expected concentration of amlodipine in this extract was 1,000 µg/mL. The organic extract was then centrifuged at 12,000 rpm for 3 minutes, filtered using an Agilent syringe filter (Agilent Econofilter 25/0.2 µm PTFE), and used for GC/MS analysis. To evaluate the accuracy and recovery of the newly developed GC/Q-TOF method for the efficient quantitation of impurities, a known quantity of the analytes have been added (500 ng/mL of impurities, which is 0.05 % with respect to amlodipine) to the extracted drug product matrix solution.

GC/Q-TOF Instrumentation and software

Analyses were performed using an Agilent 7890A GC equipped with a multimode (MM) inlet. Injection was performed using an Agilent 7693A Automatic Liquid Sampler. The GC was coupled to an Agilent 7200A Q-TOF Mass Spectrometer. Agilent J&W DB-5ms, (p/n 122-5532) 30 m × 250 µm, 0.25 µm column was connected from MM inlet and Aux PCM and Agilent uncoated deactivated fused silica tube (p/n 160-2625) 0.7 m × 150 µm column was used to connect the Aux PCM and MS detector. A 2-µL sample volume was injected in cold splitless mode at 100 °C. After a 0.2-minute hold time, the injector temperature was raised to 250 °C with a ramp of 150 °C/min. The gas saver was on, with a value of 20 mL/min after 3 minutes. The carrier gas was helium at 1.9 mL/min constant flow. The oven was programmed from 60 °C (hold time 1 minute) at 10 °C/min to 250 °C (hold time 5 minutes). The transfer line was set at 260 °C.

The 7200A Q-TOF was operated in MS and MS/MS mode using electron impact (EI) ionization. The source temperature was 230 °C. The acquisition rate was 5 spectra/second in 2 GHz extended dynamic range (EDR) mode. The mass range for MS was 50 to 600 Da. No internal mass referencing was used, but the instrument was mass calibrated before each run using the keyword command (MassCal) in the sequence. The analyte molecular ion and most abundant fragment ions were selected for time segmented MS/MS analysis. Additionally, for MS/MS analysis, acquisition time was 200 ms/spectrum, and collision energy was 10 V using three or four precursors per target analyte.

Agilent MassHunter GC/MS Acquisition Software (Version: B.07.02) was used for the data acquisition. All qualitative data processing was performed using Agilent MassHunter Qualitative Analysis Workstation Software (Version: B.07.00), and quantitative analysis was performed using Agilent MassHunter Quantitative Analysis Workstation Software (Version: B.07.00). NIST 2014 Mass Spectral Search Program (Version: 2.2) was used for the spectral library search. MSC software (Version: B.07.00) uses accurate mass MS/MS data to predict and evaluate possible structures of the ions of interest. It can be used to mine databases, for example, ChemSpider to extract structures corresponding to the empirical formula of the molecular ion or fragment ions and rank them according to a compatibility score.

Procedure

The method validation strategy was intended to evaluate the reproducibility of the proposed GC/Q-TOF method. To accomplish this, selectivity, detection limits, and linearity ranges for each of the analytes, as well as the precision and accuracy of the instrumental technique were evaluated. The prepared linearity levels were injected in five replicates for the limits of detection (LOD), limits of quantification (LOQ), and linearity determination. The acetonitrile extract of amlodipine drug product was used to perform the selectivity of the Q-TOF. Selectivity was evaluated by extracting the accurate mass of analyte molecular ions and fragments with various extraction windows from drug product matrix. System precision was evaluated by measuring relative standard deviations (RSDs) of retention time (RT) and area from the replicate injections of the standard preparations. Spiked and unspiked drug product extract was injected to calculate accuracy and recovery of the GC/Q-TOF method.

Results and discussion

Accuracy

The DB-5ms column was able to resolve the analytes into two well-separated sharp peaks. The total ion chromatogram (TIC) obtained for the analysis of the 1,000 ng/mL standard mix is shown in Figure 2. The accurate mass spectra of MBS and EBS are shown in Figure 3.

Using the formula generator option, the molecular ion peaks were correctly assigned as $[C_7H_8O_3S]^+$ and $[C_8H_{10}O_3S]^+$. For MBS and EBS, the mass error value was found to be < 4 ppm, with an isotopic abundance and spacing score of > 97 for the predicted formula (Figure 4).

