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Analysis of Benzenesulfonic Acid and P-Toluenesufonic Acid Esters in Genotox Monitoring using UPLC-UV-MS

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Abstract

This paper demonstrates the improvements obtained using sub-2-µm UltraPerformance LC (UPLC) column packing materials UPLC/UV-MS for the analysis of benzenesulfonic acid, p-toluenesulfonic acid, and their alkyl esters.

Benefits

UPLC combines with PDA and mass detection provides an excellent solution to the analysis of alkyl arylsulfonate esters in drug substances and drug products for genotoxic impurity monitoring. UPLC provides both high resolution and a highthroughput analysis, reducing analysis times from as much as 30 minutes to only 5 minutes. For applications where sensitivity and selectivity are not as demanding, this UPLC/UV system allows for a simple, low cost solution to the analysis of these genotoxic impurities.

Introduction

Alkyl esters of sulfonic acids, particularly methanesulfonic, benzenesulfonic, and p-toluenesulfonic acid esters, are a common class of reagents used in the pharmaceutical industry as alkylating agents, catalysts, and in purification steps of the chemical synthesis of a drug substance. In addition, sulfonic acids are often used as the final salt form of the drug substance due to improved chemical properties or bioavailability. The presence of any residual alcohols from synthetic reaction or recrystalization steps may result in the formation of alkyl esters of the sulfonic acids. Many of these mesylate, besylate, or tosylate esters are known to be genotoxic, while others are potentially genotoxic, requiring monitoring in the drug substance and drug product.

The U.S. FDA draft guidance[1] and the EMEA guidelines[2,3] require that any possible genotoxic impurities in a drug substance or drug product that have not been shown to be removed during early synthesis steps be monitored to ensure that the levels are below the Threshold for Toxicological Concern (TTC) of 1.5 µg/day based upon the maximum daily dosage of the pharmaceutical compound.

Depending upon the particular active pharmaceutical ingredient (API), the sensitivity requirements for the analytical method can be quite challenging. For a drug product with a maximum daily dose of 1000 mg, any genotoxic impurity must be analyzed to be less than the TTC level:

[1.5 µg/day] / 1.000 gm = 1.5 ppm

Therefore, the genotoxic impurity must be at a level less than 1.5 ppm in the drug product or drug substance.[4,5]

The most common analytical techniques for monitoring alkyl sulfonate esters have been GC-MS or HPLC/UV-MS with derivatization using pentafluorothiophenol. More recently, HPLC-MS has been shown to give good results without the need for a complicated derivatization step; however, run times on the order of 20 to 30 minutes are required to achieve sufficient resolution from the API.[6]

This paper demonstrates the improvements obtained using sub-2-µm UltraPerformance LC (UPLC) column packing materials UPLC/UV-MS for the analysis of benzenesulfonic acid, p-toluenesulfonic acid, and their alkyl esters. Analysis times of less than 5 minutes with sufficient sensitivity to meet FDA and EMEA requirements are demonstrated without the need for pre- or post-column derivatization.

Experimental

LC conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH Phenyl Column 2.1 x 50 mm, 1.7 µm
Column temp.:	50 °C
Flow rate:	600 µL/min
Mobile phase A:	5 mM Ammonium Formate, pH 9.0
Mobile phase B:	Methanol

Gradient:	10 to 98% B/5 min
PDA detection:	210 to 300nm, 1.2 nm resolution, 40 pts/sec

MS conditions

MS System:	Waters SQ Mass Detector
Ionization Mode:	ESI positive
Capillary Voltage:	3200 V
Cone Voltage:	24 V
Desolvation temp.:	400 °C
Source temp.:	130 °C
Desolvation gas:	800 L/Hr
Cone gas flow:	50 L/Hr
MS method:	- ESI+ / SIR

Results and Discussion

A method scouting approach was utilized to quickly develop a chromatographic method for the analysis of methyl benzenesulfonate, ethyl benzenesulfonate, methyl p-toluenesulfonate, and ethyl p-toluenesulfonate utilizing an ACQUITY UPLC System with an ACQUITY UPLC PDA detector and a SQ Mass Detector. The MS data was collected in ESI+ mode using timed SIR functions to maximize the dwell time and therefore sensitivity of the analysis. A simple 5-minute gradient from 10% to 98% methanol resulted in a rapid separation with resolution of 1.9 or better for all analytes with sufficient sensitivity to meet FDA and EMEA

Genotoxic Impurities Guideline requirements.

UV detection

A mixture of 1 ppm each of the alkylsulfonate ester standards was analyzed in less than 3 minutes and the UV chromatograms (220 nm) for three replicate injections are shown (Figure 1). All of the alkylsulfonate esters analyzed exhibited good linearity with correlation coefficients greater than 0.9997 for calibration curves covering the concentration range from 0.01 to 10 ppm (Figure 2). This is sufficiently sensitive to monitor these genotoxic and potentially genotoxic impurities (PGIs) in drug substances and drug products at the levels required by FDA and EMEA guidelines as long as the analytes can be well resolved from the active pharmaceutical ingredient and excipients.

