APPLICATION BRIEF

Chemical/Industrial

Following the Synthesis of a Sulfa Drug Using UHPLC/SQ MS

Sulfonamide drugs were the first antimicrobial drugs, discovered in 1935, that led to the antibiotic revolution in medicine. Sulfonamides are sulfa-related antibiotics which are used to treat bacterial and some fungal infections. Sulfonamides are prepared by the reaction of a sulfonyl chloride with ammonia or an amine. Thousands of molecules containing the sulfanilamide structure have been created since its discovery, yielding improved formulations with greater effectiveness and less toxicity.

Experimental Conditions

Target Analytes: Acetanilide, *p*-acetamidobenzene sulfonamide and sulfanilamide

Sample Preparation Conditions

Stage 1 – Synthesis of *p*-acetamidobenzene sulfonamide from acetanilide

Acetanilide in an Erlenmeyer flask was heated to melt and spread in a thin film over the flask. The flask was then cooled in an ice bath. Chlorosulfonic acid was added into flask. The flask was connected to a trap to collect the HCl produced during the reaction. The flask was then heated on a steam bath for ~15 minutes to dissolve the solid, resulting in an oily substance. The flask was then removed from heat and ice added to the product. A thick white precipitate of *p*-acetamidobenzenesulfonyl chloride was observed to form. The white precipitate was filtered after mixing with ammonium hydroxide solution. The mixture was warmed in a steam bath and then cooled. A white pasty solid of *p*-acetamidobenzene sulfonamide was observed to form. ~1 mg of the wet precipitate was dissolved in MeOH, diluted 1:100 in water and analyzed by UHPLC/ SQ MS to identify the intermediate compound formed.

Stage 2 – Synthesis of sulfonamide from *p*-acetamidobenzene sulfonamide

The white precipitate of p-acetamidobenzene sulfonamide from Stage 1 was filtered and washed with water into a 50 mL flask. HCl was added to the precipitate. The flask was connected to a reflux water condenser and heated on a steam bath for ~20 min.

An aliquot of the mixture was retrieved from the flask every 5 min through the reaction, neutralized with $NaHCO_3$ and analyzed by UHPLC/SQ MS.

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Liquid Chromatography Conditions

Pump Type:	PerkinElmer® Flexar™ FX-15
Column:	PerkinElmer Brownlee™ HRes PFPP column (2.1 mm x 100 mm, 1.9 µm)
Mobile Phase:	A: water containing 0.1% formic acid B: acetonitrile containing 0.1% formic acid
Flow Rate:	0.4 mL/min
Injection Volume:	2 µL in partial fill mode

Gradient:

Time (min)	%A	%B
0	100	0
2	100	0
3	95	5
2	70	30
4	30	70
3	30	70

Mass Spectrometer Conditions

Ionization:	Ultraspray™ ESI – Positive mode
Scan Range:	65-400 m/z
Scan Rate:	1000 u/sec
Capillary Exit Voltage:	100 V

Results

A qualitative UHPLC/SQ MS method is presented to detect and identify the reaction products of a multi-step chemical synthesis (Figure 1) using a single quadrupole MS detector and its front-end Collision Induced Dissociation (CID) capability.

The soft ionization provided by the Flexar SQ 300 MS Ultraspray ESI, combined with the low capillary exit voltage values used, allowed the molecular ion and fragmentation information of the intermediate product p-acetamidobenzene sulfonamide (Figure 2) and the final product sulfanilamide (Figure 3) to be obtained.

This method has also been used for a real-time monitoring of the formation of the sulfanilamide during the acid refluxing-2nd stage synthesis (Figure 4).

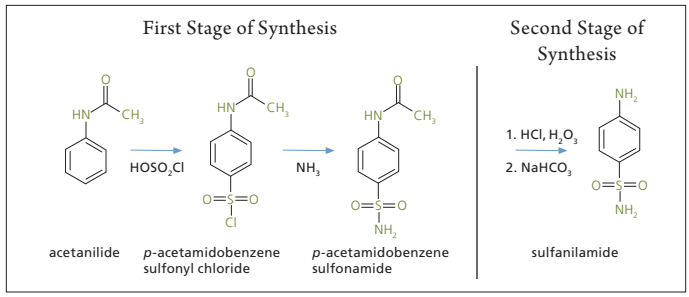

Figure 1. Schematic of the two stage synthesis steps for the formation of the sulfa drug, sulfanilamide.