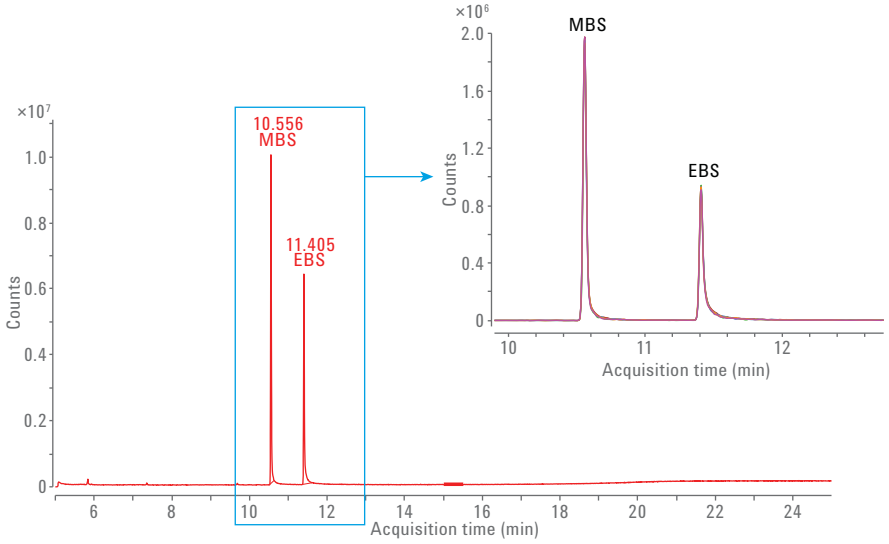


Figure 2. Elution profile of MBS and EBS. Overlay of five replicate injections are included in the inset to demonstrate the injection reproducibility.

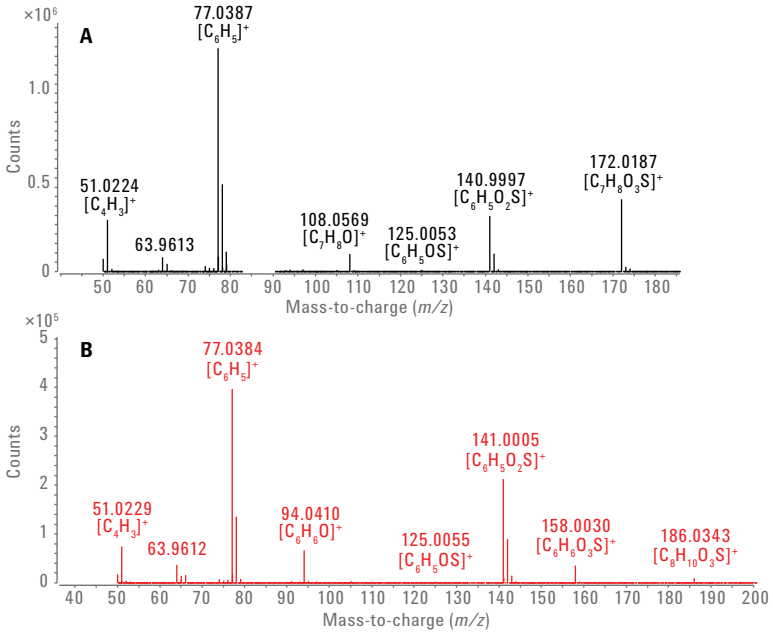


Figure 3. High resolution accurate mass spectra of MBS (A) and EBS (B) labeled with the formula generated from the **generate formula** algorithm.

Selectivity

To assess the selectivity of the method, the accurate mass of targeted analyte molecular ions and fragments were extracted from the amlodipine drug matrix with various extraction windows. This is illustrated in Figure 5. In Figure 5A, the TIC of the drug product matrix is shown. Figure 5B contains the extracted ion chromatogram (EIC) of the analytes with a single quadrupule extraction window of ± 0.5 Da. The EICs of analytes extracted at a mass accuracy of ± 20 ppm and ± 10 ppm are shown in Figures 5C and 5D, respectively. An EIC with a ± 10 ppm extraction window offered selective detection of target analytes with minimum background interference. This confirmed that the Q-TOF method is selective for the simultaneous separation and quantitation of MBS and EBS.

Figure 4. Mass Error values for MBS (A) and EBS (B). Low mass error values (< 4 ppm) are highlighted.

