

APPLICATIONS

Successful Separation of Six Alkyl Sulfonate Esters on Luna[®] Omega Polar C18 Without the Use of Ion-pairing Additives

Laura Snow, Ryan Splitstone, and Genevieve Hodson
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA



Laura Snow
Senior Application Scientist
Outside of the lab, Laura enjoys spoiling her dog Maggie and subjecting her husband to novel methods of torture, such as end-less playlists of sad songs and long walks on the beach to catch Pokémon.

Introduction

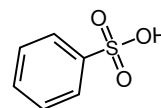
Sulfonic acids are often used as the final salt form of a drug substance to improve chemical properties or bioavailability. However, the presence of any residual alcohol and sulfonic acids, within the associated drug manufacturing steps can potentially lead to the formation of the genotoxic impurities known as alkyl esters. Therefore, a drug substance in the form of a sulfonate, as well as the resulting drug products must be monitored for the potential presence of alkyl sulfonic acids. The US Food and Drug Administration (FDA) and European Medicines Authority (EMA) have established guidelines requiring that any possible genotoxic impurities in a drug substance or drug product be monitored to ensure that the levels are below the established Threshold for Toxicological Concern (TTC) of 1.5 µg/day based upon the maximum daily dosage of the pharmaceutical compound. This can result in significant challenges in the development of analytical methods to attain sensitivities as low as 1 ppm.

Therefore, a fast and reliable analytical method is needed for the retention and identification of common alkyl sulfonic acids (benzenesulfonic acid, p-toluenesulfonic acid, methyl benzenesulfonate, ethyl benzenesulfonate, methyl p-toluenesulfonate, and ethyl p-toluenesulfonate). Previously proposed HPLC methods have required the use of either a pre-column derivatization step or the addition of an ion-pair reagent to ensure adequate retention and selectivity of the polar alkyl sulfonate esters. However, the increase in method complexity, associated irreproducibility issues, and the incompatibility with certain detection methods limit the application of both derivatization and ion-pairing procedures.

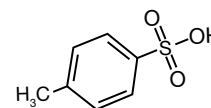
This technical note will explore the use of different mobile phases with the Luna Omega Polar C18 UHPLC column in order to develop a method that provides good analyte retention and peak shape without the use of harsh mobile phase additives for the analysis of six commonly monitored alkyl sulfonate esters.

Analytes

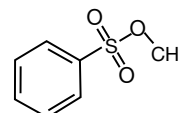
Benzenesulfonic acid



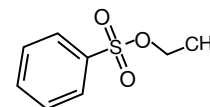
p-toluenesulfonic acid



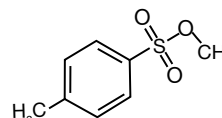
Methyl benzenesulfonate



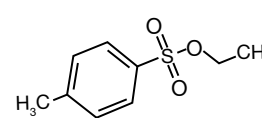
Ethyl benzenesulfonate



Methyl p-toluenesulfonate

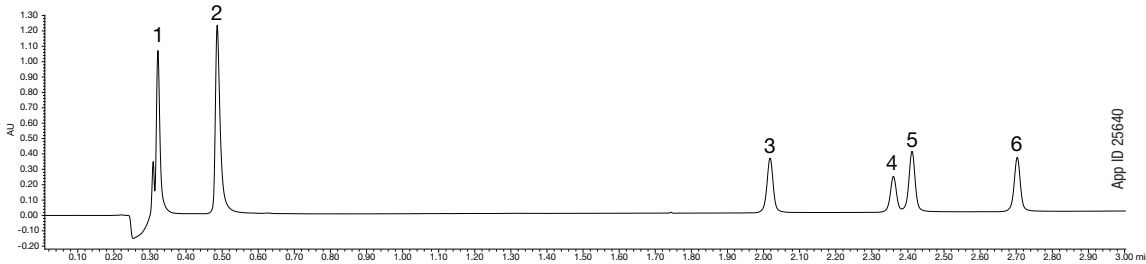


Ethyl p-toluenesulfonate



All analytical standards were purchased from Sigma-Aldrich[®]

