APPLICATIONS

Sample Preparation Method to Reduce Surfactant Interference for the Quantitative Estimation of Analytes from a Pharmaceutical Drug Formulation

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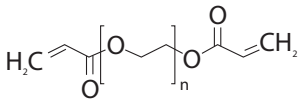
Introduction

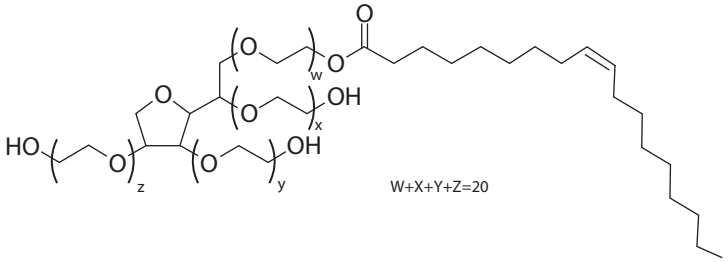
Surfactants are the key ingredients of a formulation buffer that are non-toxic, non-antigenic, and highly soluble in water. The surfactant-drug conjugates have several advantages: a prolonged residence in the body, a decreased degradation of metabolic enzymes, and a reduction or elimination of protein immunogenicity. Their biocompatibility, functional entities help maintain the solubility, stability and bioavailability feature of a drug molecule. All these attributes playing a critical role as formulation excipient and as drug conjugate, playing a critical role in the improvement of therapeutic values [1-3]. PEGylation is a process of chemical conjugation between polyethylene glycol and a drug molecule. However, analysis of samples comprising these surfactants could be complex mixtures due to the presence of the different variety of polymeric species and their homologs. They may result in ion suppression and analytical instrument downtime due to a possible buildup of the excipients. They pose challenges and may become an issue for mass spectrometric analysis if not removed from the sample before injection. Therefore, there is a need for sample preparation methods that can effectively eliminate these excipients and enabling a quantitative estimation of drugs present in a pharmaceutical formulation. We investigated a few popular surfactants such as Polyethylene glycol (PEG 600, 1000 and 1500), Triton™ X-100 and Polysorbate 80 (TWEEN®) to target a comprehensive sample cleanup procedure. Two separate solid phase extraction (SPE) sorbents, the cationic Strata®-X-C and anionic Strata-X-A (for basic and acidic analytes respectively) were chosen to take advantage of the aggressive organic wash that ion-exchange sorbents can withstand. The optimized extraction method was extended to a proprietary formulation buffer, that results in the significant reduction of the background noise while maintaining a reliable and consistent recovery of the analytes across the board.

Surfactants structures

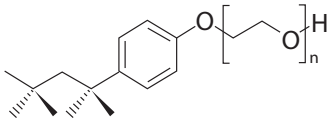
Polyethylene glycol (PEG)



Polysorbate (TWEEN 80)



Triton X-100



Materials and Methods

Reagents and Chemicals

Analytical reference standards, internal standards were purchased from Cerilliant® Corporation (Round Rock, TX,). Proprietary formulation buffer courtesy of Novartis® Pharmaceuticals, (Basel, Switzerland). All other chemicals, were obtained from the Sigma-Aldrich® (St. Louis, MO). Ultrapure D.I. water was obtained from Sartorius arium® comfort II, courtesy of Sartorius Corporation (Bohemia, NY).

For this investigation the LC system utilized was an Agilent® 1260 (Palo Alto, CA) and a SCIEX® 4000 QTRAP® (Framingham, MA) for detection. A Kinetex® 5 µm Phenyl-Hexyl column was used for basic and acidic analytes, and a Kinetex 5 µm C18 column was used for Q1 qualitative conformation.

Sample Pre-treatment: Basic Analytes

A surfactant working solution consisting of 0.4 mL of PEG (50 ppm of PEG 600, 1000, and 1500), Triton™ X-100 (100 ppm of Triton X-100) and TWEEN® (100 ppm of TWEEN 80) and 0.8 mL of 1 % Formic acid was combined for a final volume of 1.2 mL. The solution was then mixed/vortexed for 5 – 10 seconds.

