APPLICATIONS

Preventing Analyte Loss by Skipping the Dry Down Step using Microelution Solid Phase Extraction (SPE)

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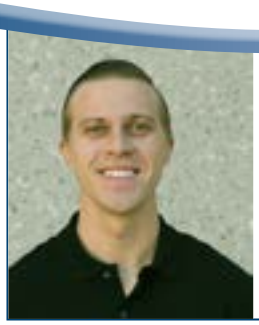

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Matt Brusius is an avid ice hockey player. He likes skating backwards and taking slapshots from the point.

Introduction

Peptide therapeutics have recently gained popularity as more and more peptide based therapeutics receive approval to enter the pharmaceutical market. As the research and use of these therapeutics increases, laboratories must adapt their traditional small molecule analyses to accommodate the unique challenges that are inherent with peptide work. Perhaps one of the most frustrating challenges encountered when working with peptides, is their extreme "stickiness" or affinity for a variety of plastics and glassware, which makes it challenging to extract and analyze peptides from biological matrices such as serum[i]. The extraction step is crucial to HPLC analysis because interferences must be removed from the sample and the peptide must be concentrated to ensure accurate quantitation, however, many extraction procedures include a dry down step which risks peptide loss because the peptide's contact with plastics and glassware is extended during this step. This work will focus on two extraction methods using solid phase extraction (SPE), comparing the recovery of DALDA C8 (a central nervous system peptide therapeutic[ii, iii]) from serum using a traditional 10 mg 96-well SPE plate versus a Strata®-X microelution 96-well SPE plate. The Strata-X microelution 96-well plate allowed us to elute our peptide in only 50 µL which eliminated the need to further concentrate using a dry down step. By skipping this dry down step, the Strata-X microelution 96-well SPE plate resulted in peptide recoveries of 95% as compared to the traditional 10 mg 96-well SPE plate which resulted in recoveries of ~50%.

Experimental Conditions

Extraction Procedure

To demonstrate the improved recovery that can be achieved, we extracted DALDA C8 from serum using two different polymeric reversed phase SPE formats: a 10 mg 96-well plate and a 2 mg microelution 96-well plate. The same amount of serum was loaded onto the sorbents and the same solvents were used to perform the condition, equilibration, wash, and elution steps however the solvent volumes differed to accommodate the recommendations for each product. After the extraction, the eluent from the 10 mg 96-well SPE plate was dried down and reconstituted in elution solvent to bring the sample to the same volume (50 µL) as the eluent from the microelution 96-well plate. The eluent from the microelution 96-well plate was not dried down and was direct injected onto the LC/MS/MS. **Table 1** outlines the procedures performed for each SPE format.

HPLC Conditions

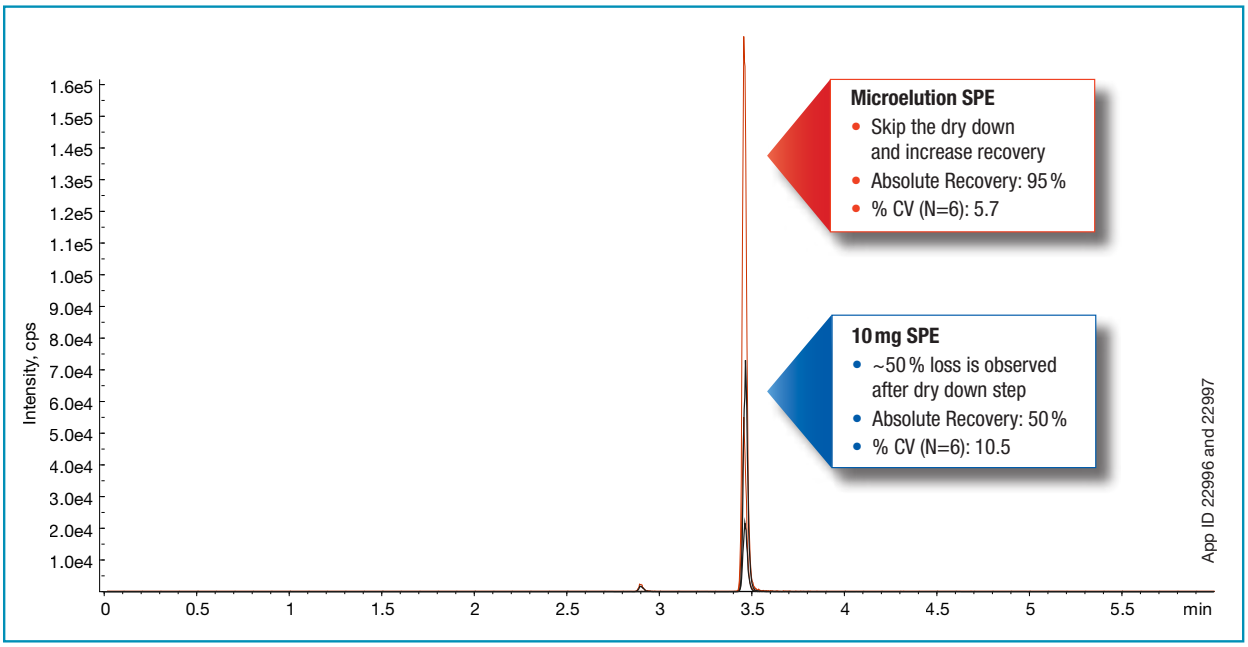
After cleanup by SPE, 10 µL of each extraction was injected onto a Kinetex® 5 µm C8 HPLC column and the resulting concentrations were determined by LC/MS/MS.