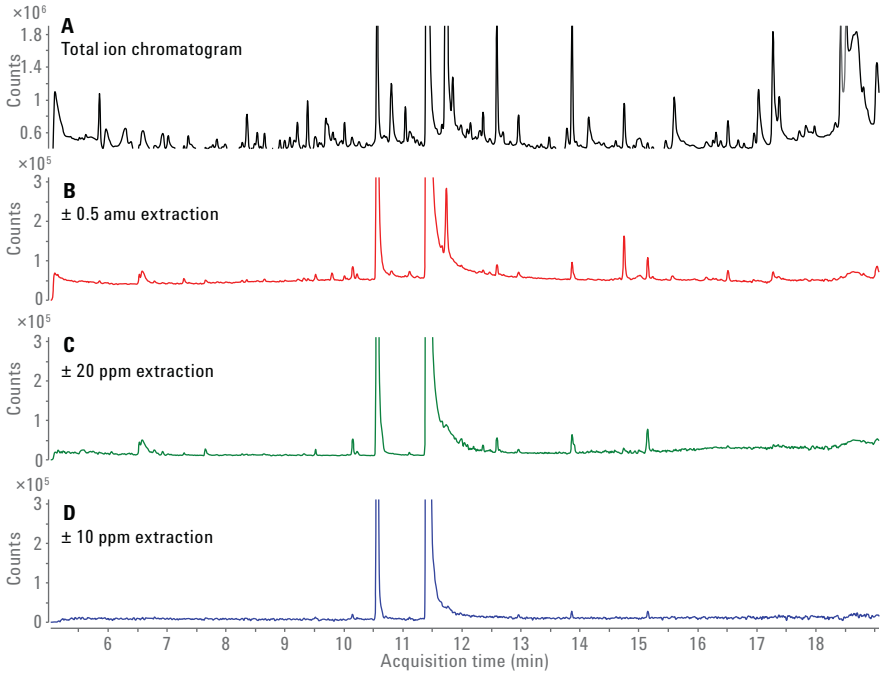


Figure 5. Q-TOF TIC and EIC of analytes from amlodipine drug product injection. The EIC was generated by extracting targeted impurity molecular ions and most abundant fragment ions (*m/z* 172.0187, 140.9997, 142.0075, 77.0387, 78.0456, 51.0224, 94.0410, 158.0030, 186.0343) using various extraction windows, as labeled.

LOD, LOQ, and linearity

LOD and LOQ were calculated from the replicate injections of lower linearity level preparations. The lowest concentration of each analyte peak with a signal-to-noise (S/N) ratio of at least 10:1 was recorded as the LOQ, and with a S/N ratio of 3:1 as the LOD. The LOD of MBS was 10 ng/mL and the LOD of EBS was 20 ng/mL. The linearity curves for MBS and EBS were plotted using the peak area versus concentration range from LOQ to 5,000 ng/mL. To determine the best regression response function, various regression models were evaluated by plotting the data and performing statistical analysis. The best calibration model to fit the data within the desired dynamic range was determined to be a linear regression model when fitted to a 1/x weighting. The linearity experiment results showed that mass spectrometric responses are proportional to their concentration within the range of 20–5,000 ng/mL for MBS and 50–5,000 ng/mL for EBS. The correlation coefficient was obtained more than 0.99 for both the analytes. The results for LOD, LOQ, and linearity thus obtained for both analytes are summarized in Table 1.

Table 1. LOD and LOQ results for MBS and EBS.

Parameter	MBS	EBS
LOD		
Concentration (ng/mL)	10	20
S/N	7	3.2
RSD of peak area (%)	10.7	13.7
LOQ		
Concentration (ng/mL)	20	50
S/N	10.4	10.1
RSD of peak area (%)	4.9	8.4

Precision

Precision was determined by calculating the RSDs of RT and peak area for both analytes from replicate injections at all concentration levels. These are tabulated in Table 2. Area RSD values for MBS at all concentration levels were less than 5.0 %. Area RSD values for EBS at all concentration levels except for LOQ were less than 5.0 %, and for LOQ this value was 8.4 %. Excellent RT precision was observed for both analytes throughout the calibration range, providing a secondary evidence of method robustness. RT RSDs were less than 0.2 % for MBS and EBS at all concentrations. Therefore, the measurements made using the GC/Q-TOF instrument setup were found to be accurate and precise across repeated injections, and met the requirements for analysis of MBS and EBS.

Degraded amlodipine tablet analysis

The method was then used to analyze samples derived from the degraded amlodipine drug product and the amount of MBS and EBS formed using the linearity equations. Approximately 344 ng/mL of MBS and 3,687 ng/mL of EBS was present in the degraded sample. This GC-Q-TOF method is more than 10 times more sensitive for the detection of these trace level degradation impurities. The TOF-MS provides high mass accuracy for compound identification, which reduces false positives when analyzing samples in complex matrices. EI mass spectra of impurities were searched against the standard NIST 2014 library, and resulted in a forward match factor > 850 for both targeted analytes.

Table 2. RSD values of area and RT for MBS and EBS across calibration levels.