SPE Protocol

Cartridge:	Strata®-X-C 30 mg/3 mL
Part No.:	8B-S029-TBJ
Condition:	1 mL Methanol
Equilibration:	1 mL Water
Load:	1.2 mL Pre-treated sample
Wash 1 (weak):	1 mL Water
Wash 2 (strong):	1 mL 50 % Acetonitrile followed by 50% Methanol. * (see below for complete list of tested wash solvents)
Dry:	3-4 minutes at maximum vacuum
Elute:	2 x 500 µL of 5 % Ammonium hydroxide in Methanol/Acetonitrile (1:1)
Dry Down:	Evaporate to dryness under gentle stream of N_2 at 45-50 °C
Reconstitute:	400 µL initial mobile phase

Sample Pre-treatment: Acidic Analytes

A surfactant working solution consisting of 0.4 mL of PEG (50 ppm of PEG 600, 1000, and 1500), Triton X-100 (100 ppm of Triton X-100) and TWEEN (100 ppm of TWEEN 80) and 0.8 mL of 1 % Ammonium hydroxide was combined for a final volume of 1.2 mL. The solution was then mixed/vortexed for 5 – 10 seconds.

SPE Protocol

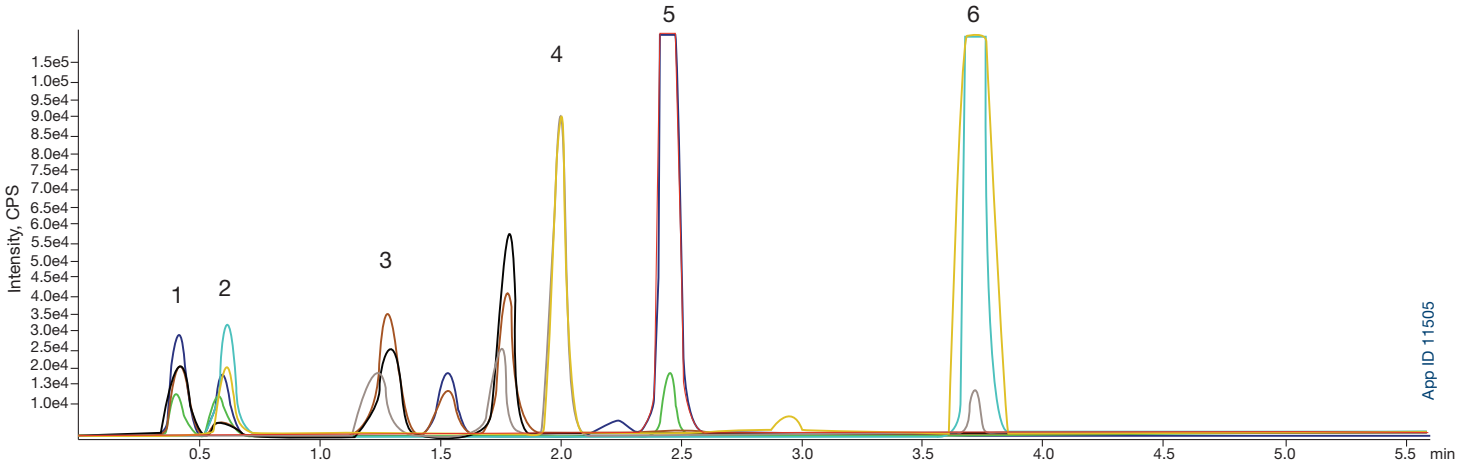
Cartridge:	Strata-X-A (30 mg/3 mL)
Part No.:	8B-S123-TBJ
Condition:	1 mL Methanol
Equilibration:	1 mL Water
Load:	1.2 mL Pre-treated sample
Wash 1 (weak):	1 mL Water
Wash 2 (strong):	1 mL 50 % Acetonitrile followed by 50 % Methanol * (see below for complete list of tested wash solvents)
Dry:	3-4 Minutes at maximum vacuum
Elute:	2 x 500 µL of 5 % Formic acid in Methanol/Acetonitrile (1:1)
Dry Down:	Evaporate to dryness under a gentle stream of N_2 at 45-50 °C
Reconstitute:	400 µL initial Mobile Phase

*Wash 2 solvents tested

1. 50 % Acetone
2. 50 % Acetone followed by 50 % Methanol
3. 50 % Acetone followed by Methanol/Dichloromethane (1:1)
4. 50 % Acetonitrile
5. Methanol/Dichloromethane (1:1)
6. 50 % Acetonitrile followed by 50 % Methanol

Results

Figure 1.
Representative LC/MS-MS chromatogram (+ve ionization) for the basic and zwitterionic analytes on a Kinetex® 5 µm Phenyl-Hexyl column.



