Coupling Mass Detection with UV for the Analysis of Esters of Benzenesulfonic and p-Toluenesulfonic Acids in Genotoxic Impurities Monitoring

Margaret Maziarz and Mark Wrona
Waters Corporation, Milford, MA

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INTRODUCTION

Genotoxic impurities (GTIs) are intermediates or reactants that have the potential to react with DNA, consequently produce carcinogenic response and cancer.

Sulfonic acids are commonly used as counter ions with Active Pharmaceutical Ingredients (APIs) to form salts. Sulfonic acids can interact with residual alcohols to generate alkyl esters, which are considered potential genotoxic impurities.

In this work, we present the development of a robust and quick dual detection (UV and MS) UPLC method for the analysis of esters of benzenesulfonic and p-toluenesulfonic acids.

METHODS

Solutions Preparation

Separate stock solutions of esters of benzenesulfonic and p-toluenesulfonic acids were prepared in methanol at 1.0 mg/mL. An equal volume of each stock solution was transferred to one vial and diluted with standard diluent (20:80 methanol/5 mM ammonium acetate) to make a mixture solution containing 50 µg/mL of each analyte. The mixture solution was serially diluted with standard diluent to make linearity standard solutions.

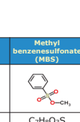
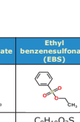
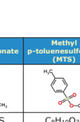
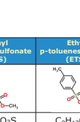
Parameter	Methyl benzenesulfonate (MBS)	Ethyl benzenesulfonate (EBS)	Methyl p-toluenesulfonate (MTS)	Ethyl p-toluenesulfonate (ETS)
Structure				
Molecular formula	$C_7H_8O_3S$	$C_8H_{10}O_3S$	$C_8H_{10}O_3S$	$C_9H_{12}O_3S$
Monoisotopic mass (Da)	172.0	186.0	186.0	200.0
QDa detection (Da)	$[M + NH_4]^+$ = 190.0	$[M + NH_4]^+$ = 204.0	$[M + NH_4]^+$ = 204.0	$[M + NH_4]^+$ = 218.0

Table 1. Genotoxic impurities for method development.

UPLC Method

LC System	ACQUITY UPLC® H-Class with PDA & ACQUITY QDa Detectors
Solvents	A: 5 mM Ammonium acetate in water B: Methanol
Column	ACQUITY UPLC® CSH C_{18} (1.7-µm, 2.1 x 50 mm)
Flow Rate	0.6 mL/min
Column Temp.	40 °C
Injection Vol.	8.0 µL
Sample Temp.	10 °C
Gradient	Step 1: Initial, 90.0, 10.0, Initial Step 2: 3.5, 10.0, 90.0, 6 Step 3: 4.0, 10.0, 90.0, 6 Step 4: 4.5, 90.0, 10.0, 6 Step 5: 6.5, 90.0, 10.0, 6 (Time (minutes); Solvent A (%); Solvent B (%); Curve)
PDA Detection	200 – 400 (derived at 220 nm)
MS Detetion	Ionization mode: ESI+, ESI- MS Acquisition range: 100 - 250 Da SIR(+): 190.0, 204.0 & 218.0 Da Sampling rate: 10 pts/sec Capillary voltage: Pos: 1.4 kV, Neg: 0.8 kV Cone voltage: 6 V Probe temperature: 300°C

Table 2. UPLC method conditions. Parameters were optimized to 1 mM ammonium acetate & 10.0 µL injection volume for methyl ester to improve sensitivity with MS detection.

RESULTS

Method Development

We investigated different chromatographic parameters such as column chemistry, pH and mobile phase. Furthermore, we studied the effect of different mobile phases and tuning parameters of the MS detector on the sensitivity of the MS method.

Effect of mobile phase

Each mobile phase investigated in this study (Figure 1) provided an acceptable separation between all components but resulted in different mass spectral data. With 0.1% formic acid (Figure 1A), we see formation of different adduct ions including protonated, ammonium and sodium. Under ammonium hydroxide conditions, we observed formation of ammonium adducts for all peaks and sodium adduct for methyl benzenesulfonate ester (Figure 1B). Finally, with ammonium acetate (Figure 1C), ammonium adduct ions are generated for all esters.

