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Analysis of Potential Genotoxic Impurities (PGI) in Active Pharmaceutical Ingredients (API) by GCMS/MS

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Introduction

In pharmaceutical products, impurities are defined as substances that provide no therapeutic benefit, but do have the potential to cause adverse health effects. According to ICH guidelines, genotoxic impurities form a special case that poses a significant safety risk, even at low concentrations. These impurities may be mutagenic and are therefore potentially damaging to DNA. As a result they can lead to mutations or cause cancer. Chemicals like Methanesulfonic acid (mesylate), Benzenesulfonic acid (besylate) and p-Toluenesulfonic acid (tosylate) used in the process of API synthesis are likely to generate sulfonic acid esters (Figure 1) as a reaction by-products. These compounds are potential genotoxic impurities[1] (PGI) and are of great concern to pharmaceutical manufacturers.

A TTC (Threshold of Toxicological concern) based acceptable intake of a mutagenic impurity of 1.5 µg per person per day is considered to be associated with a negligible risk and can, in general, be used for most pharmaceuticals as a default value, to derive an acceptable limit for control[2][3].

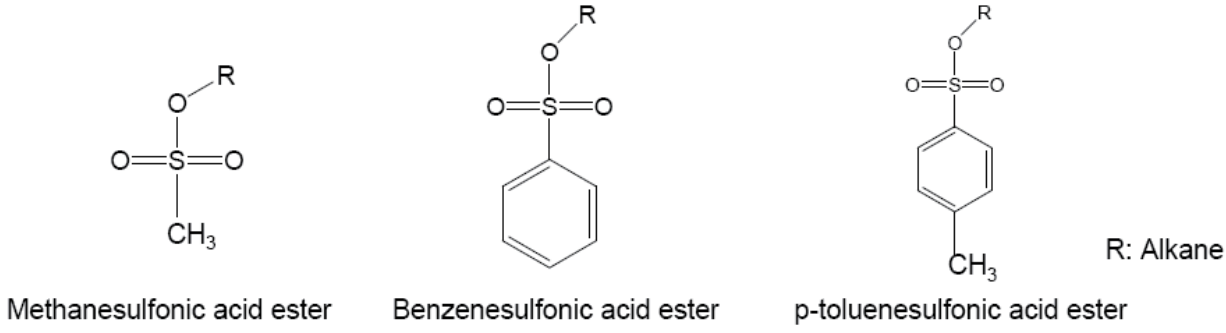


Figure 1. Structural formulae for sulfonic acid esters

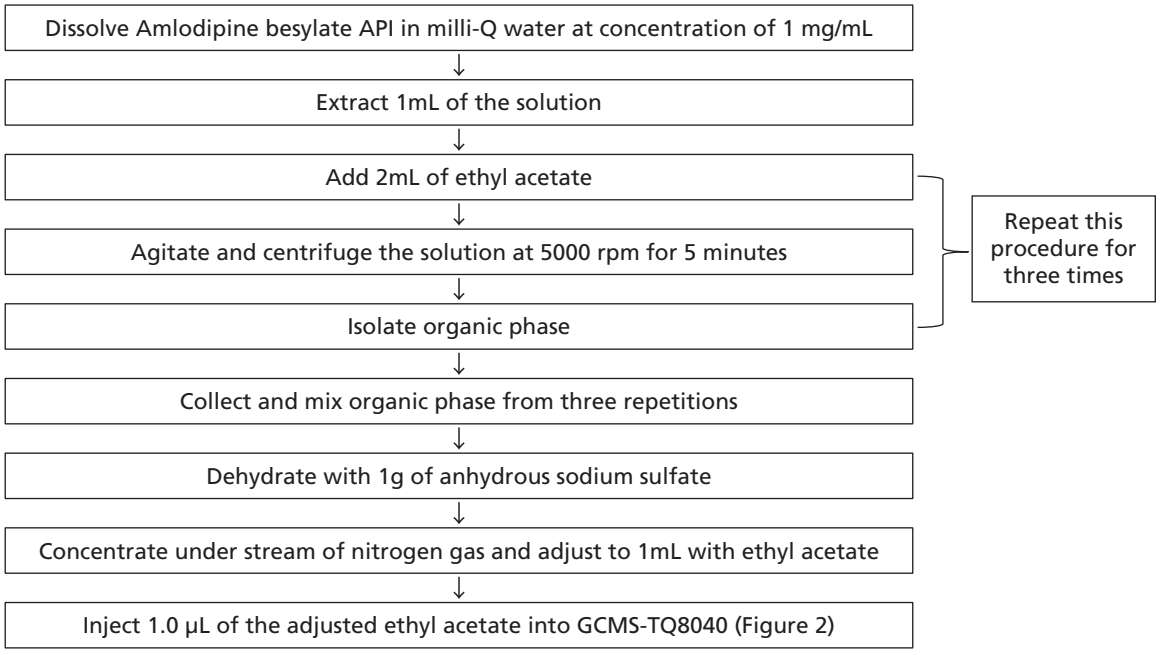
A highly sensitive Gas Chromatography-Mass Spectrometry Triple Quadrupole (GCMS/MS) method was developed for the determination of twelve PGI's viz. methanesulfonic acid esters (4 compounds), benzenesulfonic acid esters (3 compounds) and toluenesulfonic acid esters (5 compounds) in Amlodipine besylate API.