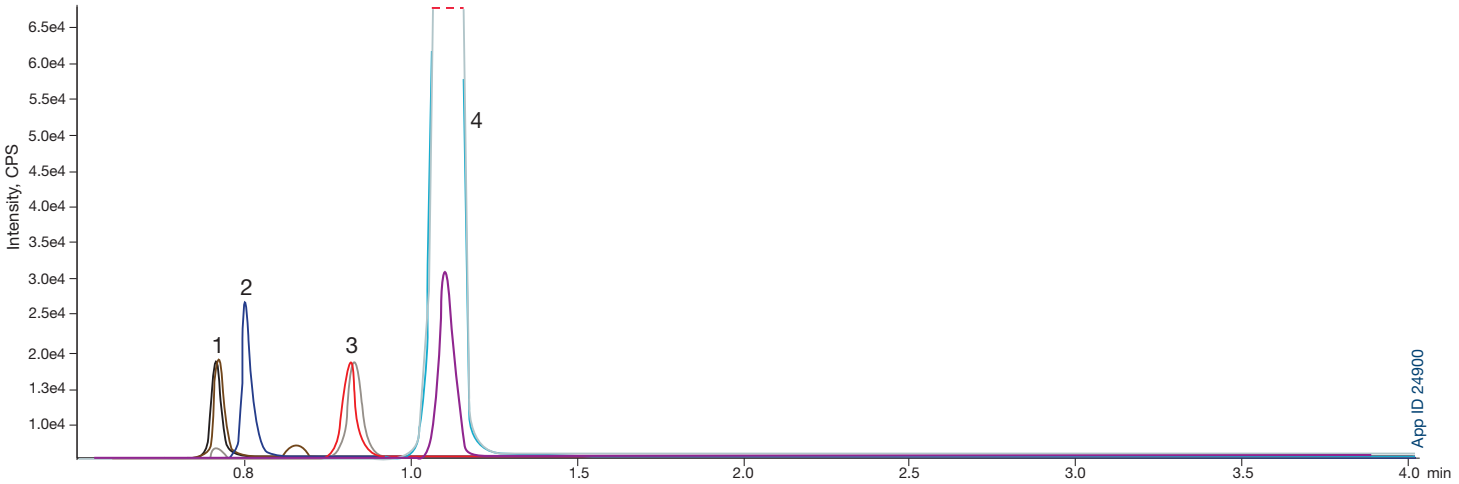
Column: Kinetex 5 µm Phenyl-Hexyl
Dimensions: 50 x 2.1 mm
Part No.: 00B-4603-AN
Mobile Phase: A: Water with 0.1 % Formic Acid
B: Methanol with 0.1 % Formic Acid

Gradient:

Time (min)	% B
0	10
4	95
5.5	95
5.51	10
7	10

Flow Rate: 0.5 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detector: MS/MS (SCIEX® 4000 QTRAP®), ESI+
Sample: 1. Morphine
2. Gabapentin
3. Codeine
4. Ritanilic acid
5. Benzoylecgonine
6. Methadone

Figure 2.
Representative LC-MS/MS chromatogram (-ve ionization) for the acidic analytes on a Kinetex 5 µm Phenyl-Hexyl column.



Column: Kinetex 5 µm Phenyl-Hexyl
Dimensions: 50 x 2.1 mm
Part No.: 00B-4603-AN
Mobile Phase: A: Water with 10 mM Ammonium formate
B: Methanol

Gradient:

Time (min)	% B
0	50
0.2	50
2.5	95
4	95
4.1	50
6	50

Flow Rate: 0.5 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detector: MS/MS (SCIEX 4000 QTRAP), ESI
Sample: 1. Phenobarbital
2. Butalbital
3. Secobarbital
4. Warfarin

Figure 3.
Representative overlaid chromatograms (A) comparing neat PEG buffer against Strata®-X-C extracted samples under various wash conditions (enlarged view from 0 to 7.5 minutes in chromatogram B).