Figure 1.
Traditional Ion-Pairing Mobile Phase



Experimental Conditions

LC conditions for Figure 1

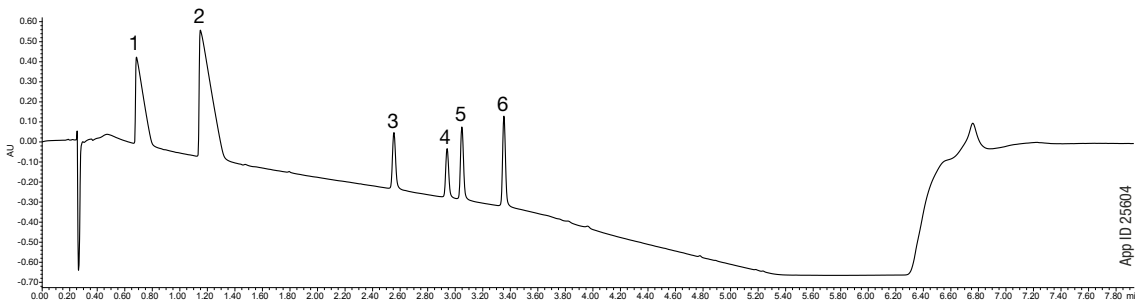
Column: Luna[®] Omega 1.6 µm Polar C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4748-AN
Mobile Phase: A: Water with 0.1 % TFA
 B: Acetonitrile with 0.1 % TFA
Gradient:

Time (min)	% B
0	10
5.0	90
5.1	90
6.0	10
7.0	10

Flow Rate: 0.6 mL/min
Column Temperature: 25 °C
Instrument: Waters[®] ACQUITY[®] I-Class UPLC
Detector: PDA @ 220 nm
Analytes:

1. Benzenesulfonic acid
2. p-Toluenesulfonate acid
3. Methyl benzenesulfonate
4. Ethyl benzenesulfonate
5. Methyl p-toluenesulfonate
6. Ethyl p-toluenesulfonate

Figure 2.
Mobile Phase A: Water with 0.1 % Formic Acid



Experimental Conditions

LC conditions for Figures 2 and 3

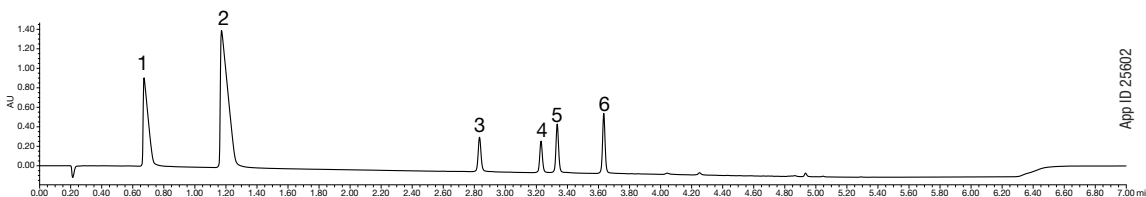
Column: Luna Omega 1.6 µm Polar C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4748-AN
Mobile Phase: A: Water with 0.1 % Formic acid or
 Water with 10 mM Ammonium formate
 (as noted)
 B: Methanol
Gradient:

Time (min)	% B
0	2
5.0	90
5.1	90
6.0	2
7.0	2

Flow Rate: 0.6 mL/min
Temperature: 25 °C
Instrument: Waters ACQUITY I-Class UPLC
Detector: PDA @ 220 nm
Analytes:

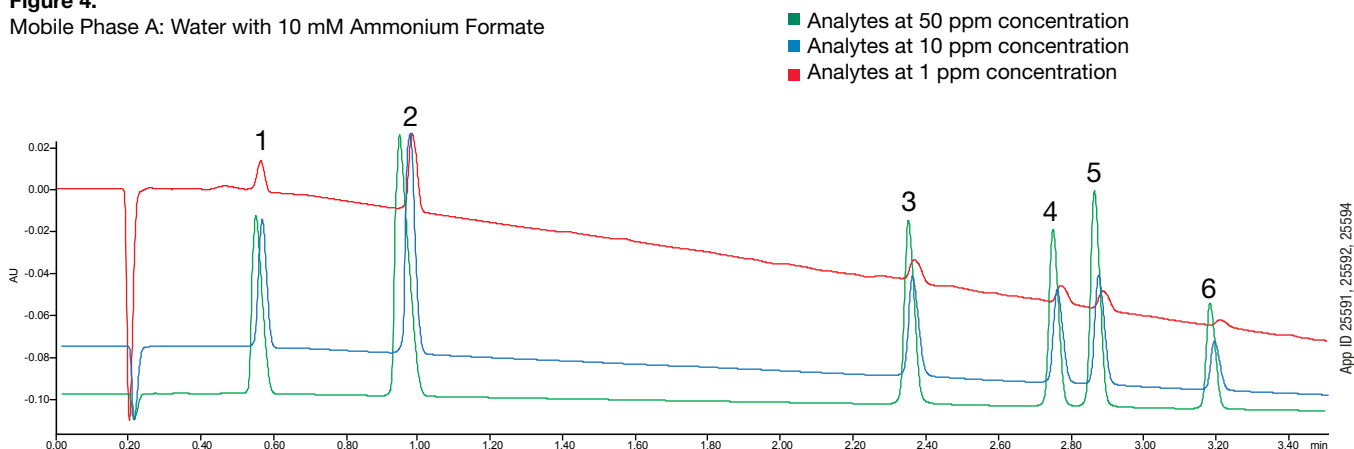
1. Benzenesulfonic acid
2. p-Toluenesulfonic acid
3. Methyl benzenesulfonate
4. Ethyl benzenesulfonate
5. Methyl p-toluenesulfonate
6. Ethyl p-toluenesulfonate

Figure 3.
Mobile Phase A: Water with 10 mM Ammonium Formate



*All neat standards were prepared in water.

Figure 4.
Mobile Phase A: Water with 10 mM Ammonium Formate



Results and Discussion

Alkyl sulfonate esters vary in their degrees of hydrophobicity, with benzenesulfonic acid and p-toluenesulfonic acid being quite hydrophilic and poorly retained under reversed phase conditions in the absence of an ion-pairing reagent in the mobile phase.

Figure 1 represents the typical chromatogram of alkyl sulfonate esters obtained using an ion-pairing reagent trifluoroacetic acid (TFA) in the mobile phase. While good retention and peak shape are obtained, the mobile phase is not mass spectrometry (MS) detection friendly. To address this limitation, formic acid and acetic acid were tried as alternatives in place of TFA.

Several different Phenomenex columns were initially screened for this analysis and the best peak shape and retention for the polar benzenesulfonic acid and p-toluenesulfonic acid with TFA in the mobile phase was achieved using the Luna® Omega PS C18 and the Luna Omega Polar C18. However, Luna Omega Polar C18 provided much better peak shape in the absence of the TFA ion-pairing mobile phase, so the Luna Omega PS C18 was excluded from the remainder of this work. The Luna Omega Polar C18 is stable under 100 % aqueous conditions and provides increased polar retention due to its polar modified surface; this allowed the initial percent organic for the gradient conditions to be lowered from 10 % to 2 % and the organic was changed from acetonitrile to methanol for the remaining analysis.

Figure 2 was obtained with 0.1 % formic acid in water as mobile phase A and resulted in broad peaks compared to the TFA for benzenesulfonic acid and p-toluenesulfonic acid. Although the peak shape worsened for the first two peaks, the peak shape and resolution for methyl benzenesulfonate, ethyl benzenesulfonate, methyl p-toluenesulfonate and, ethyl p-toluenesulfonate

moderately improved from the ion-pair mobile phase conditions. Additionally, 0.1 % acetic acid was tried but formic acid yielded slightly better peak shape. Note that the drop in percent organic allowed additional retention of benzenesulfonic acid and p-toluenesulfonic acid.

