

Application Data Sheet

No.40

GCMS

Gas Chromatograph Mass Spectrometer

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (2)

This Application Datasheet introduces evaluation results with respect to quantification in the GC/MS analysis of sulfonate esters, so-called PGI (potential genotoxic impurities), in active pharmaceutical ingredients (API). Please refer to GC-MS Application Datasheet No. 18, "Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients," for the analysis conditions and the total ion current chromatogram for the sulfonate esters.

Experiment

Gabexate mesylate and amlodipine besilate, commercially-available research reagents, were used as the API. Solvent extraction was utilized as the extraction method, and the other pretreatment procedures are shown in Fig. 1. The API were dissolved in Milli-Q water to a concentration of 10 mg/mL, and 1 mL of the solution was extracted. 2 mL of ethyl acetate was added. After agitation, the sample was centrifuged for 5 minutes at 2,000 rpm, and the organic phase was isolated. This extraction procedure was repeated a total of 3 times, and all of the organic phase extracts were mixed together. Afterwards, 1 g of anhydrous sodium sulfate was added to the organic phase to dehydrate it. After concentration under a stream of nitrogen gas, it was adjusted to 1 mL using acetic ether to arrive at the experimental solution.

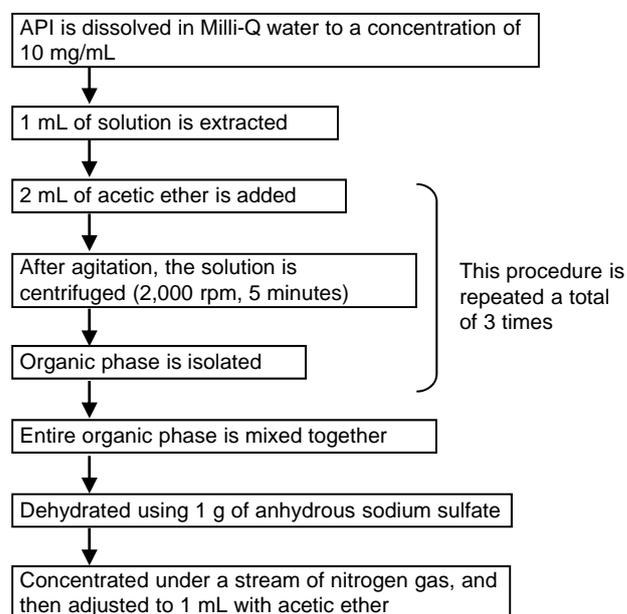


Fig. 1: Pretreatment Procedure

Linearity of the Calibration Curve

Utilizing the analysis conditions shown in GC-MS Application Datasheet 18, a calibration curve was created with the standard sulfonate ester solutions at a concentration of 0.01 $\mu\text{g/mL}$ to 10 $\mu\text{g/mL}$. If pretreatment is performed as shown above, this concentration range is equivalent to 1 ng/mg to 1,000 ng/mg of API. The calibration curve correlation coefficient (R) was at least 0.9996, indicating that favorable linearity was obtained.

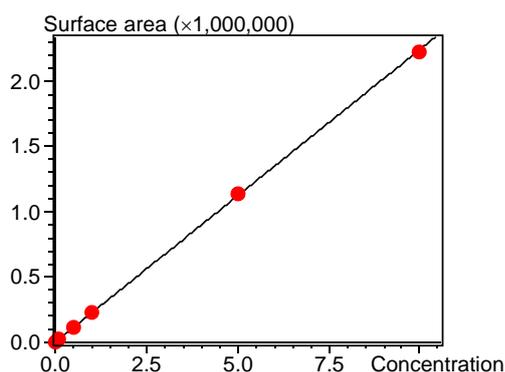


Fig. 2. Methyl Methanesulfonate Ester Calibration Curve

Table 1: Calibration Curve Correlation Coefficients
(Concentration: 0.01 $\mu\text{g/mL}$ to 10 $\mu\text{g/mL}$)

Name of Compound	R (Correlation Coefficient)
Methyl methanesulfonate ester	0.9999
Ethyl methanesulfonate ester	0.9999
Methanesulfonic acid isopropyl ester	0.9999
Methanesulfonic acid n-propyl ester	0.9999
Benzenesulfonic acid methyl ester	0.9999
Benzenesulfonic acid ethyl ester	0.9999
<i>p</i> - toluenesulfonic acid methyl ester	0.9998
<i>p</i> - toluenesulfonic acid ethyl ester	0.9998
Benzenesulfonic acid isopropyl ester	0.9996
Benzenesulfonic acid butyl ester	0.9998
<i>p</i> - toluenesulfonic acid n-propyl ester	0.9998
<i>p</i> - toluenesulfonic acid butyl ester	0.9996

Calibration Curve Weighting: 1/C (Concentration)

Spiked Recovery Test

To perform the spiked recovery test, 1 mL of gabexate mesylate solution (concentration: 10 mg/mL), which was confirmed to not contain the target compound, was extracted, and pretreatment was performed by adding 100 ng of the sulfonic esters. In this case, the concentration of the sulfonic esters in the API was 10 ng/mg. The spiked recovery test was repeated 5 times, and the recovery ratio and recovery ratio repeatability were calculated (Table 2). Favorable results were obtained, with an average recovery ratio in the range of 90.8% to 116.6%, and a recovery ratio repeatability (%RSD) of 3.4% max. for 5 repetitions.