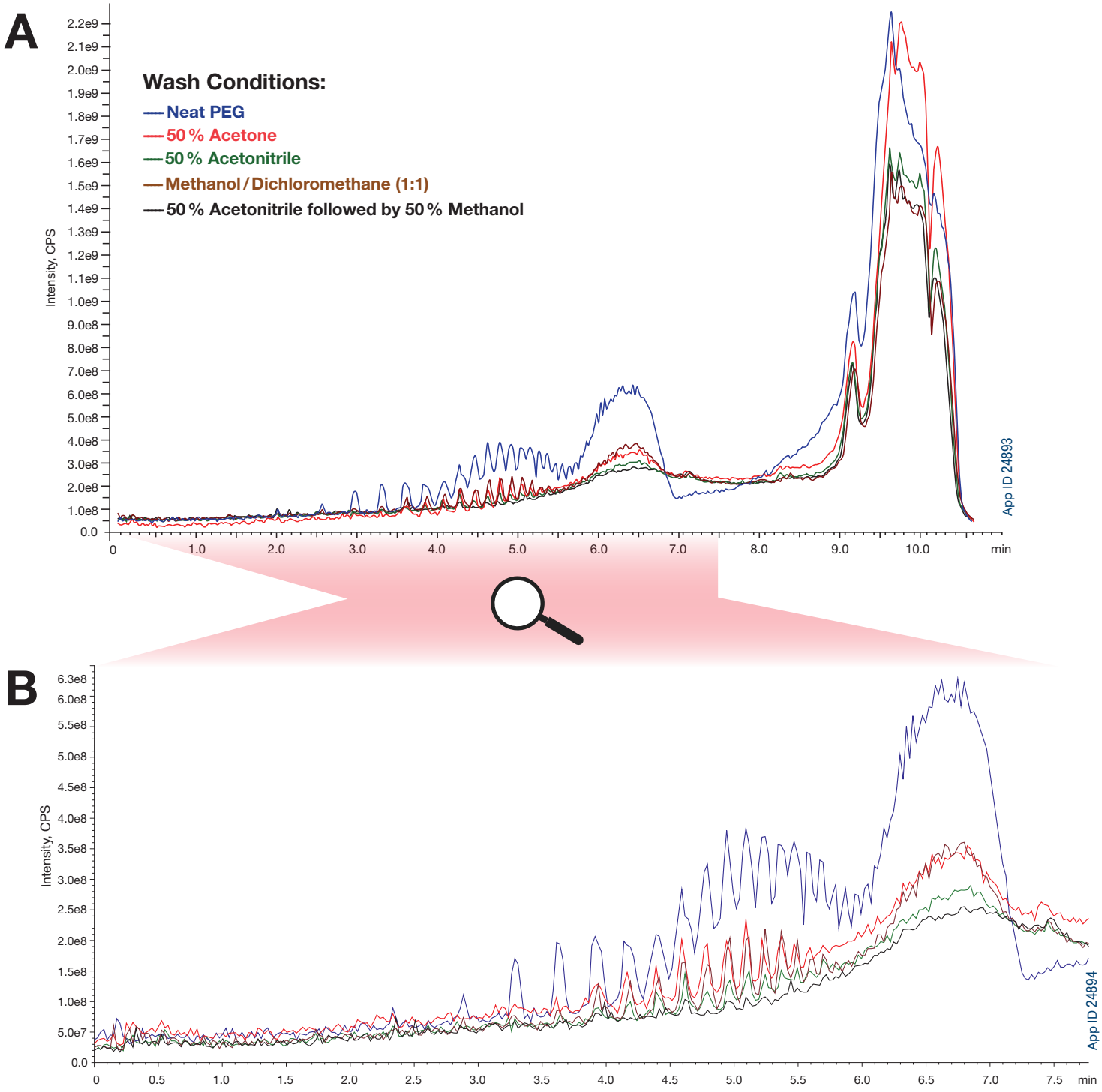


Figure 4.
Representative overlaid chromatograms comparing neat TWEEN 80 buffer against Strata®-X-C extracted samples under various wash conditions.