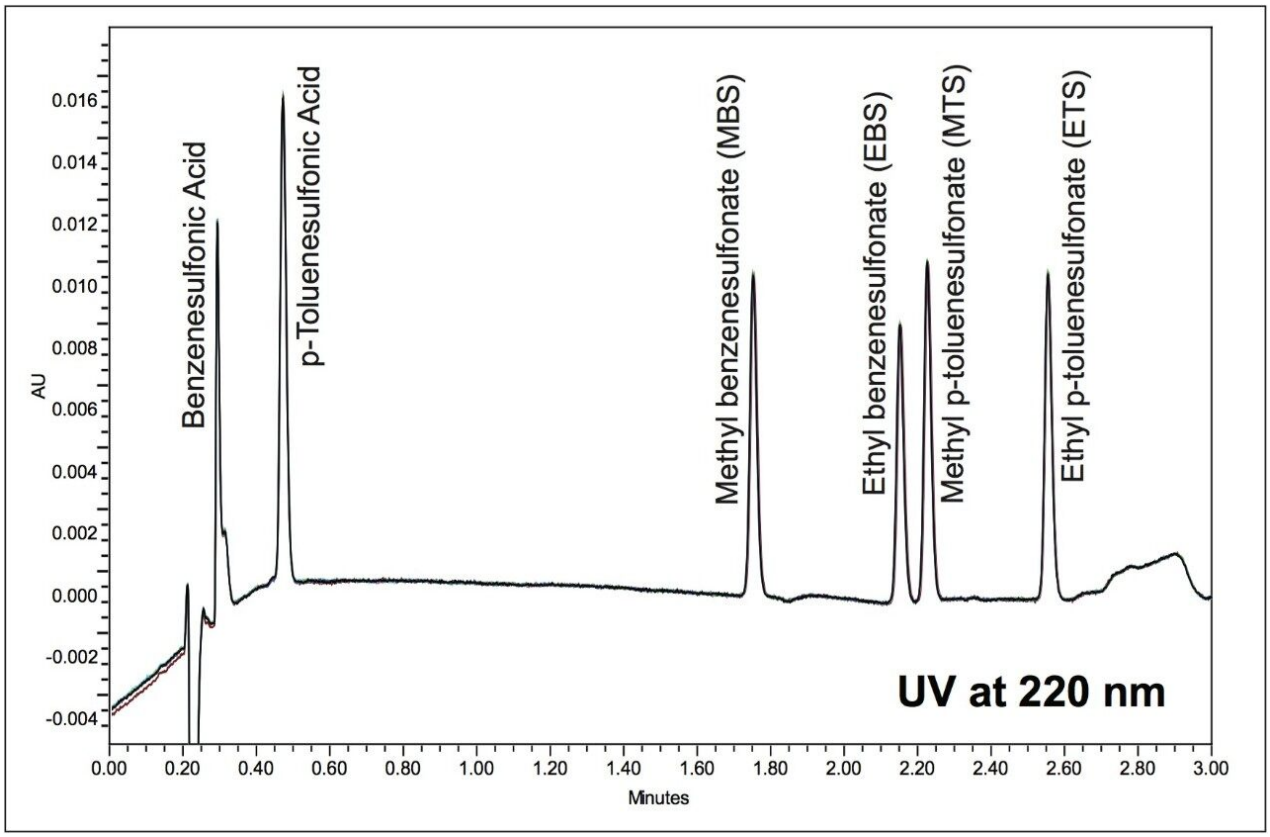


Figure 1. Overlay of three replicate injections (10 µL) of 1 ppm alkylsulfonate ester standards with UV detection at 220 nm. Benzenesulfonic acid and p-toluenesulfonic acid standards (1 ppm) are shown for reference.

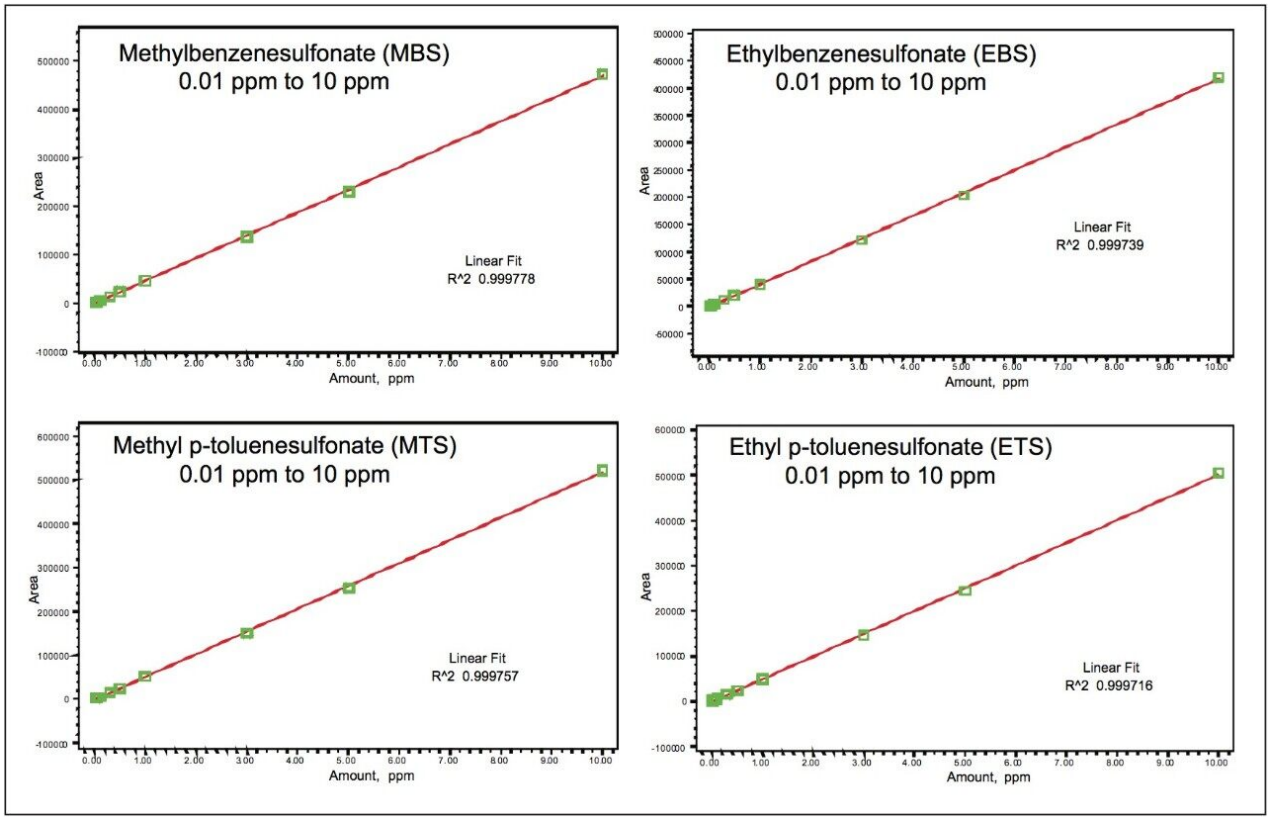


Figure 2. UV results at 220 nm demonstrates good linearity over a range of approximately 0.01 ppm to 10 ppm with correlation coefficients greater than 0.9997 for all analytes.

