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Instant Sensitivity Gains Without the Need to Dry Down and Reconstitute Using Microelution Solid Phase Extraction (SPE)

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Introduction

Solid Phase Extraction (SPE) is an excellent cleanup solution for bioanalytical samples because it is selective, reproducible, and results in ultra-clean and concentrated samples. While the technique is effective, traditional formats such as 10 mg 96-well plates or tubes pose challenges for small or limited sample volumes and can result in dilute eluents if the sample is not dried down and reconstituted after the extraction procedure. This dry down step can require 30 or more minutes, adding a significant amount of time to the procedure. To overcome these challenges, the microelution 96-well SPE plate format was developed. The microelution SPE plates contain significantly less sorbent as compared to a traditional 10 mg 96-well SPE plate, allowing analysts to elute in volumes as low as 25 µL. These low elution volumes result in ultra-concentrated samples that do not need to be dried down. In this study, we compared the microelution 96-well SPE plate to a traditional 10 mg 96-well SPE plate to determine the amount of sensitivity gain that can be achieved by moving a 10 mg SPE method to the microelution format.

Experimental Conditions

Extraction Procedures

Timolol and procaine were extracted from serum using a traditional 10 mg 96-well SPE plate and a 2 mg microelution 96-well SPE plate, each of which was packed with the same polymeric strong cation-exchange chemistry, Strata®-X-C. The same volume of sample was loaded onto each SPE plate and the condition, equilibration, wash, and elution solvents were the same across both extractions to standardize the extraction protocols.

Table 1 .
SPE Extraction of Timolol and Procaine from Serum

	Strata-X-C 96-Well SPE Plate, 10 mg/well	Strata-X-C Microelution 96-Well SPE Plate, 2 mg/well
Condition	500 µL Methanol	200 µL Methanol
Equilibrate	500 µL Water	200 µL Water
Load	750 µL diluted serum (375 µL serum diuted 1:1 with 4 % Phosphoric acid in water)	750 µL diluted serum (375 µL serum diuted 1:1 with 4 % Phosphoric acid in water)
Wash 1	500 µL 2 % Formic acid in water	200 µL 2 % Formic acid in water
Wash 2	500 µL Methanol	200 µL Methanol
Elute	375 µL (3x 125 µL) 5 % Ammonium hydroxide in acetonitrile/methanol (60:40)	25 µL 5 % Ammonium hydroxide in acetonitrile/methanol (60:40)
Inject	1 µL	1 µL

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HPLC Conditions

After cleanup by SPE, 1 µL of each extraction was injected onto a Kinetex® 2.6 µm Biphenyl core-shell HPLC column and the resulting concentrations were determined by LC/MS/MS.

Column:	Kinetex 2.6 µm Biphenyl
Dimensions:	50 x 2.1 mm
Part No.:	00B-4462-AN
Mobile Phase:	A: 0.1 % Formic acid in Water
	B: 0.1 % Formic acid in Acetonitrile

Gradient:	**Time (min)**	**B (%)**
	0.00	5
	0.50	5
	3.00	95
	3.50	95
	3.51	5
	5.50	5

Flow Rate:	500 µL/min
Temperature:	Ambient
Detection:	MS/MS, API 5000™ (AB SCIEX)

Results and Discussion

Both timolol and procaine were extracted from serum using two different SPE formats; a traditional 10 mg 96-well SPE plate and a 2 mg microelution 96-well SPE plate. The microelution format is designed to allow analysts to elute in small sample volumes which results in ultra-concentrated samples without the need to perform a dry down step. To standardize our comparison, the same polymeric strong cation-exchange sorbent, Strata®-X-C, was packed in each 96-well SPE plate and the same method was performed however the solvent volumes were reduced for the microelution method (**Table 1**). While both extraction methods were extremely effective and resulted in clean samples, the microelution format resulted in 15x more concentrated samples (**Figures 1** and **2**) when 1 µL of the eluent was injected onto the LC/MS/MS.