Method of analysis

Sample preparation

Extraction of PGI's was done by Liquid-Liquid Extraction (LLE) method, as given below[4].

Table 1. Sample pretreatment procedure



Standard preparation

Standards of sulfonic acid ester were procured from Tokyo Chemical Industries Co. Ltd. and Sigma-Aldrich®. Stock solutions for individual standards were prepared in ethyl acetate. These individual stocks were further mixed to prepare linearity solutions of 1.0 ppb, 2.5 ppb, 5.0 ppb, 7.5 ppb, 10 ppb, 25 ppb, 50 ppb and 100 ppb.

Pre-extraction spike sample preparation

Extraction efficiency of the method was studied by spiking sample with standard prior to LLE. For this, 100 ppb and 250 ppb of solvent standard prepared in acetonitrile was used to spike 1 mg/mL sample prior to the extraction. Pre-extraction spike at the concentration levels of 10 ppb and 25 ppb were analyzed to study the recoveries.

MRM Method development

For MRM optimization, about 10 ppm individual standard mixtures were analyzed separately using scan mode. Customized n-alkane standard mixture was analyzed to set the retention indices for target compounds. For individual components, precursor ions were selected. Using selected precursor ions, product ion scan was performed with different Collision Energies (CE). Intensity of each MRM transition of each component against range of CEs was studied to plot CE optimization graph as shown in Figure 3. All the above steps were simplified with the help of Smart MRM optimization tool. These MRM transitions along with individual retention index were registered to Smart Database and the final MRM method with optimum segments was generated. MRM chromatogram acquired using this method is shown in Figure 4.

Figure 2. GCMS-TQ8040 Triple quadrupole system by Shimadzu

Key Features of GCMS-TQ8040

1. Smart Productivity : Analysis of 400 pesticides that used to require 2 or 3 methods, can now be accomplished in a single acquisition method by the new firmware protocol.
2. Smart Operation : Smart MRM technology creates optimal MRM methods automatically. The "MRM Optimization Tool" automates best MRM transitions for new compounds.
3. Smart Performance : ASSP achieves high sensitivity at scan speeds of 20,000 u/second. Fastest MRM 800 trans/sec. Single GC/MS mode with the maximum possible sensitivity and repeatability.

GCMS/MS Analytical Conditions

The analysis was carried out on Shimadzu GCMS-TQ8040 as per the conditions given below.

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Table 2. Analytical condition

Chromatographic parameters

Column	: SH-Rtx™-1701 (30 m L x 0.25 mm I.D. x 0.25 µm)
Injection Mode	: Split
Split Ratio	: 5.0
Carrier Gas	: Helium
Flow Control Mode	: Linear Velocity
Linear Velocity	: 40.2 cm/sec
Column Flow	: 1.20 mL/min
Injection Volume	: 1.0 µL

Column Temp. Program :

Rate (°C /min)	Temperature (°C)	Hold time (min)
	70.0	2.00
25.00	150.0	10.00
15.00	200.0	0.00
30.00	260.0	9.50