Calibration level (ng/mL)	MBS		EBS	
	RT RSD (%)	Area RSD (%)	RT RSD (%)	Area RSD (%)
20	0.01	4.87		
50	0.01	3.91	0.16	8.38
100	0.01	3.14	0.03	3.66
200	0.01	0.68	0.01	3.63
500	0.01	0.77	0.01	0.30
1,000	0.01	0.63	0.01	3.08
2,000	0.01	2.69	0.01	0.86
5,000	0.00	0.43	0.02	0.90

Accuracy

A separate 500 ng/mL amount of MBS and EBS standard was spiked into the degraded amlodipine drug product, and analyzed to evaluate recovery. The added amount corresponded to 0.05 % of each impurity with respect to the main drug concentration. The accuracy of the analytes was calculated by comparing the response/area increment of each peak, and back-calculating using the linearity equations. The amount recovered for MBS and EBS were 520 and 565 ng/mL, respectively. The results confirm recovery within 100 ± 13 %.

Impurity substructure elucidation/fragmentation mechanism using MSC software

MS/MS analysis was performed on molecular ions, and on most abundant fragment ions of both MBS and EBS to elucidate parent structure and their fragmentation mechanism. Figures 6 and 7 show the MS/MS data of MBS and EBS. Using the **Find by targeted MS/MS** algorithm, the features were extracted. Using the **generate formula** algorithm, the molecular ion peaks were correctly assigned as $[C_7H_8O_3S]^+$ and $[C_8H_{10}O_3S]^+$. The accurate mass information from the precursor and fragment ions of the impurities was uploaded to the MSC software using a Compound Exchange Format (.CEF) file, and searched against the ChemSpider database to retrieve all possible structures.

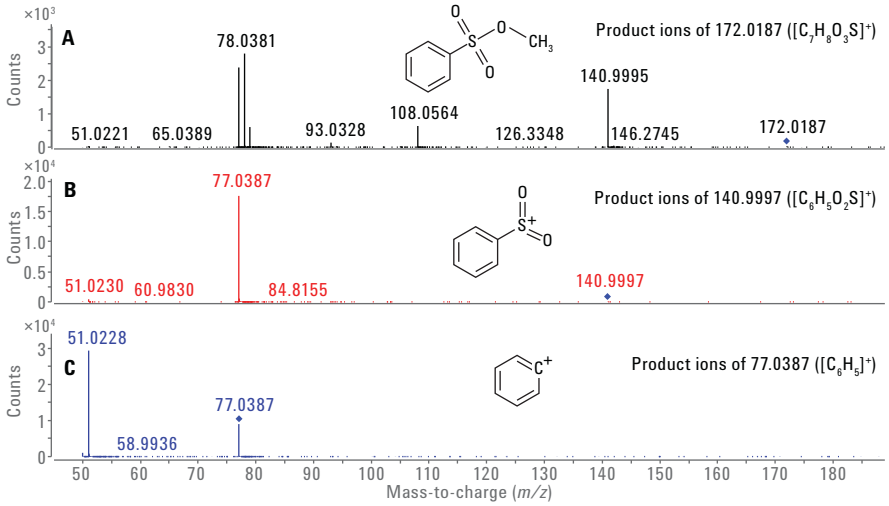


Figure 6. MS/MS data of MBS (dissociated ions of most abundant fragmented ions).

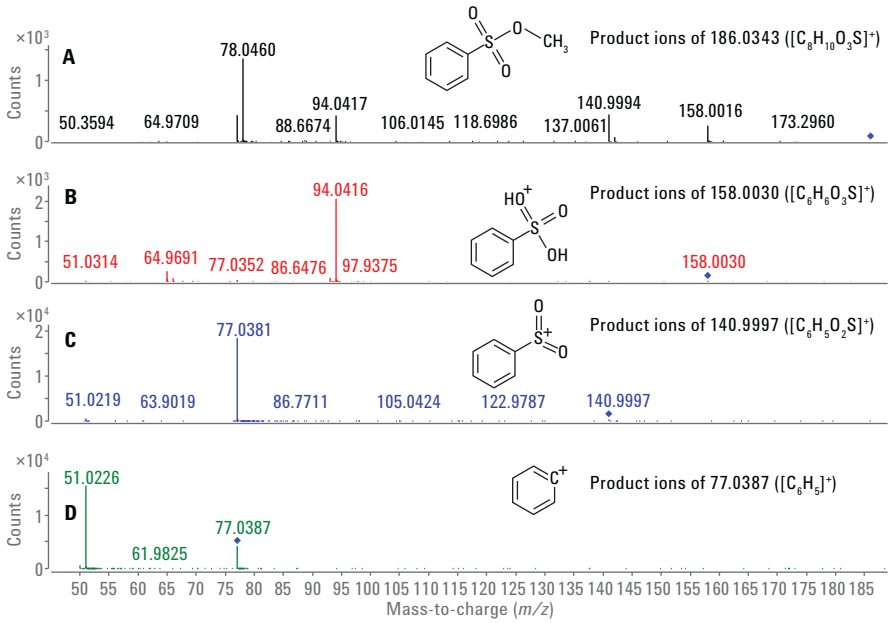


Figure 7. MS/MS data of EBS (dissociated ions of most abundant fragmented ions).