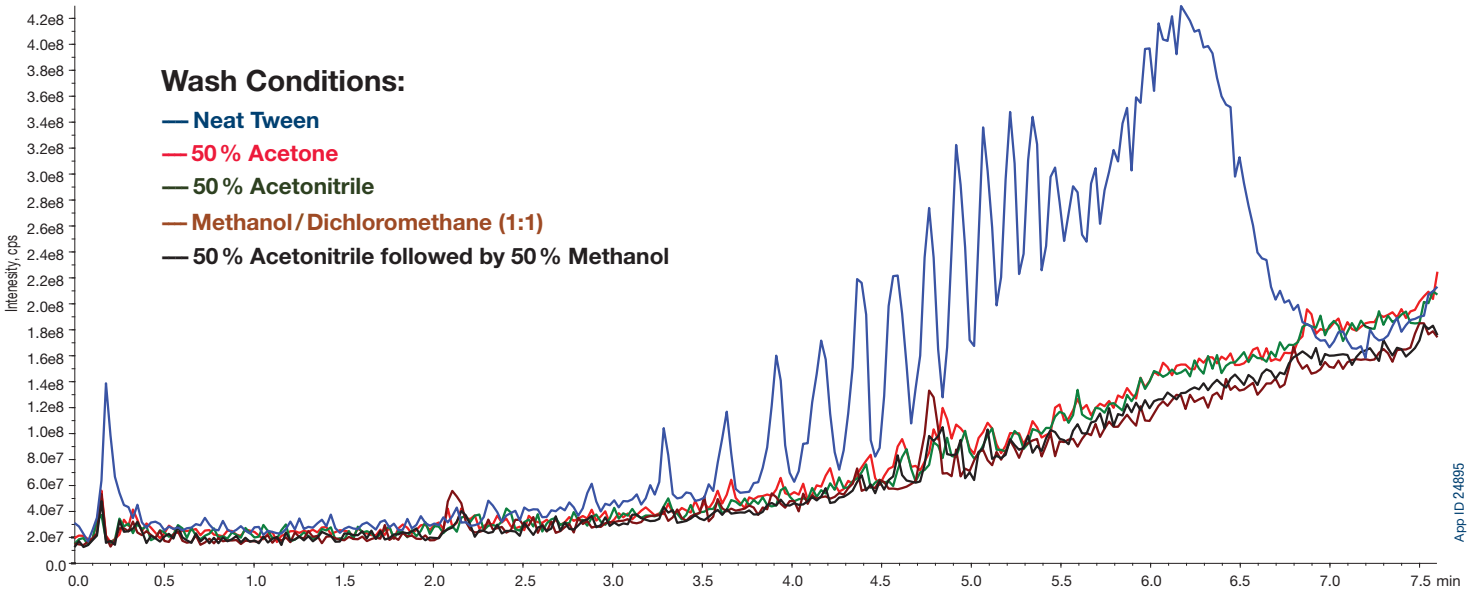


Figure 5.
Representative overlaid chromatograms comparing neat Triton™ X-100 buffer against Strata-X-C extracted samples under various wash conditions.

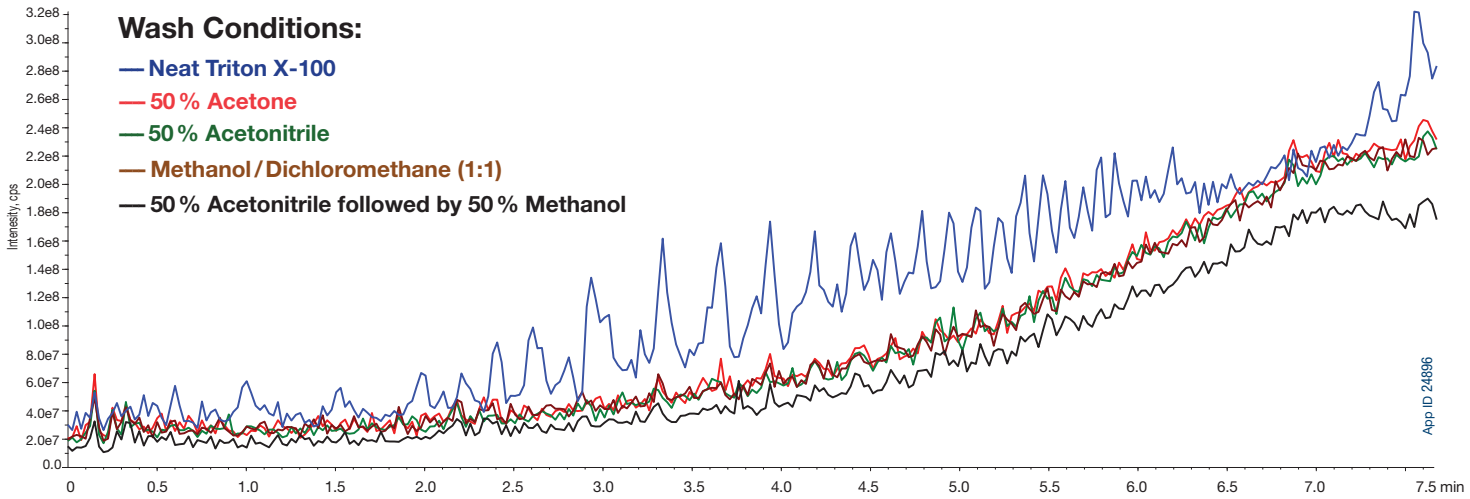


Figure 6.