Table 2: Recovery Ratio and Repeatability Results for the Spiked Recovery Test

Name of Compound	Recovery Ratio (%)					Average Recovery Ratio	Recovery Ratio Repeatability %RSD
	NO.1	NO.2	NO.3	NO.4	NO.5		
Methyl methanesulfonate ester	96.4	98.6	93.7	97.3	98.7	96.9	2.1
Ethyl methanesulfonate ester	89.5	89.6	84.4	89.4	89.5	88.5	2.6
Methanesulfonic acid isopropyl ester	92.1	93.4	89.0	94.0	94.1	92.5	2.3
Methanesulfonic acid n-propyl ester	101.7	100.3	99.9	101.8	102.0	101.1	1.0
Benzenesulfonic acid methyl ester	116.3	115.6	112.3	111.7	111.1	113.4	2.1
Benzenesulfonic acid ethyl ester	119.6	116.2	116.2	117.4	113.5	116.6	1.9
p - toluenesulfonic acid methyl ester	107.1	104.0	101.7	105.2	103.9	104.4	1.9
p - toluenesulfonic acid ethyl ester	99.4	96.0	93.2	90.9	95.7	95.0	3.4
Benzenesulfonic acid isopropyl ester	93.8	91.9	88.5	92.0	88.0	90.8	2.7
Benzenesulfonic acid butyl ester	104.8	102.6	100.2	99.1	99.3	101.2	2.4
p - toluenesulfonic acid n-propyl ester	108.1	107.1	104.6	100.5	102.9	104.6	3.0
p - toluenesulfonic acid butyl ester	103.6	105.2	102.4	97.7	100.2	101.8	2.9

Measurement of the API Sample

Pretreatment was performed using commercially-available amlodipine besilate as the research reagent. Fig. 3 shows the total ion current chromatogram and mass chromatograms for the sulfonic esters detected. Four sulfonic esters were detected.

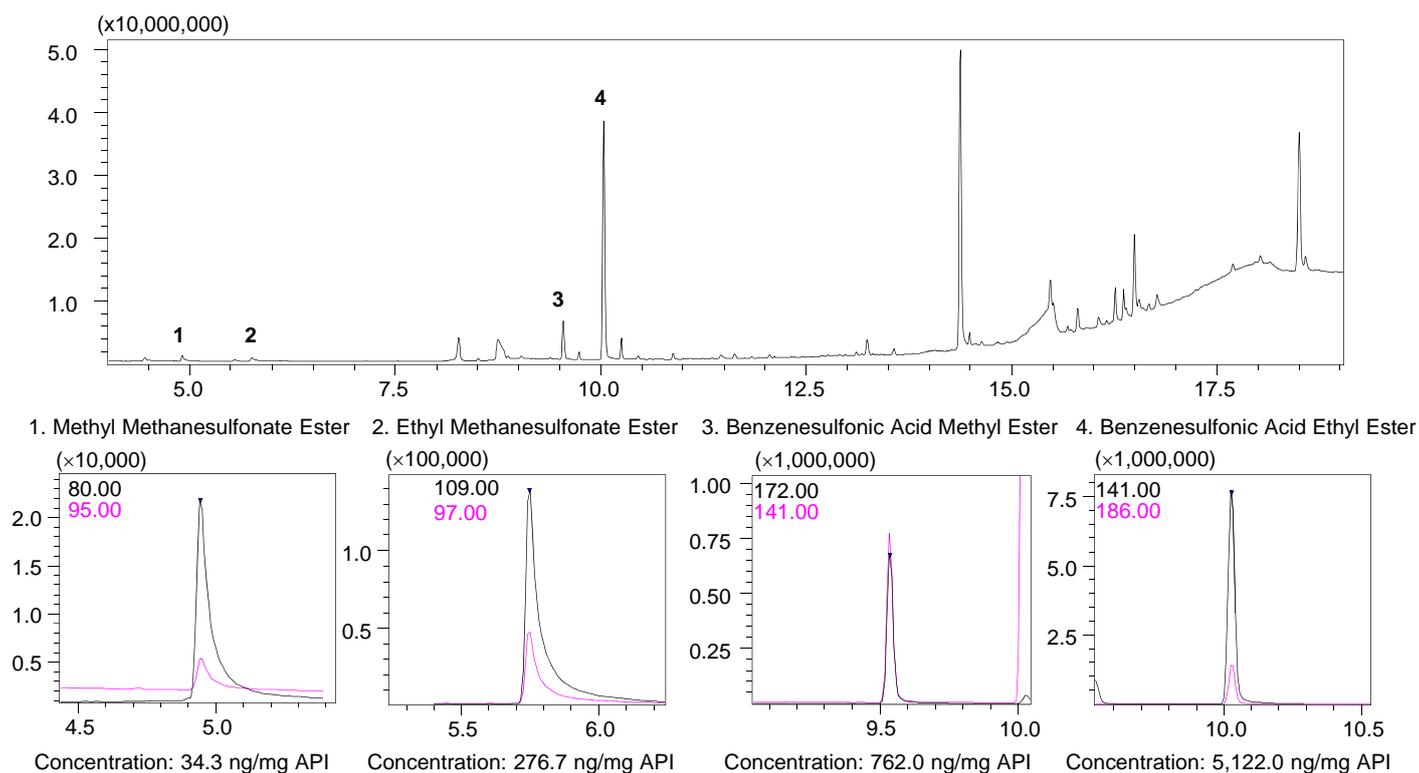


Fig. 3: Total Ion Current Chromatogram and Mass Chromatograms for the Sulfonic Esters Detected

