MONITORING AND QUANTITATING GENOTOXIC IMPURITIES USING MASS DETECTION AND UV – SENSITIVE ANALYSIS OF ARYL SULFONIC ACID ESTERS

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INTRODUCTION

Genotoxic impurities (GTIs) are intermediates or reactants that are known to react with DNA and pose significant health risks[1]. They can develop during the synthesis, formulation or storage of a drug product. Aryl sulfonic acids such as benzenesulfonic acid (besylate) and p-toluenesulfonic acid (tosylate) are commonly used as counter ions with Active Pharmaceutical Ingredients (APIs). These sulfonic acids can interact with residual alcohols to generate aryl esters, which are considered potential genotoxic impurities. MS detection enables the detection and identification of these impurities early and accurately in the drug development process. An integrated UV-MS-UPLC system with a compliant Chromatography Data Software (CDS, Empower) allows accurate discrimination and sensitive quantification of genotoxic impurities, while remaining suitable for use in a regulated environment.

In this work, we present the development of a UPLC method coupled with both UV and MS detection for analysis of benzenesulfonic and p-toluenesulfonic acid esters. We will demonstrate that by employing mass detection we can enhance sensitivity and selectivity of the method for analysis of low level impurities in pharmaceutical samples.

METHODS

The chemical structures of esters of benzenesulfonic and p-toluenesulfonic acids are shown in Table 1.

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 investigated in this study: esters of benzenesulfonic and p-toluenesulfonic acids.

EXPERIMENTAL

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 (20:80 methanol/5 mM ammonium acetate) to make linearity standard solutions.

Linearity standards for UV detection were prepared at the following concentrations: 50, 100, 500, 1,000, 2,500, 5,000, 7,500 and 10,000 ng/mL. Linearity standards for MS detection included: 1.5, 7.5, 15, 25, 50, 75, 100, 250 and 500 ng/mL.

Final UPLC method Conditions

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 / Time (minutes) / Solvent A (%) / Solvent B (%) / Curve 1 / Initial / 90.0 / 10.0 / Initial 2 / 1.5 / 10.0 / 90.0 / 6 3 / 4.0 / 10.0 / 90.0 / 6 4 / 4.5 / 90.0 / 10.0 / 6 5 / 6.5 / 90.0 / 10.0 / 6
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

A quick and robust UPLC method was developed for the dual detection analysis of esters of benzenesulfonic and p-toluenesulfonic acids. The UV and mass detection using an ACQUITY QDa detector was utilized for fast and accurate monitoring of genotoxic impurities and peak homogeneity determination.

Method Development

During the development process we investigated the effect of column chemistry, pH and mobile phase on the chromatographic separation between all components. Furthermore, we studied the effect of different mobile phases and tuning parameters of the MS detector on the quality of the MS spectral data and sensitivity of the MS method.

Effect of mobile phase

The mobile phase investigated in this study included 0.1% formic acid in water, 0.1% ammonium hydroxide in water, and 5 mM ammonium acetate in water. Separation was performed using a standard gradient of 5—90% of methanol over 5 minutes.

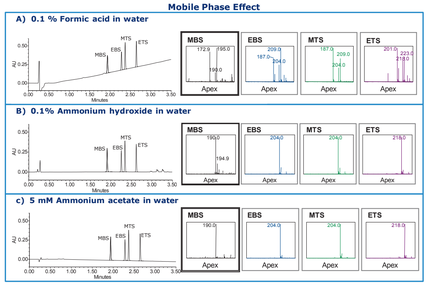

Figure 1. Mobile phase effect on the separation (UV at 220 nm) and MS spectral data. An acceptable separation was achieved with each mobile phase but ammonium acetate resulted in best quality of the MS data.

Each mobile phase provided an acceptable separation between all components of the mixture (Figure 1) 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.

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 benzenesulfonic and p-toluenesulfonic 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.

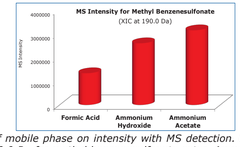

Figure 2. Effect of mobile phase on intensity with MS detection. Data processed using MS XIC (+) at 190.0 Da for methyl benzenesulfonate ester. Ammonium acetate mobile phase resulted in greatest MS intensity.

Optimizing Tuning Parameters of MS Detector

The MS tuning parameters of the ACQUITY QDa Detector were optimized to improve sensitivity of the method. The parameters included probe temperature, cone and capillary voltages (Figure 3).

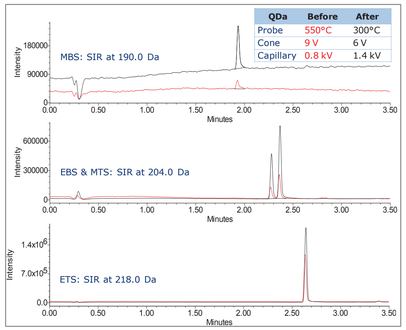

Figure 3. Optimizing tuning parameters of the ACQUITY QDa Detector to enhance sensitivity of the MS method for esters of benzenesulfonic and p-toluenesulfonic acids. Data processed using Single Ion Recording (SIR) acquisition mode.