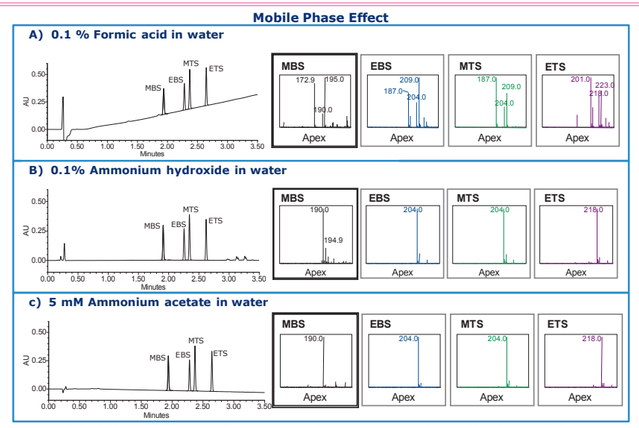


Figure 1. Mobile phase effect on the separation (UV at 220 nm) and MS spectral data. An acceptable separation was achieved with each mobile phase but ammonium acetate resulted in best MS spectral data.

ID	Analyte	$[M + H]^+$ (m/z)	$[M + NH_4]^+$ (m/z)	$[M + Na]^+$ (m/z)
MBS	Methyl benzenesulfonate	172.9	190.0	195.0
EBS	Ethyl benzenesulfonate	187.0	204.0	209.0
MTS	Methyl p-toluenesulfonate	187.0	204.0	209.0
ETS	Ethyl p-toluenesulfonate	201.0	218.0	223.0

Table 3. List of adduct ions for esters of sulfonic acids.

Furthermore, ammonium acetate mobile phase provided greatest MS intensity for our analytes (Figure 2). This is important for enhancing sensitivity of the MS method. Therefore, ammonium acetate mobile phase was selected for final method.

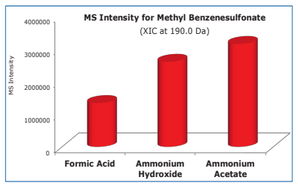


Figure 2. Effect of mobile phase on intensity with MS detection. Data processed using MS extracted ion chromatogram (XIC) at 190.0 Da for methyl benzenesulfonate ester. Ammonium acetate mobile phase resulted in greatest MS intensity.

Optimizing tuning parameters of MS detector

The MS tuning parameters of the ACQUITY QDa Detector were optimized to improve sensitivity of the method (Figure 3).

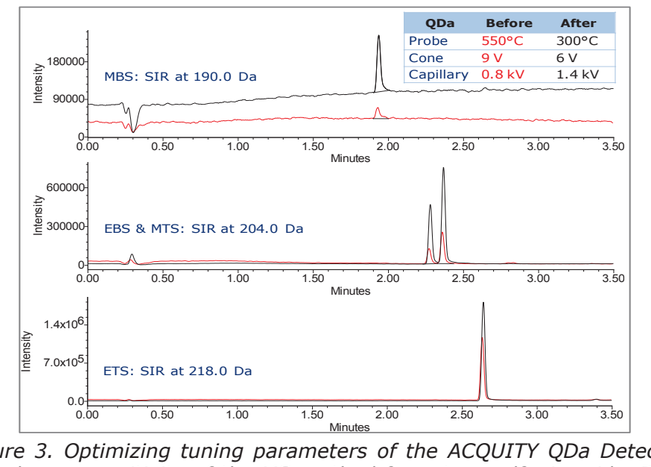


Figure 3. Optimizing tuning parameters of the ACQUITY QDa Detector to enhance sensitivity of the MS method for esters sulfonic acids. Data processed using single ion recording (SIR) acquisition mode.

Analysis of Esters

The developed UPLC method was applied for analysis of methyl, ethyl, and isopropyl esters of benzenesulfonic acid. We determined sensitivity and linearity with UV and MS detection and specificity of the method. For MS detection, we measured ammonium adduct ions using a single ion recording (SIR) mode, which determines intensity of a single ion of interest.