Figure 8 shows results from the MSC software while assigning the fragmentation mechanism for the MS/MS dissociated ions of the MBS molecular ion *m/z* 172.0187.

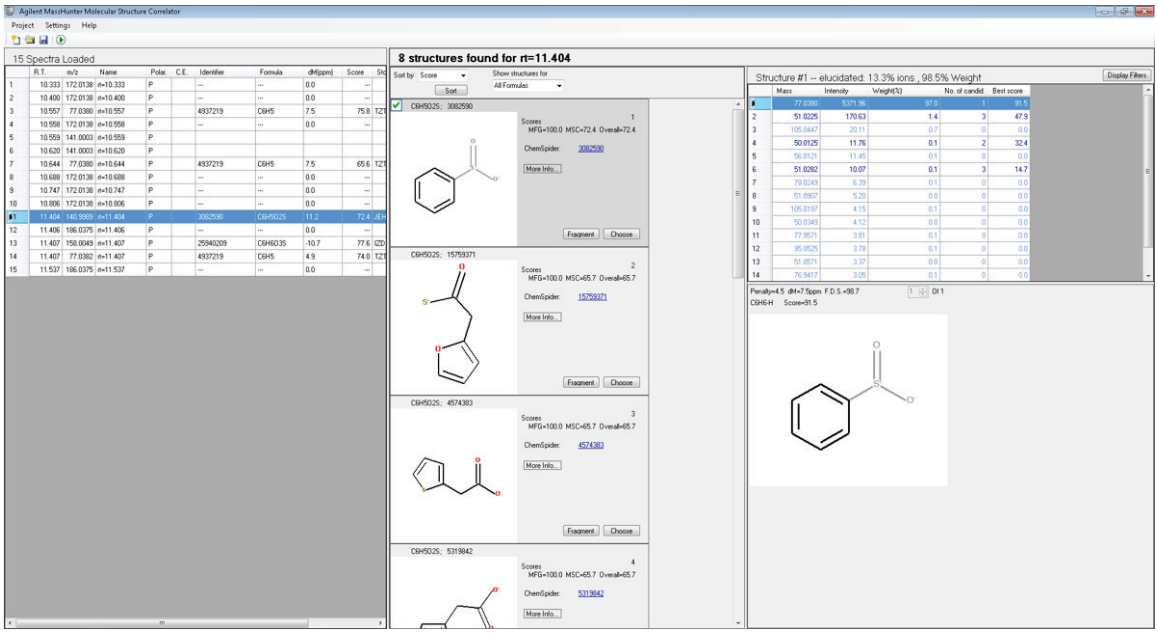


Figure 8. Screenshot of MSC results for assigning structure to the $(C_6H_5O_2S)^+$ fragment ion of MBS. The list of possible molecular structures for the precursor and candidate structure of the impurity sub-fragment is also shown.

Conclusion

A sensitive GC/Q-TOF method for the analysis of two potential genotoxic impurities, methylbenzene sulphonate and ethylbenzene sulphonate in amlodipine besylate has been developed using an Agilent GC 7890A GC coupled with an Agilent 7200A Q-TOF. Both MS and MS/MS modes were used for the identification and quantitation of these impurities. MS data were used for identification and quantitation, while MS/MS data were processed using MSC software to help determine the impurity fragment mechanism. The LOD for MBS and EBS were 10 ng/mL and 20 ng/mL, respectively.

This assay method demonstrated over two orders of dynamic range of detection with LOQs of 20 and 50 ng/mL for MBS and EBS respectively. Method recovery was evaluated from the deliberate addition of targeted impurities to the drug matrix at a concentration of 0.05 % with respect to the main drug concentration. The method was found to be reproducible, and can be used for the quantitation of MBS and EBS without the needed for chemical derivatization. The GC/Q-TOF, in combination with MSC software, can be used to elucidate the structure of unknown impurities from complex matrices.

References

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2. *IARC Monographs Program on the Evaluation of Carcinogenic Risks to Humans for ethyl methane-sulphonate 7* (**1974**) 245.

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