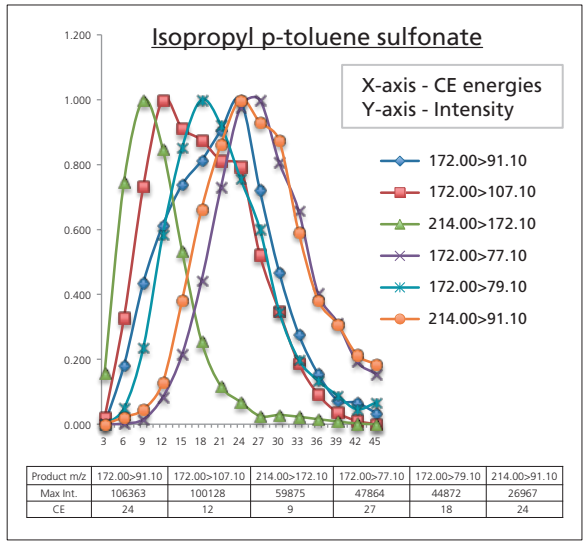
Total Program Time : 30.03 min

Mass Spectrometry parameters

Ion Source Temp.	: 230.0 °C
Interface Temp.	: 270.0 °C
Ionization Mode	: EI
Acquisition Mode	: MRM

Table 3. Method creation using GCMS-TQ8040 Smart MRM

Step.1	Step.2	Step.3	Step.4
Measure in SCAN mode and determine Pre-cursor ion	**MRM Optimization Tool:** Create batch sequence and method file of several collision energy automatically. (refer to Figure 3).	**MRM Optimization Tool:** Analyzes acquired data files and selects the best transitions and collision energy automatically. The result can be exported and Smart MRM database was created.	Method creation using Smart MRM Database



Product m/z	172.00>91.10	172.00>107.10	214.00>172.10	172.00>77.10	172.00>79.10	214.00>91.10
Max Int.	106363	100128	59875	47864	44872	26967
CE	24	12	9	27	18	24

Figure 3. CE Optimization using Smart MRM optimization tool

Results

The mixture of sulfonic acid esters (methane, benzene and p-toluene) was analyzed using the method created as mentioned in Table 3. The sulfonic acid esters were quantitatively extracted from sample and evaluated statistically with respect to precision, linearity and recovery.

Linearity for standards ranging from 1 ppb to 100 ppb concentration level was plotted with weighted linear regression (1/C). Linear response with $r^2 \geq 0.995$ was obtained (Figure 5 to 7). % RSD (n=6) at LOQ for sulfonic acid ester standard solution was less than 15% for all components (Figure 8 to 10).