Using the conditions described here with 10 µL injections, the retention time reproducibility, and the limits of detection and quantitation determined for standards with UV detection are listed in Table 1. An LOQ of 0.04 ppm corresponds to a quantitation limit of 4 ppm in a 1% solution of a drug substance or a drug product. This is sufficiently sensitive to meet regulatory guidelines for the analysis of these PGIs in pharmaceutical products with a maximum daily dose of 375 mg or less, which is adequate for a number of commercially-available pharmaceuticals. If additional sensitivity is required, preparation of the samples at higher concentrations (~5%) and/or injecting larger volumes (~20 µL) may achieve the desired LOQs, however, the use of MS detection may be necessary to meet the highest sensitivity applications.

Component	Mean RT*	%RSD RT*	LOD (3X)	LOQ (10X)
MBS	1.775	0.05%	0.01 ppm	0.04 ppm
EBS	2.175	0.05%	0.01 ppm	0.04 ppm
MTS	2.250	0.04%	0.01 ppm	0.04 ppm
ETS	2.577	0.03%	0.01 ppm	0.04 ppm

**All conc., N=30*

Table 1. Limits of detection and quantitation and reproducibility of retention times for methyl and ethyl benzenesulfonates and methyl and ethyl toluenesulfonates standards with this method using UV detection at 220 nm.

MS detection

Mass spectrometry data (SIR mode) collected for the same 1 ppm standards of the alkylsulfonate esters is shown (Figure 3). The narrow peaks observed with UPLC, which are typically 1 to 3 seconds wide, require rapid MS scanning to obtain 15 to 20 data points across the peaks necessary for accurate and reproducible integration and quantitation. The use of timed SIR functions allows for the optimization of dwell time and sensitivity for each component while maintaining sufficient data points for good integration.

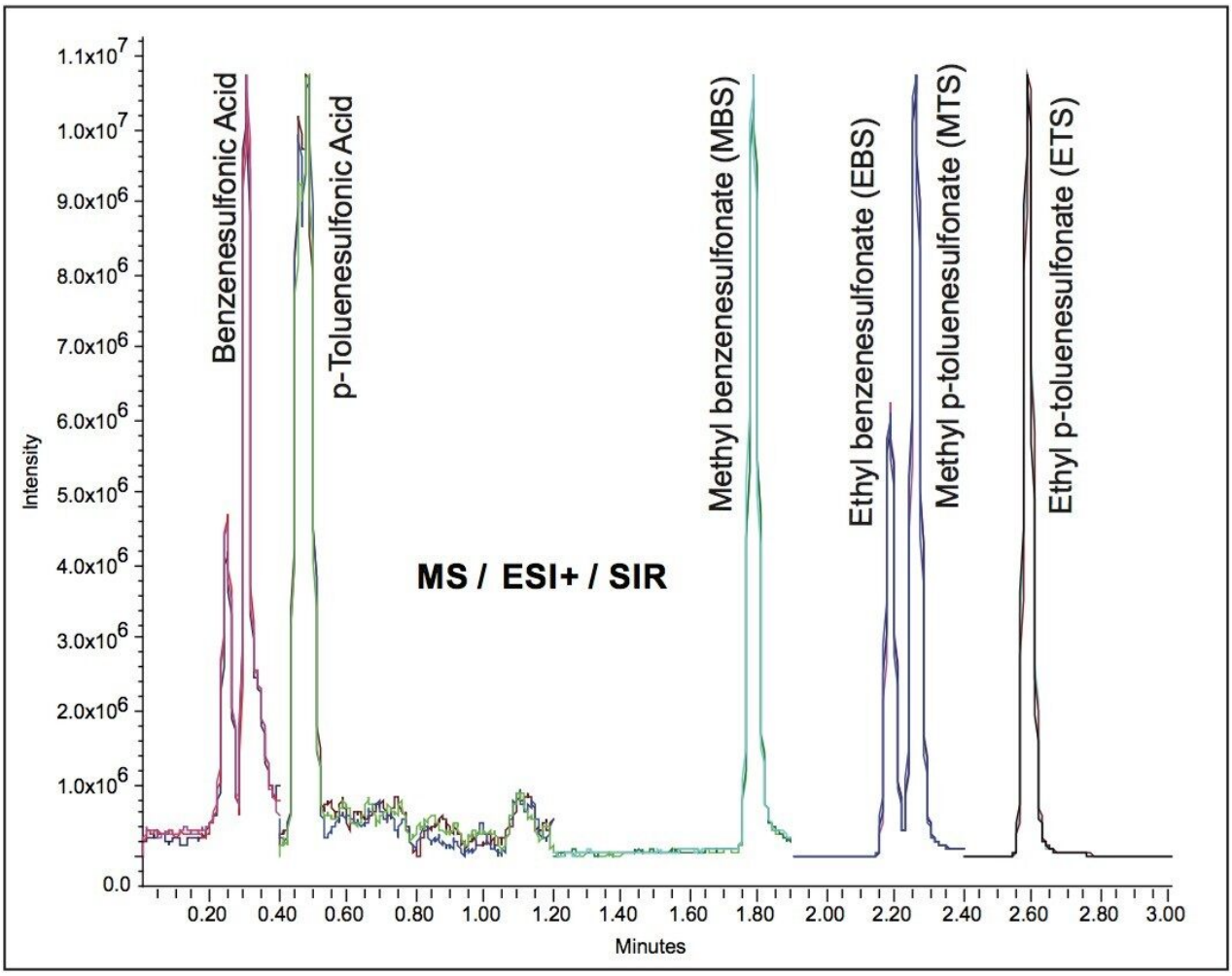
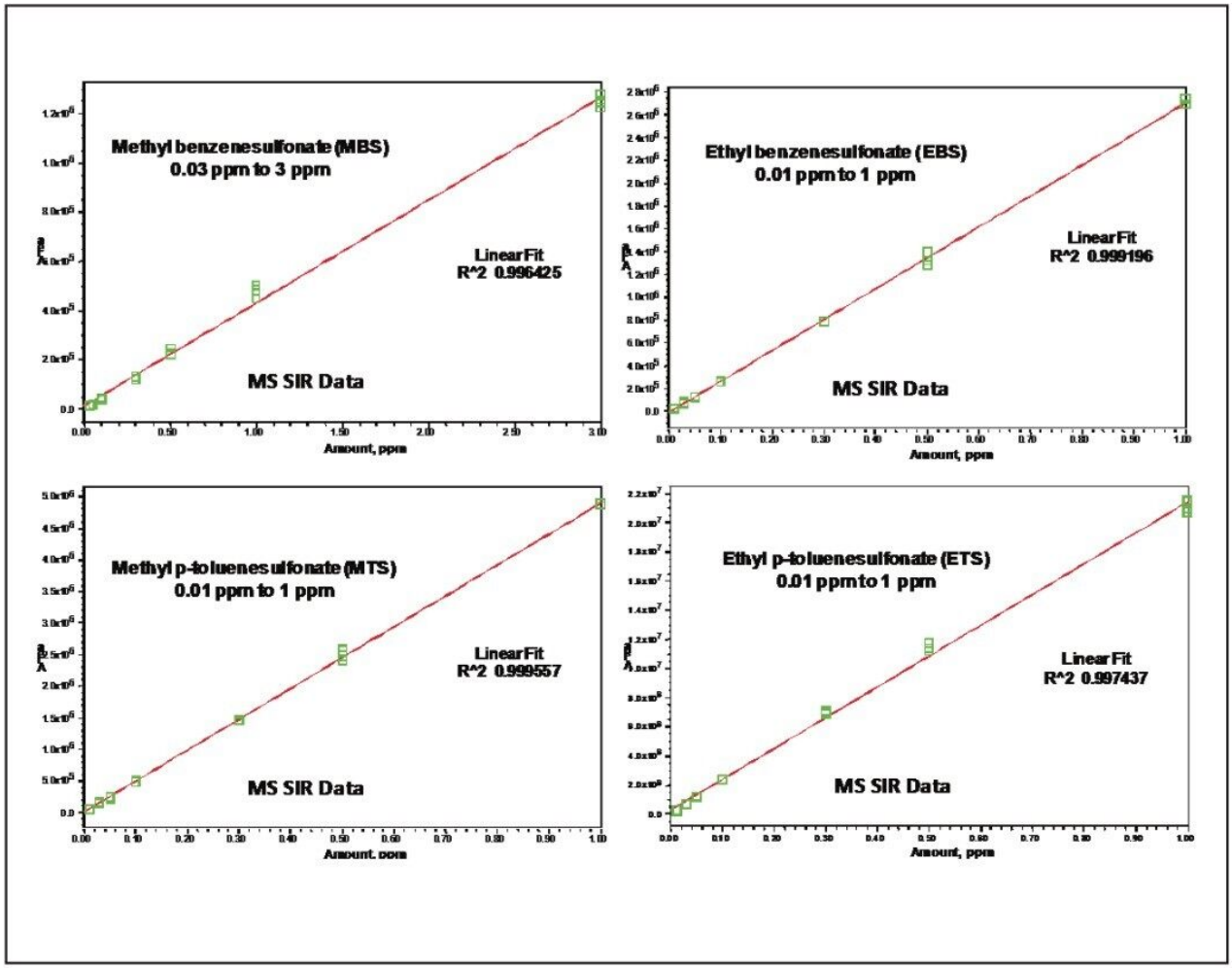