Looking to further improve the peak shape for the earlier eluting benzenesulfonic acid and p-toluenesulfonic acid, 10 mM ammonium formate was investigated next as the mobile phase. The ammonium counter ion provided significant improvement in the peak shape of both benzenesulfonic acid and p-toluenesulfonic acid (**Figure 3**) compared to the formic acid while maintaining the sharp peaks and good resolution of the last four alkyl sulfonate esters. Ammonium formate provided the method with a MS-compatible mobile phase with good peak shape and separation for all six alkyl sulfonate esters.

Figure 4 represents a concentration study with the newly developed method. Neat standards of the six alkyl sulfonate esters were diluted with water to 50, 10, and 1 ppm. The combined peak shape with the lower internal diameter of the column allows the 1 ppm concentration to be observed even with 220 nm UV detection.

Conclusion

We were able to develop a method that provided good retention, separation and peak shape for the six alkyl sulfonate esters. The new conditions avoided the use of harsh mobile phase additives, ion-pair reagents, and were able to be performed under UV detection. Improved detection could be attained using MS in place of UV detection if desired.

APPLICATIONS

Luna[®] Omega Ordering Information

1.6 µm Microbore Columns (mm)				1.6 µm Minibore Columns (mm)				SecurityGuard [™] ULTRA Cartridges [‡]	
Phases	50 x 1.0	100 x 1.0	150 x 1.0	Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Polar C18	00B-4748-A0	00D-4748-A0	00F-4748-A0	Polar C18	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJO-9505
									for 2.1 mm ID

3 µm Minibore and MidBore [™] Columns (mm)								SecurityGuard [™] Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*
Polar C18	00A-4760-AN	00B-4760-AN	00D-4760-AN	00F-4760-AN	00B-4760-YO	00D-4760-YO	00F-4760-YO	AJO-7600
								for ID: 2.0-3.0 mm

3 µm Analytical Columns (mm)					SecurityGuard [™] Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJO-7601
					for ID: 3.1-8.0 mm

5 µm Minibore and MidBore Columns (mm)								SecurityGuard [™] Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*
Polar C18	00A-4754-AN	00B-4754-AN	00D-4754-AN	00F-4754-AN	00B-4754-YO	00D-4754-YO	00F-4754-YO	AJO-7600
								for ID: 2.0 - 3.0 mm

5 µm Analytical Columns (mm)					SecurityGuard [™] Cartridges (mm)	
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*	
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJO-7601	
					for ID: 3.1-8.0 mm	

[‡] SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000
^{*} SecurityGuard Analytical Cartridges require holder, Part No.: KJO-4282
^{**} PREP SecurityGuard Cartridges require holder, Part No.: AJO-8223
[♦] PREP SecurityGuard Cartridges require holder, Part No.: AJO-8277

5 µm Axia [™] Packed Preparative Columns (mm)						SecurityGuard [™] Cartridges (mm)	
Phases	150 x 21.2	250 x 21.2	150 x 30	250 x 30	250 x 50	15 x 21.2**	15 x 30.0 [†]
Polar C18	00F-4754-P0-AX	00G-4754-P0-AX	00F-4754-U0-AX	00G-4754-U0-AX	00G-4754-V0-AX	AJO-7603	AJO-7604
						for ID: 21.2 mm	for ID: 30 mm

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: 0-0-800-4911952
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

☎ **All other countries/regions**
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com



Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks
Luna is a registered trademark and BE-HAPPY, MidBore, SecurityGuard, and Axia are trademarks of Phenomenex. Waters and ACQUITY are registered trademarks of Waters Technologies Corporation. Sigma-Aldrich is a registered trademark of Sigma-Aldrich, Inc.

Disclaimer
Comparative separations may not be representative of all applications. Phenomenex is not affiliated with Sigma-Aldrich, Inc. or Waters Corporation. Axia column and packing technology is patented by Phenomenex. U.S. Patent No. 7,674,383
SecurityGuard is patented by Phenomenex. U.S. Patent No. 6,162,362
CAUTION: this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP, or ULTRA holders, or to any cartridges.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.
© 2019 Phenomenex, Inc. All rights reserved.