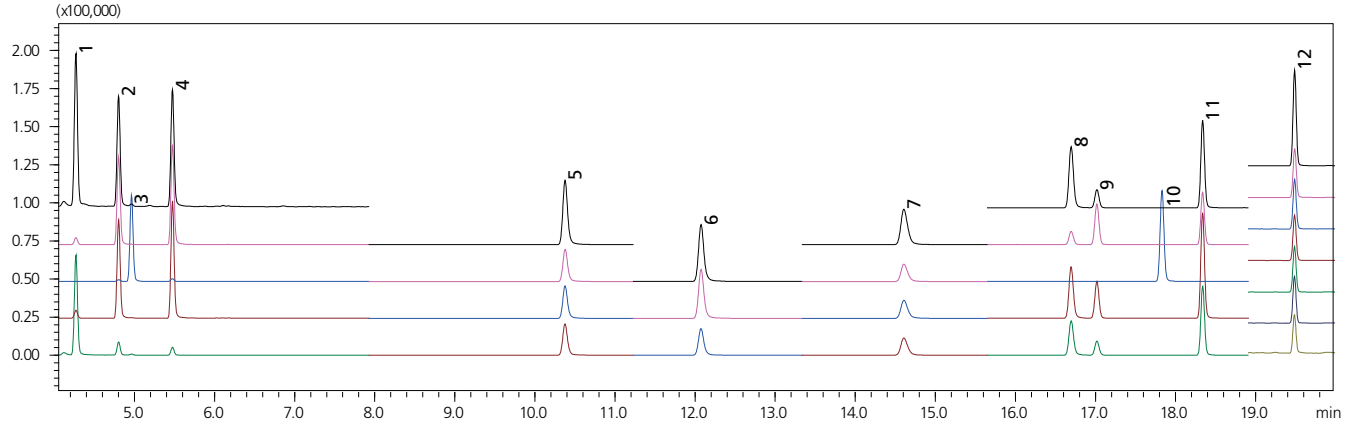


Figure 4. MRM chromatogram for 100 ppb standard mixture

Table 4. Result table

ID	Name	Index	Target MRM (m/z)	Calibration Range (ppb)	r^2	LOQ (ppb)	LOQ % RSD (n=6)	% Recovery at 10 ppb (n=3)	% Recovery at 25 ppb (n=3)
1	Methyl methanesulfonate	1078	80.00>65.00	1 - 100	0.9993	1.0	13.5	105.0	86.4
2	Ethyl methanesulfonate	1148	109.00>79.00	1 - 100	0.9995	1.0	7.8	84.0	84.4
3	Isopropyl methanesulfonate	1170	123.00>79.00	1 - 100	0.9992	1.0	8.5	91.0	87.2
4	n-propyl methanesulfonate	1240	109.00>79.00	1 - 100	0.9995	1.0	7.9	96.0	86.0
5	Methyl benzenesulfonate	1614	141.00>77.10	1 - 100	0.9983	1.0	6.7	103.8	97.8
6	Ethyl benzenesulfonate	1672	141.00>77.10	1 - 100	0.9994	1.0	5.8	107.3	105.1
7	Methyl p-toluenesulfonate	1746	155.00>91.10	2.5 – 100	0.9955	5.0	11.4	107.0	118.4
8	Ethyl p-toluenesulfonate	1804	155.00>91.10	2.5 - 100	0.9993	1.0	10.5	107.0	100.4
9	Isopropyl p-toluenesulfonate	1821	172.00>107.10	2.5 - 100	0.9961	5.0	10.7	129.0	121.6
10	Butyl benzenesulfonate	1866	141.00>77.00	1 - 100	0.9993	1.0	14.4	100.5	92.4
11	n-propyl p-toluenesulfonate	1893	155.00>91.10	1 - 100	0.9993	1.0	14.9	106.0	96.8
12	Butyl p-toluenesulfonate	1997	173.00>91.10	1 - 100	0.9998	1.0	7.8	108.0	99.6

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Due to matrix interference the content and recovery for methyl benzenesulfonate and ethyl benzenesulfonate in amlodipine besylate sample was calculated with the help of Standard Addition Method. For rest of the sulfonic acid esters recovery was calculated by using External Standard Method. For all PGI's 70 to 130 % recoveries were observed. On the basis of statistical data obtained, the method was proved to be highly sensitive, linear, accurate and reproducible.