Representative overlaid chromatograms of the extracted formulation buffer implementing the optimized Strata®-X-C SPE method.

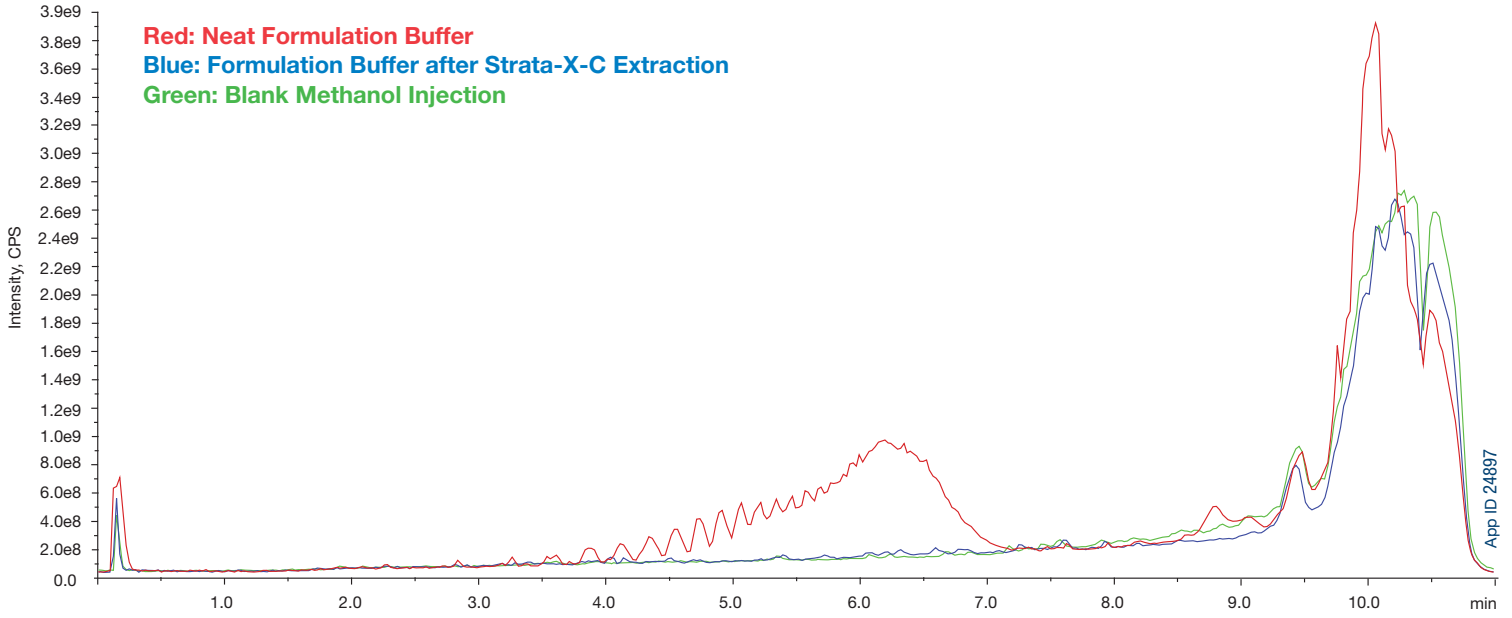
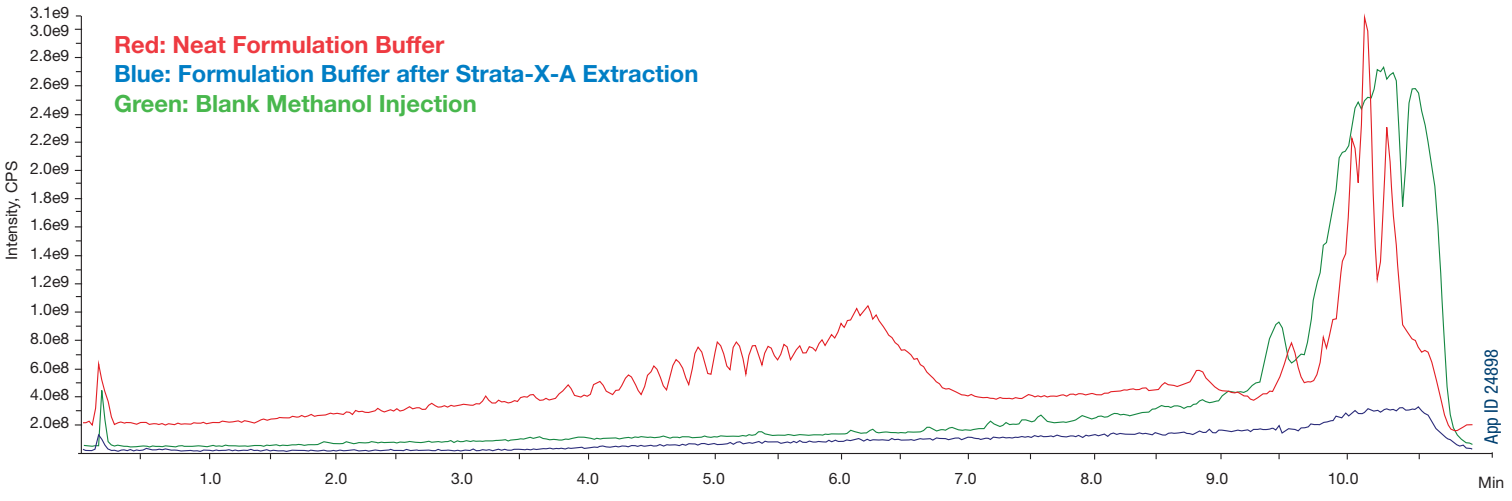