Sensitivity

Limits of detection (LOD) and quantitation (LOQ) were determined following the signal-to-noise criteria of 3:1 and 10:1, respectively (Table 4).

ID	Analyte	LOD (ng/mL)		LOQ (ng/mL)		%RSD of RT (LOQ)		%RSD of Peak Areas (LOQ)	
		UV	MS	UV	MS	UV	MS	UV	MS
MBS	Methyl benzenesulfonate	15	5.0	50	7.5	0.02	0.05	1.64	7.46
EBS	Ethyl benzenesulfonate	15	0.5	50	1.5	0.02	0.05	2.77	6.78
IBS	Isopropyl benzenesulfonate	30	0.5	100	1.5	0.01	0.04	3.50	6.56

Table 4. Limit of detection (LOD) and quantitation (LOQ) with UV and MS detection determined using data from six replicate injections. UV data at 220 nm and MS data processed using MS single ion recording (SIR) acquisition mode.

Linearity

Linearity with UV and MS detection was determined over seven concentrations levels ranging from LOQ to 10,000 ng/nL (Table 5).

ID	Analyte	UV Detection		MS Detection	
		Range (ng/mL)	R^2	Range (ng/mL)	R^2
MBS	Methyl benzenesulfonate	50 – 10,000	0.999541	15 – 1000	0.999143
EBS	Ethyl benzenesulfonate	50 – 10,000	0.999521	1.5 – 500	0.999738
IBS	Isopropyl benzenesulfonate	100 – 10,000	0.999414	1.5 – 500	0.998442

Figure 5. Method linearity with UV at 220 nm and MS detection using MS SIR data. Method exhibited an acceptable linearity.

Specificity

For specificity, we evaluated homogeneity of the peaks in the presence of the API and calculated recovery (Figures 4 and 5).

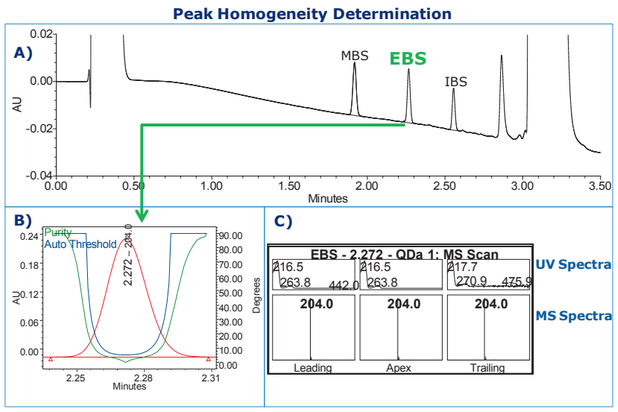


Figure 4. Peak homogeneity determination for specificity. A) Amplodipine besylate API sample at 1 mg/mL spiked with methyl, ethyl and isopropyl benzenesulfonate esters, UV at 220nm. B) Peak purity plot of ethyl ester. The purity angle is below the threshold angle, indicating peak is spectrally homogenous. C) UV and mass profile of ethyl ester at the leading, apex, and tailing edge of the peak. Presence of one mass of 204.0 Da across the peak demonstrates that ethyl ester is not co-eluting with other peaks.

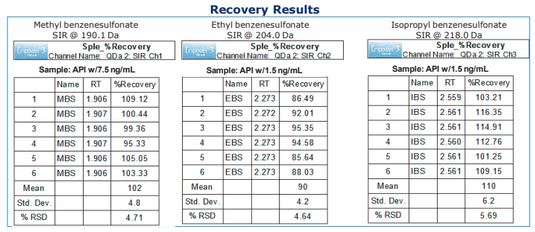


Figure 5. Recovery results for esters of benzenesulfonic acid from amlodipine besylate API sample spiked at the LOQ levels. Data processed using MS SIR acquisition mode.

CONCLUSION

- **The ACQUITY QDa enables quick and accurate determination of peak identity by mass detection**
- **The use of the mass detector and single ion recording (SIR) enhances specificity and sensitivity of the method**
- **The reproducibility and accuracy with MS detection at the quantification levels were excellent**

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