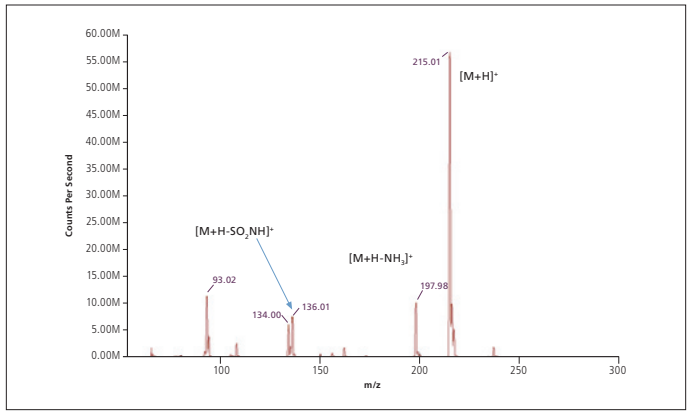

Figure 2. Mass Spectrum of p-acetamidobenzene sulfonamide after first stage of synthesis. The same spectrum has been observed in the three chromatograms shown in Figure 4 for the peak eluting at ~8.5 min.

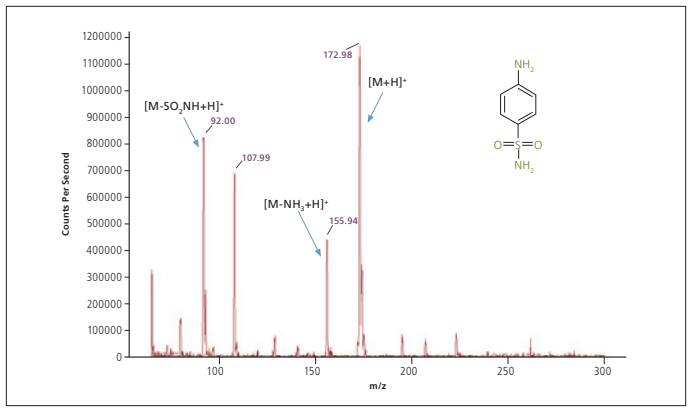

Figure 3. Mass spectrum of the synthesized sulfanilamide.

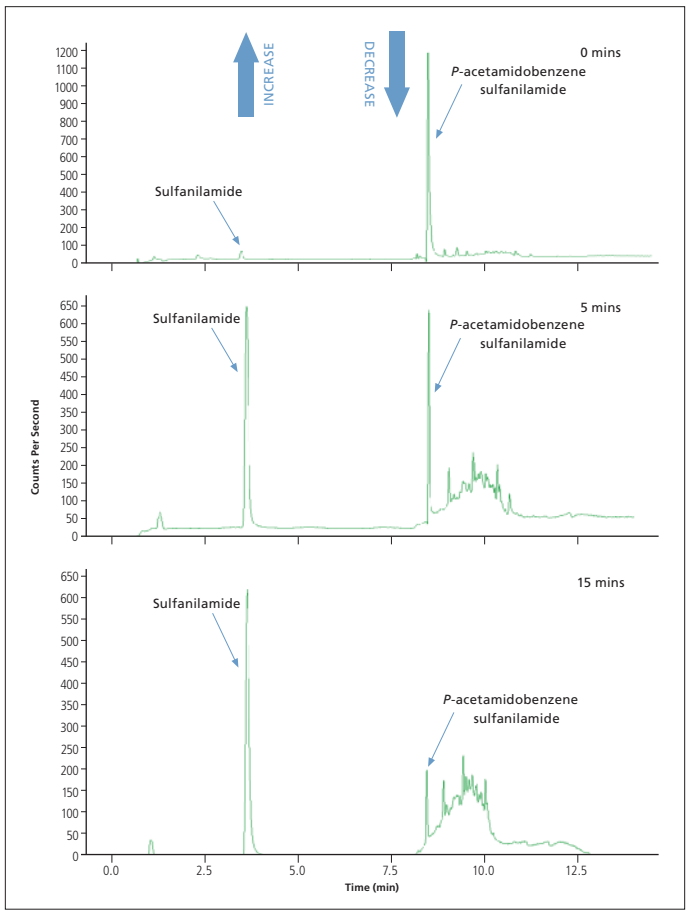

Figure 4. Real time monitoring by UHPLC/SQ MS of formation of sulfanilamide upon acid refluxing of *p*-acetamidobenzene sulfonamide during the second stage of synthesis. Formation of sulfanilamide (eluting at ~4 min) occurred with degradation of *p*-acetamidobenzene sulfonamide (eluting at ~8.5 min).

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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