Figure 7.

Representative overlaid chromatograms of the extracted formulation buffer implementing the optimized Strata-X-A SPE method.



Conditions for Figure 3-7 below:

Column: Kinetex® 5 µm, C18
Dimensions: 50 x 2.1 mm
Part No. 00B-4601-AN
Mobile Phase: A: Water with 0.1 % Formic Acid
B: Methanol with 0.1 % Formic Acid

Gradient:

Time (min)	% B
0	5
0.3	80
8.3	80
9	95
10	5
11.5	5

Flow Rate: 0.5 mL/min
Injection Volume: 10 µL
Temperature: Ambient
Detector: MS (SCIEX® 4000 QTRAP®), ESI+ Q1 scan from 100-2000 m/z (TIC Mode)
Sample: Formulation Buffer

Table 1.
Absolute recovery (%) of analyte extracted from the formulation buffer matrix.

Analyte	% Recovery	% CV (N=4)	MS Ionization	SPE sorbent
Gabapentin	108	8.5	Positive	Strata®-X-C
Morphine	90	6.5	Positive	Strata-X-C
Codeine	84	7.8	Positive	Strata-X-C
Ritanilic acid	93	10.1	Positive	Strata-X-C
Benzoylecgonine	101	5.0	Positive	Strata-X-C
Methadone	84	8.7	Positive	Strata-X-C
Secobarbital	96	1.4	Negative	Strata-X-A
Warfarin	114	0.7	Negative	Strata-X-A
Butalbital	93	6.9	Negative	Strata-X-A
Phenobarbital	96	7.1	Negative	Strata-X-A

Discussion

In an effort to seek the most effective SPE method, several wash options were tried. For better visual perception of the above, a Q1 scan (from 100 to 2000 Da, under positive and negative polarity mode) was implemented by comparing neat solution of surfactants against the extracted samples from SPE. The qualitative part of this analysis was performed on a Kinetex® 5 µm C18 50 x 2.1 mm LC column. A total of 6 different washes, 2 conditions, and a combination of different organic solvents were investigated. For visual clarity purposes, four chromatograms (out of six different wash 2 conditions) that revealed notable impact in terms of sample cleanliness, are displayed. Samples undergoing 50 % acetonitrile followed by a 50 % methanol wash (option 6 in SPE sample prep) resulted in the clean background in each case (**Figures 3**, **4** and **5** representing PEG, TWEEN and Triton X-100, respectively). The chromatographic trace and pattern generated from the Q1 scan of the surfactant solution for PEG and its homolog counterpart (PEG 600, PEG 1000, PEG 1500) appear more complex compared to other varieties (**Figures 3**-**5**) tested. The opti-mized wash 2 conditions were incorporated into a proprietary drug formulation buffer that was pre-spiked (100 ppb) with a wide range analyte. The background obtained from the Q1 scan of the extracted samples showed maximum elimination of the excipients present in the formulation while compared against blank methanol injected on the column (**Figures 6** and **7**). No chromatogram is shown for the Q1 scan under negative mode as nothing significant was captured under negative ionization. For the quantitative part, a Kinetex 5 µm Phenyl-Hexyl 50 x 2.1 mm LC column was utilized (**Figures 1** and **2**) that depicts more than 80 % recovery (% CV < 10) for all acids, bases and zwitterionic (gabapentin and ritanilic acid) compounds from the drug panel (**Table 1**).