Figure 1. Timolol (1ng/mL) Extracted from Serum Resulted in 15x More Concentrated Samples using the microelution Format

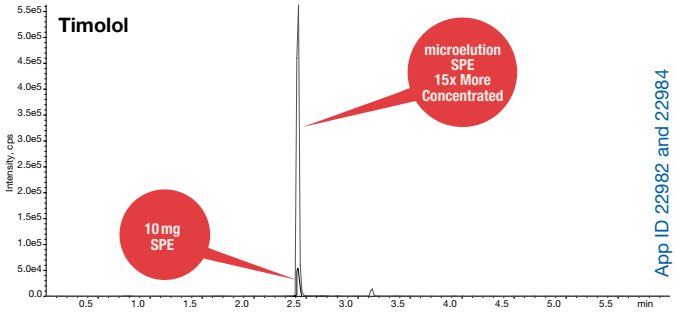
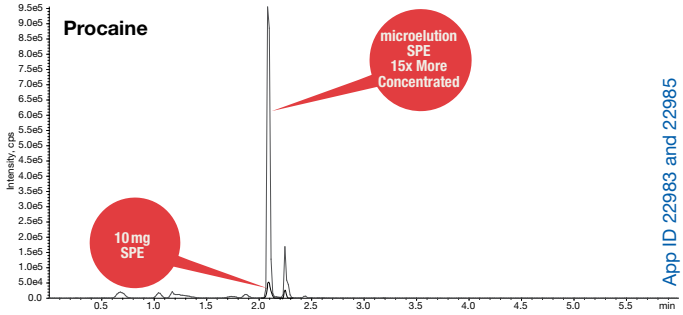


Figure 2. Procaine (1ng/mL) Extracted from Serum Resulted in 15x More Concentrated Samples using the microelution Format



Conclusion

While both the 10 mg 96-well SPE plate and the 2 mg microelution 96-well SPE plate were both extremely effective at cleaning up our serum samples, the microelution format allowed us to produce ultra-concentrated samples without the need to dry down and reconstitute. By skipping this step we were able to save 30-45 minutes without losing any sensitivity. These time savings essentially doubled our productivity as the total cleanup time using the microelution format took approximately 30 minutes. In addition to the time savings, the microelution format is also amenable to small or limited sample volumes (as low as 10 µL) which provides an excellent solution in the event that our sample size decreases.

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Ordering Information

Strata®-X Microelution 96-Well SPE Plates

Part No.	Description	Unit
8M-S035-4GA	Strata-X-CW 33 µm Polymeric Weak Cation-Exchange Microelution 96-Well Plate, 2 mg/well	1/pk
8M-S029-4GA	Strata-X-C 33 µm Polymeric Strong Cation-Exchange Microelution 96-Well Plate, 2 mg/well	1/pk
8M-S100-4GA	Strata-X 33 µm Polymeric Reversed Phase Microelution 96-Well Plate, 2 mg/well	1/pk
8M-S123-4GA	Strata-X-A 33 µm Polymeric Strong Anion-Exchange Microelution 96-Well Plate, 2 mg/well	1/pk
8M-S038-4GA	Strata-X-AW 33 µm Polymeric Weak Anion-Exchange Microelution 96-Well Plate, 2 mg/well	1/pk

Kinetex® Core-Shell HPLC/UHPLC Columns

5 µm Minibore Columns (mm)			SecurityGuard™ ULTRA Cartridges‡
Phase	50 x 2.1	100 x 2.1	3/pk
Biphenyl	00B-4627-AN	00D-4627-AN	AJ0-9209 for 2.1 mm ID

5 µm MidBore™ Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phase	50 x 3.0	100 x 3.0	3/pk
Biphenyl	00B-4627-Y0	00D-4627-Y0	AJ0-9208 for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phase	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207 for 4.6 mm ID

2.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phase	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00A-4622-AN	00B-4622-AN	00D-4622-AN	00F-4622-AN	AJ0-9209 for 2.1 mm ID

2.6 µm MidBore Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phase	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Biphenyl	00B-4622-Y0	00D-4622-Y0	00F-4622-Y0	AJ0-9208 for 3.0 mm ID

2.6 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phase	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Biphenyl	00B-4622-E0	00D-4622-E0	00F-4622-E0	AJ0-9207 for 4.6 mm ID

1.7 µm Minibore Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phase	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJ0-9209 for 2.1 mm ID

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

guarantee

If Phenomenex products in this technical note do not provide at least equivalent separations as compared to other products of the same phase, size and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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