Figure 3. Overlay of three replicate injections (10 µL) of 1 ppm alkylsulfonate ester standards with MS detection using ESI+ and SIR mode. Benzenesulfonic acid and p-toluenesulfonic acid standards (1 ppm) are shown for reference.

MS data for all of the alkylsulfonate esters analyzed exhibited good linearity with correlation coefficients greater than 0.9960 for calibration curves covering the concentration range from 0.01 to 3 ppm (Figure 4). Using the conditions described here, the retention time reproducibility and the limits of detection and quantitation obtained are listed in Table 2. An LOQ of 6 ppb (MTS) corresponds to a quantitation limit of 0.6 ppm in a 1% solution of a drug substance or a drug product and meets the sensitivity level required for a pharmaceutical product with a maximum daily dose of 2500 mg or less, which is adequate for most commercially available pharmaceuticals.

Higher sample concentrations and larger injection volumes may be employed to achieve additional sensitivity for particularly difficult samples.



Amlodipine Besylate

The first example shown uses the drug substance Amlodipine Besylate, which is the benzenesulfonic acid salt of Amlodipine. If this material is exposed to residual alcohols from purification/recrystalization steps or any other source, the potential exists for the formation of the potentially genotoxic alcohol esters of the benzenesulfonic acid. This drug substance must therefore be monitored for the presence of these compounds.

Amlodipine Besylate is a long-acting calcium channel blocker use for the treatment of high blood pressure. It is usually administered orally and has a maximum daily dose of 10 mg, which corresponds to a TTC level of 150 ppm for each of the genotoxic impurities. Amlodipine Besylate was obtained from U.S. Pharmacopeia (Rockville, MD) and was analyzed as a 1% solution. Methyl benzenesulfonate (MBS) and ethyl benzenesulfonate (EBS) were not detected in this sample (less than 0.2 ppm) as demonstrated in Figure 5 (MS data).