Conclusion

In this technical note, we utilized a dual extraction method that successfully eliminates the detrimental presence of surfactants in pharmaceutical analysis and results in a high and consistent recovery for a comprehensive drug panel. The dual extraction method utilizing the cationic (Strata-X-C) and anionic (Strata-X-A) SPE provided maximum cleanliness, which can increase MS sensitivity and reduce downtime due to column and system maintenance.

References

1. F. Veronese, G. Pasut. PEGylation, successful approach to drug delivery. *Drug Discovery Today*. **2005**, 10(21), 1451-1458.
2. H. Joshi, R. Tejwani, M. Davidovich, V. Sahasrabudhe, A. Serajuddin. Bioavailability enhancement of a poorly water-soluble drug by solid dispersion in polyethylene glycol–polysorbate 80 mixture. *International Journal of Pharmaceutics*. **2004**, 269, 1(9), 251-258.
3. L. Snow, S. Huq, S. Orlowicz and S. Sadjadi. More than Recovery-Cleanliness: A Through Approach to Oral Fluid LC/MS Analysis with OFC Devices; www.phenomenex.com/MSACLOralFluidPoster.

APPLICATIONS

Ordering Information

Kinetex® Core-Shell HPLC/UHPLC Columns

5 µm Minibore Columns (mm)			SecurityGuard™ ULTRA Cartridges‡
Phases	50 x 2.1	100 x 2.1	3/pk
C18	00B-4601-AN	00D-4601-AN	AJ0-8782
Phenyl-Hexyl	00B-4603-AN	00D-4603-AN	AJ0-8788
			for 2.1 mm ID

5 µm MidBore™ Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	3/pk
C18	00B-4601-Y0	00D-4601-Y0	AJ0-8775
Phenyl-Hexyl	00B-4603-Y0	00D-4603-Y0	AJ0-8781
			for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
Phenyl-Hexyl	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774
					for 4.6 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

Strata®-X-C Solid Phase Extraction (SPE)

Format	Sorbent Mass	Part Number	Unit
Microelution 96-Well			
	2 mg	8M-S029-4GA	1 Plate/Box
96-Well Plate			
	10 mg	8E-S029-AGB	2 Plates/Box
	30 mg	8E-S029-TGB	2 Plates/Box
	60 mg	8E-S029-UGB	2 Plates/Box
Tube			
	30 mg	8B-S029-TAK*	1 mL(100/box)
	30 mg	8B-S029-TBJ	3 mL (50/box)
	60 mg	8B-S029-UBJ*	3 mL (50/box)
	100 mg	8B-S029-EBJ	3 mL (50/box)
	100 mg	8B-S029-ECH	6 mL (30/box)
	200 mg	8B-S029-FBJ	3 mL (50/box)
	200 mg	8B-S029-FCH	6 mL (30/box)
	500 mg	8B-S029-HBJ	3 mL (50/box)
	500 mg	8B-S029-HCH	6 mL (30/box)

*tab-less tubes available

Strata-X-A

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S123-TAK**	1 mL (100/box)
	30 mg	8B-S123-TBJ	3 mL (50/box)
	60 mg	8B-S123-UBJ	3 mL (50/box)
	100 mg	8B-S123-EBJ	3 mL (50/box)
	100 mg	8B-S123-ECH	6 mL (30/box)
	200 mg	8B-S123-FBJ	3 mL (50/box)
	200 mg	8B-S123-FCH	6 mL (30/box)
	500 mg	8B-S123-HBJ	3 mL (50/box)
	500 mg	8B-S123-HCH	6 mL (30/box)
96-Well Plate			
	10 mg	8E-S123-AGB	2 Plates/Box
	30 mg	8E-S123-TGB	2 Plates/Box
	60 mg	8E-S123-UGB	2 Plates/Box
96-Well Microelution Plate			
	2 mg	8M-S123-4GA	ea

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