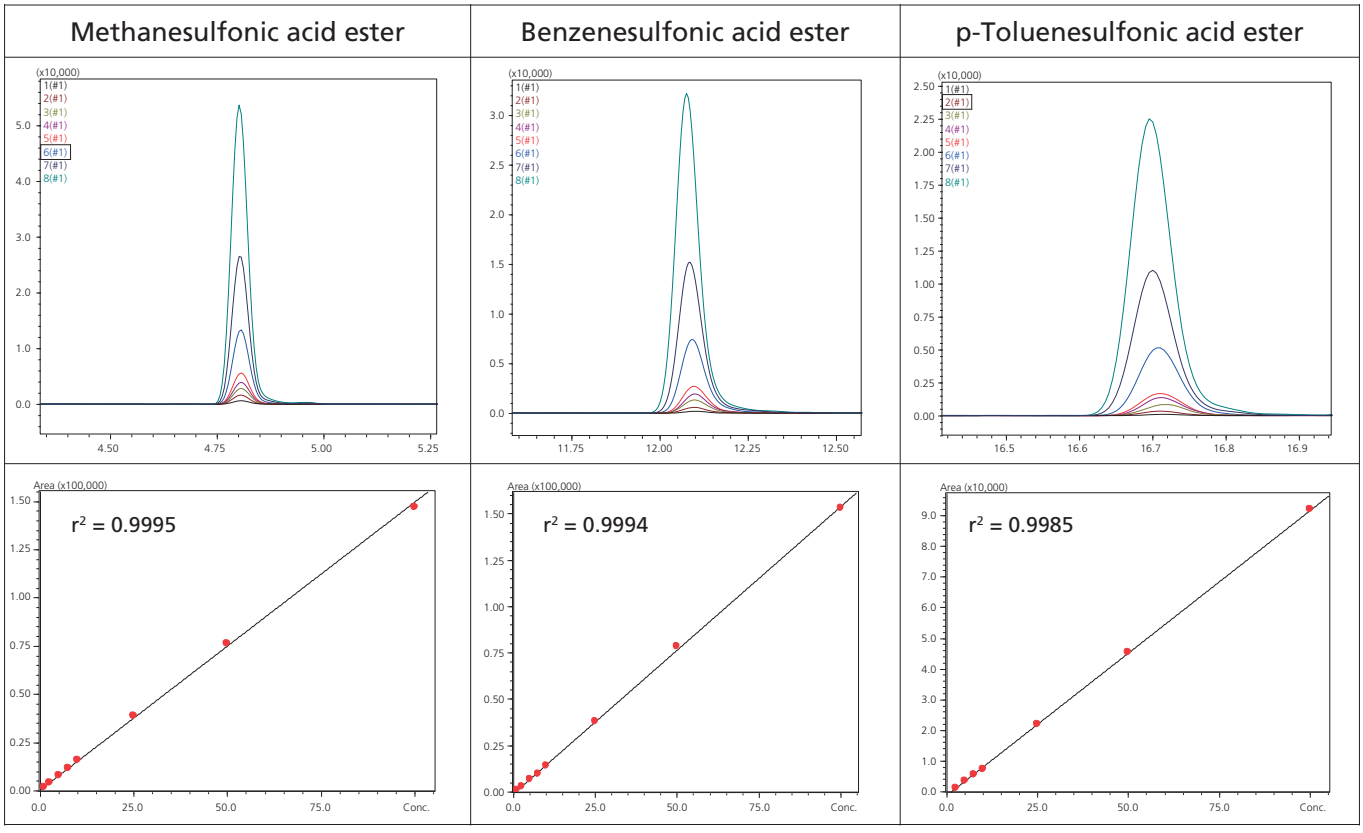

Figure 5. Linearity overlay, and graph for Ethyl methanesulfonate

Figure 6. Linearity overlay, and graph for Ethyl benzenesulfonate

Figure 7. Linearity overlay, and graph for Ethyl p-toluenesulfonate

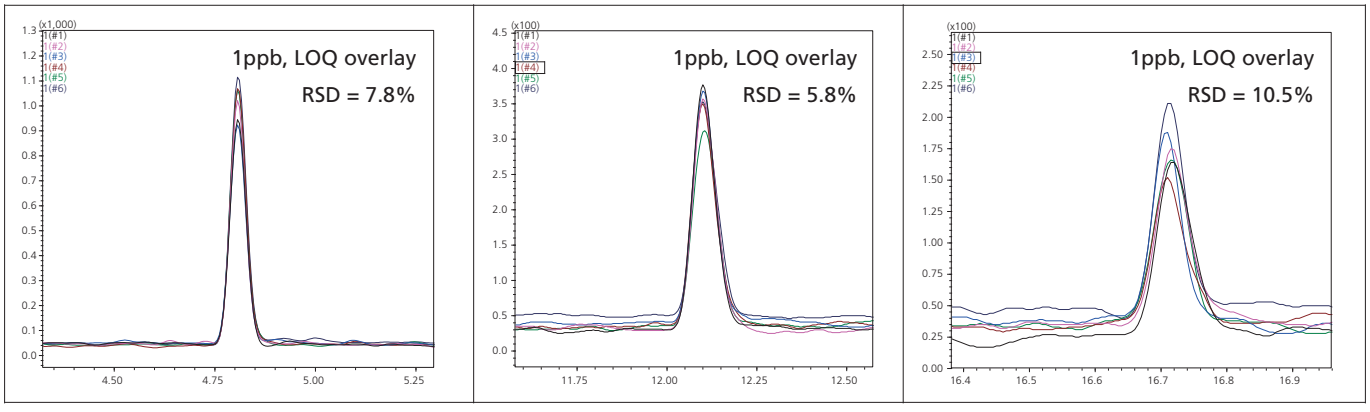

Figure 8. LOQ precision overlay for Ethyl methanesulfonate

Figure 9. LOQ precision overlay for for Ethyl benzenesulfonate

Figure 10. LOQ precision overlay for for Ethyl p-toluenesulfonate

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Conclusion

- A highly sensitive and selective method was developed for PGI's like sulfonic acid esters by using Shimadzu GCMS-TQ8040.
- Developed MRM method can be used for screening and quantification of sulfonic esters in various Pharmaceutical products.
- Ultra Fast scanning, UFsweeper® and ASSP™ features enabled sensitive, selective and reproducible method of analysis.

References

[1] Elder, D. P. & Snodin, D. J, Journal of Pharmacy and Pharmacology, Vol. 61, (2009), 269-278.
[2] EMEA/CHMP/QWP/251344/2006.
[3] M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to limit potential carcinogenic risk guidance for industry (2015), International Council of Harmonization(ICH).
[4] Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (2), Application data sheet no.40, (2011).

Disclaimer: GCMS-TQ8040 is intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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