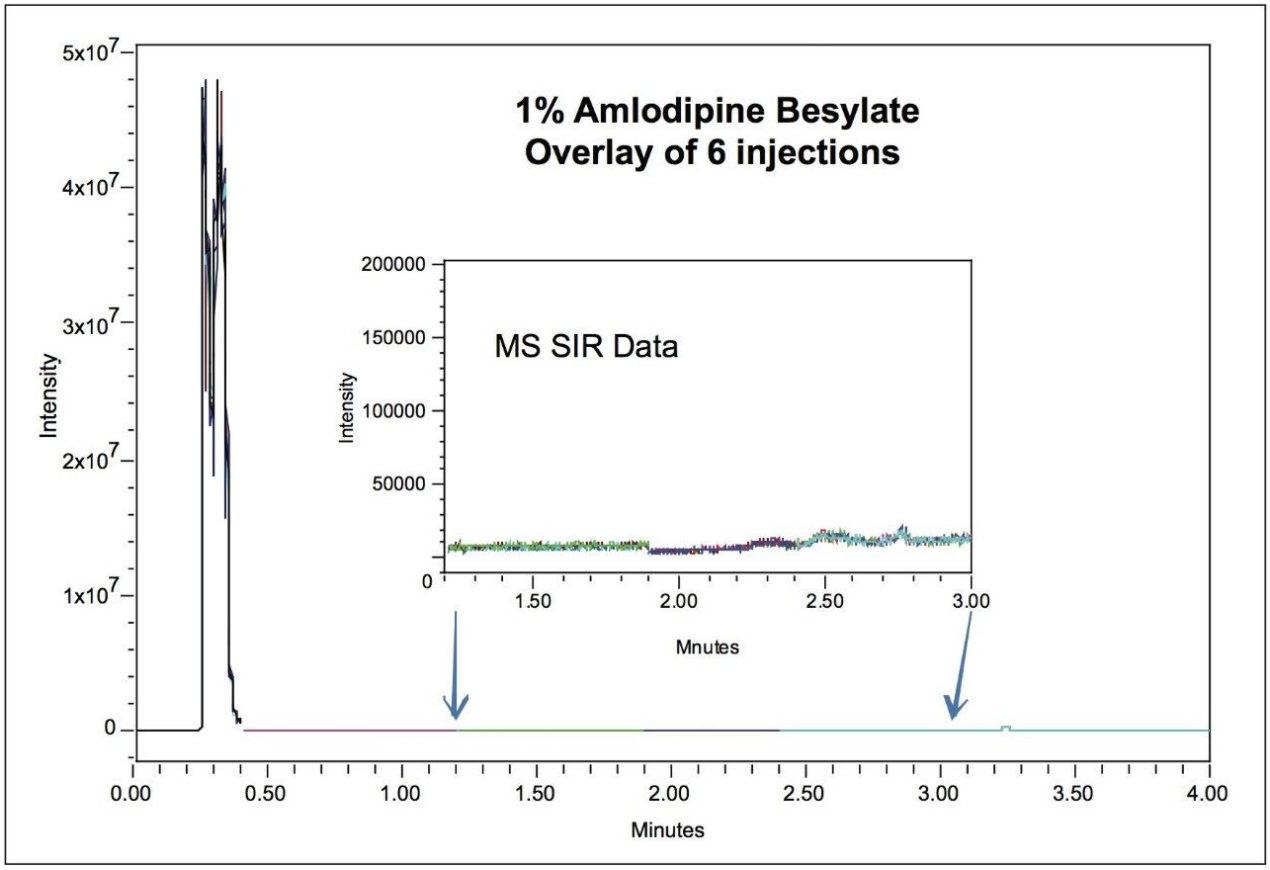


Figure 5. MS analysis of alkyl toluenesulfonates in Amlodipine Besylate.

A second sample was prepared and spiked with methyl benzenesulfonate and ethyl benzenesulfonate at 15 ppm each relative to the solid Amlodipine Besylate representing 1/10th the regulatory requirements for the limits of these genotoxins in this particular drug substance. This sample was analyzed and the resulting MS chromatograms for six replicate injections (Figure 6) demonstrate the excellent sensitivity and reproducibility of the chromatographic method. The separation of the analytes of interest away from the free sulfonic acids ensures that ionization of the sulfonate esters will not be suppressed by the excess of the benzenesulfonic acid. The quantitative MS results (Table 3) display the accuracy and precision of the method with RSDs of 3.5 to 8% and recoveries of 103% and 122% for the spiked MBS and EBS components respectively.

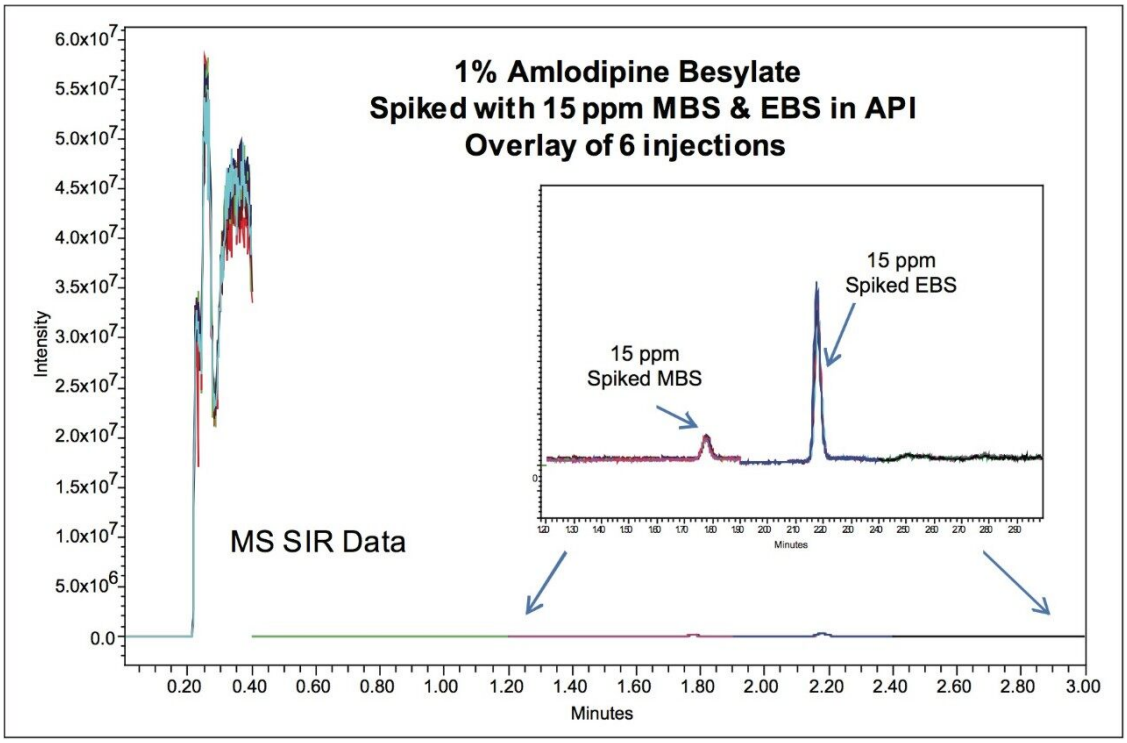


Figure 6. MS analysis of Amlodipine Besylate spiked with 15 ppm methyl benzenesulfonate and ethyl benzenesulfonate.