Analysis of Esters

The developed UPLC method was applied for analysis of methyl, ethyl, and isopropyl esters of benzenesulfonic acid (Figure 4). 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. The SIR mode enhances method sensitivity, and simplifies analysis and quantification.

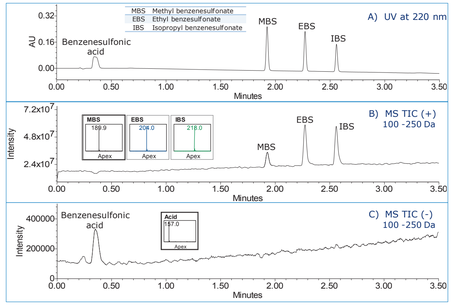

Figure 4. UV and MS chromatographic data for the separation of methyl, ethyl and isopropyl esters of benzenesulfonic acid. A) UV trace at 220 nm, B) MS Total Ion Chromatogram (TIC) data in ESI+. C) MS TIC data in ESI-.

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). Data from six replicate injections of standard was evaluated to establish and to verify performance at the LOD and LOQ limits.

ID	Analyte	LOD (ng/mL) UV	LOD (ng/mL) MS	LOQ (ng/mL) UV	LOQ (ng/mL) MS	%RSD of RT (LOQ) UV	%RSD of RT (LOQ) MS	%RSD of Peak Areas (LOQ) UV	%RSD of Peak Areas (LOQ) 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. MBS: 190.0 Da, EBS: 204.0 Da, IBS: 218.0 Da. MS detection improved sensitivity of the UPLC method.

Linearity

Linearity with UV and MS detection was determined over seven concentrations levels ranging from LOQ to 10,000 ng/mL.

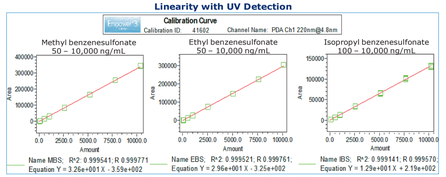

Figure 5. Method linearity with UV at 220 nm. Method exhibited an acceptable linearity.

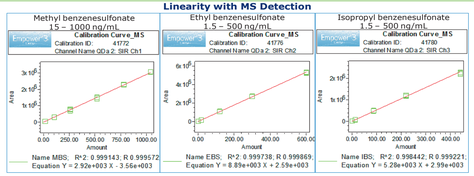

Figure 6. Method linearity with MS detection. MS data processed using MS Single Ion Recording (SIR) acquisition mode. MBS: 190.0 Da, EBS: 204.0 Da, IBS: 218.0 Da. Method exhibited an acceptable linearity.

Specificity

To demonstrate specificity, we spiked amplodipine besylate API sample with methyl, ethyl and isopropyl esters. We evaluated homogeneity of the peaks in the presence of sample matrix and calculated recovery.

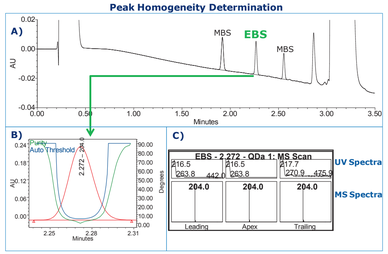

Figure 7. Peak homogeneity determination for specificity. A) Amplodipine besylate API sample at 1 mg/mL spiked with methyl, ethyl and isopropyl benzenesulfonate esters, UV at 220nm. B) Peak purity plot of ethyl ester. The purity angle is below the threshold angle, indicating peak is spectrally homogenous. 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.

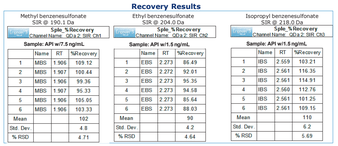

Figure 8. Recovery results for methyl, ethyl and isopropyl benzenesulfonate esters from amlodipine besylate API sample spiked at the LOQ levels. Data processed using MS SIR acquisition. The average recoveries ranged from 90 - 110%.

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 UPLC method required for low level analysis**
- **The reproducibility and accuracy of the MS method at the quantification levels were excellent**
- **The ACQUITY UPLC H-Class System coupled with UV and MS detectors and a compliant-ready Empower 3 software is suitable for use in a regulated environment**

References

1. Liu D.Q., Sun M., Kord A.S., Recent Advances in Trance Analysis of Pharmaceutical Genetoxic Analysis, *Journal of Pharmaceutical and Biomedical Analysis*, 2010, 999-1014

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