Sample	Mean MBS*	%RSD	Mean EBS*	%RSD
Amlodipine Besylate	N/D	——	N/D	——
Amlodipine Besylate spiked with 15 ppm (in API)	15.4 ppm	3.4%	18.3 ppm	8.1%

**Average of 6 inj.*

Table 3. Analytical MS results for Amlodipine Besylate sample and sample spiked with 15 ppm of methyl benzenesulfonate and ethyl benzenesulfonate.

UV detection has adequate sensitivity to analyze these PGIs in many drug products providing that the TTC levels are sufficiently high and that the analytes of interest are well resolved from the API, the free acid, and any excipients present in the drug product. The analysis using UV detection at 220 nm of the Amlodipine Besylate sample spiked with 15 ppm of MBS and EBS (Figure 7) demonstrates the resolution of the analytes from the benzenesulfonic acid, the late eluting Amlodipine, and other impurities present in the sample. These

results illustrate the capability of UV detection to achieve the detection levels required by the FDA and the EMEA for MBS and EBS in this particular sample.

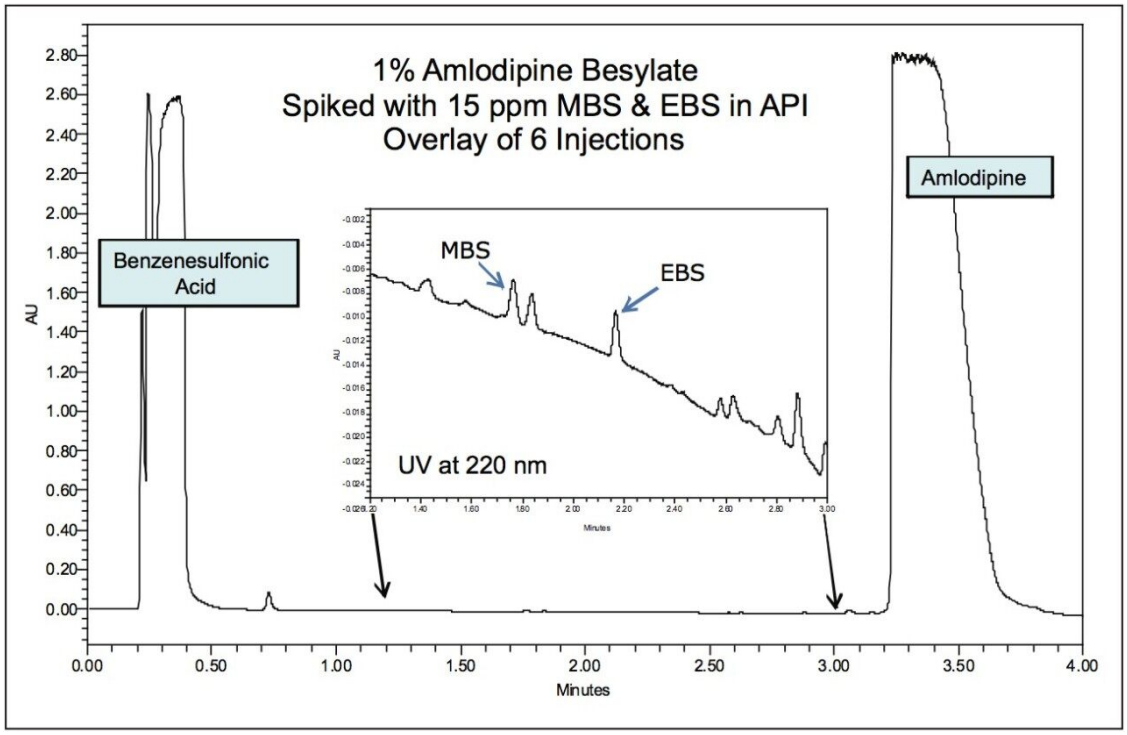


Figure 7. UV Analysis of Amlodipine Besylate spiked with 15 ppm methyl benzenesulfonate and ethyl benzenesulfonate.

Bretylium Tosylate

The second example shown uses the drug substance Bretylium Tosylate, which is the p-toluenesulfonic acid salt of Bretylium. Bretylium Tosylate is an antifibrillatory and antiarrhythmic agent that is normally administered by IV and has a maximum daily dose of approximately 2 to 3 gm, which corresponds to a TTC level of 0.5 ppm (for 3 gm dose) for each of the genotoxic impurities. Bretylium Tosylate was obtained from U.S. Pharmacopeia (Rockville, MD) and was analyzed as a 1% solution. Methyl toluenesulfonate (MTS) and ethyl toluenesulfonate (ETS) were not detected in this sample (less than 0.2 ppm) as demonstrated in Figure 8.

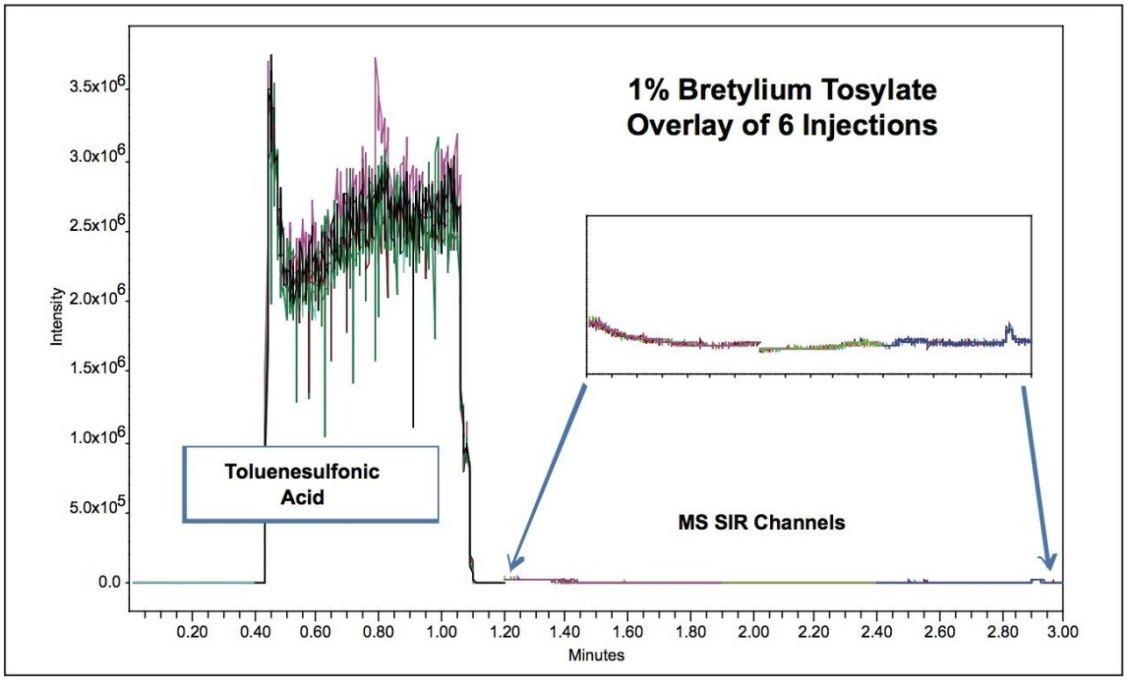


Figure 8. Analysis of alkyl toluenesulfonates in Bretylium Tosylate.

A second sample was prepared and spiked with methyl toluenesulfonate and ethyl toluenesulfonate at 0.5 ppm each relative to the solid Bretylium Tosylate representing the regulatory requirements for the limits of these genotoxins in this particular drug substance. This sample was analyzed and the resulting chromatograms for six replicate injections (Figure 9) demonstrate the excellent sensitivity and reproducibility of the chromatographic method. The separation of the analytes of interest away from the free sulfonic acids ensures that ionization of the sulfonate esters will not be suppressed by the excess of the toluenesulfonic acid. The quantitative results (Table 4) display the accuracy and precision of the method with RSDs of 4 to 7% and recoveries of 94% and 104% for the spiked MTS and ETS components respectively.

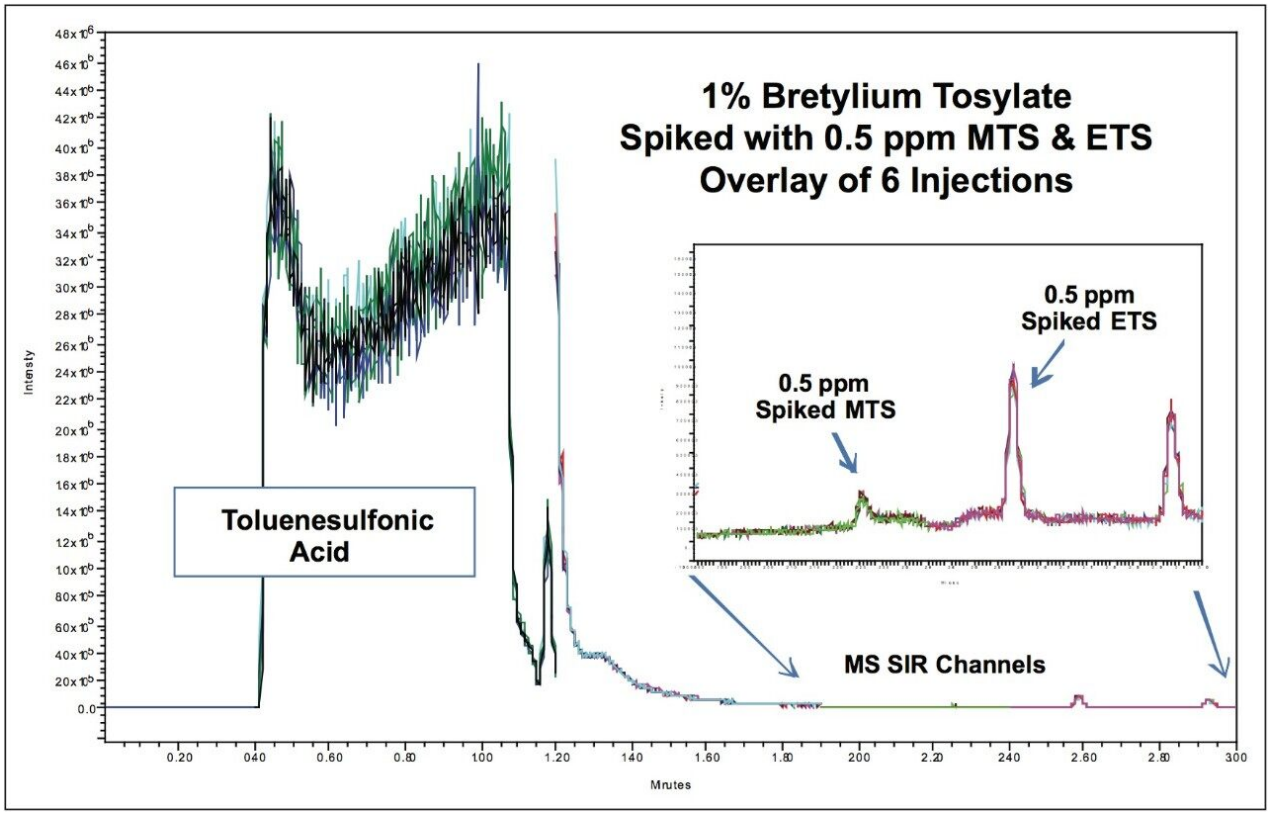


Figure 9. Analysis of Bretylium Tosylate spiked with 0.5 ppm methyl toluenesulfonate and ethyl toluenesulfonate.

Sample	Mean MTS*	%RSD	Mean ETS*	%RSD
Bretylium Tosylate	N/D	——	N/D	——
Bretylium Tosylate spiked with 0.5 ppm *Average of 6 inj.	0.47 ppm	7.3%	0.52 ppm	4.1%

Table 4. Analytical MS results for Bretylium Tosylate sample and sample spiked with 0.5 ppm of methyl toluenesulfonate and ethyl toluenesulfonate.

UV data obtained for this sample (Figure 10) demonstrates the limitations of UV detection for many of these types of genotoxic impurity analyses. Due to the inherent combination of lower sensitivity and specificity of UV detection relative to MS detection, UV detection may be unsuitable for a number of drug products or drug substances with high daily doses that require very high sensitivity or those that are not separated chromatographically from the analytes of interest. Bretylium elutes much earlier in the chromatogram than

Amlodipine and tails into the region where the MTS and ETS peaks elute swamping out the analytes.

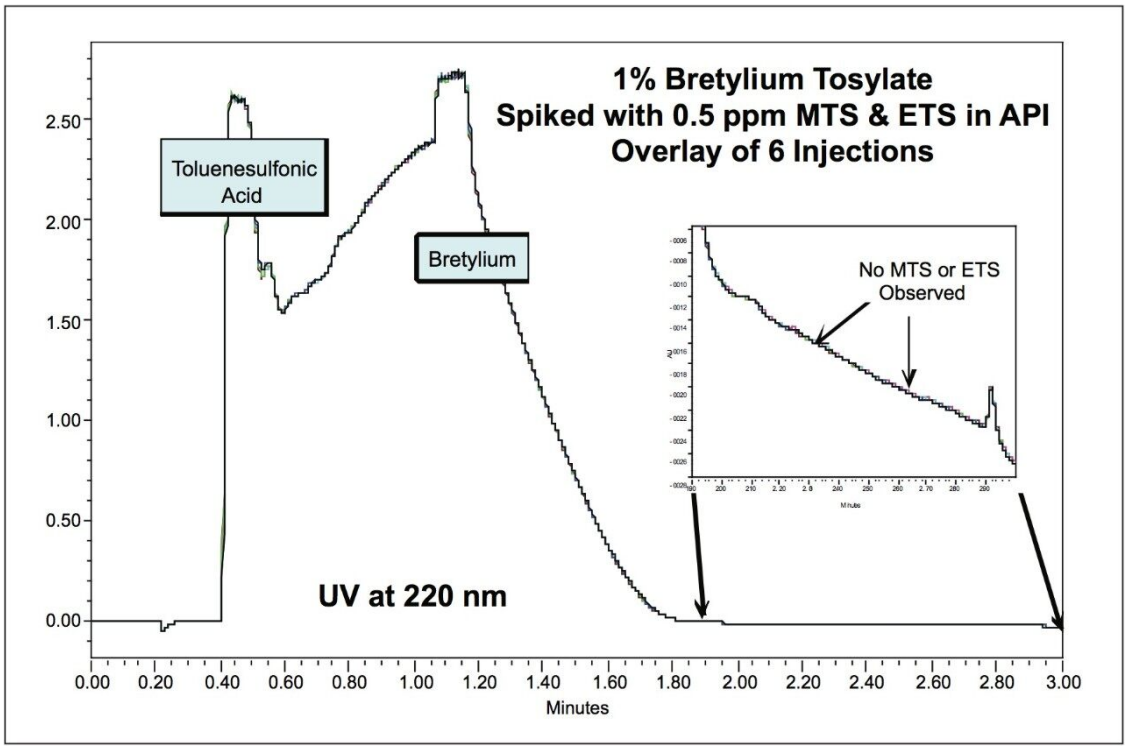


Figure 10. Analysis of Bretylium Tosylate spiked with 0.5 ppm methyl toluenesulfonate and ethyl toluenesulfonate.

In addition, the low TTC levels required for this analysis (0.5 ppm in API) are below the quantitation limits of the UV method. Sensitivity could be improved somewhat by injecting a larger volume (20 µL) of a sample concentration of 5%, however, interference of the analytes with the Bretylium peak would still be an issue.

Overall, UPLC with MS detection using SIR mode offers the most sensitive method with the best specificity for the analysis of alkyl arylsulfonates in drug substances and drug products. Despite the limitations of UV detection, UPLC with UV may be used successfully for the analysis of GTIs in a number of pharmaceutical compounds, particularly those with low maximum daily doses that do not require the utmost sensitivity afforded by MS detection.

Conclusion

The ACQUITY UPLC System paired with the ACQUITY UPLC PDA Detector and the SQ Mass Detector provides an excellent system solution to the analysis of alkyl arylsulfonate esters in drug substances and drug products for genotoxic impurity monitoring. UPLC allows for a high resolution and high throughput analysis, reducing analysis times from as much as 30 minutes to only 5 minutes, increasing laboratory productivity while decreasing solvent consumption and the generation of waste solvents.

The use of a single quadrupole mass spectrometer with single ion recording (SIR) methods achieves the specificity and sensitivity necessary to analyze very low levels of impurities in the presence of 1 to 10% concentrations of drug substances or drug products, to meet today's demanding regulatory requirements for genotoxic impurity analysis. For particular applications where sensitivity and selectivity are not as demanding, UPLC combined with UV detection allows for a simple, low cost solution to the analysis of these genotoxic impurities.

References

1. US Food and Drug Administration, Guidance for Industry, Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches, Draft Guidance. Dec. 2008.
2. The European Medicines Agency [EMEA], Committee for Medicinal Products for Human Use [CHMP], Guidelines on the Limits of Genotoxic Impurities, CPMP/SWP/5199/02.
3. The European Medicines Agency [EMEA], Committee for Medicinal Products for Human Use [CHMP], Questions and Answers on the CHMP Guideline on the Limits of Genotoxic Impurities, CPMP/SWP/431994/07.
4. Glowienke S, Frieauff W, Allmendinger T, Martus H, Suter W, Mueller L. Mutat. Res. 581 (2005) 23.
5. Muller L, et al. Regulatory Toxicology and Pharmacology. 2006; 44: 198-211.
6. Taylor GE, Gosling M, Pearce A. *J. Chromotogr A*. 2006; 1119: 231-237.

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