Column:	Kinetex 5 µm C8	
Dimensions:	50 x 4.6 mm	
Part No.:	00B-4608-E0	
Mobile Phase:	A: 0.1 % Formic acid in Water	
	B: 0.1 % Formic acid in Methanol	
Gradient:	**Time (min)**	**% B**
	0.00	10
	3.00	90
	4.00	90
	4.01	10
	6.00	10
Flow Rate:	600 µL/min	
Temperature:	Ambient	
Detection:	MS/MS, Triple Quad™ 4500 (AB SCIEX), ESI+	
Sample:	DALDA C8 (peptide), spiked at 10 ng/mL	

Table 1. SPE Extraction of DALDA C8 (peptide) from Serum

	Strata-X 96-Well SPE Plate, 10 mg/well	Strata-X Microelution 96-Well SPE Plate, 2 mg/well
Condition	400 µL Methanol	200 µL Methanol
Equilibrate	400 µL Water	200 µL Water
Load	400 µL diluted serum (200 µL serum diluted 1:1 with 4 % Phosphoric acid in water)	400 µL diluted serum (200 µL serum diluted 1:1 with 4 % Phosphoric acid in water)
Wash 1	400 µL 2 % Formic acid in water	200 µL 2 % Formic acid in water
Wash 2	400 µL 20 % Acetonitrile in water	200 µL 20 % Acetonitrile in water
Elute	2x 175 µL Trifluoroacetic acid/Acetonitrile/Water (1:74:25)	2x 25 µL Trifluoroacetic acid/Acetonitrile/Water (1:74:25)
Dry Down	Dry down under a gentle stream of Nitrogen and reconstitute in 50 µL Trifluoroacetic acid/ Acetonitrile/Water (1:74:25)	**NOT REQUIRED**
Inject	10 µL	10 µL

Figure 1. DALDA C8 (peptide) Extracted from Serum using 10 mg SPE and Microelution SPE



Results and Discussion

To study the effect that a dry down step played on analyte recovery of a peptide therapeutic, two different SPE formats were used to extract DALDA C8 from serum, each of which was packed with the same polymeric reversed phase sorbent, Strata®-X, to ensure consistency. The first format was a traditional 10 mg 96-well SPE plate. This format required an elution volume of 350 µL to completely elute the peptide from the sorbent. While effective, this elution volume resulted in a dilute sample which required a dry down step in order to produce a concentrated sample. The sample was dried down under a stream of nitrogen and was reconstituted in 50 µL of elution solvent **(Table 1)**.

A second SPE format, the Strata-X microelution 96-well plate, was used to extract DALDA C8 from the same sample volume as the 10 mg 96-well plate. While this format was able to process the same volume of serum as the 10 mg 96-well plate, it required half the volume of condition, equilibration, and wash solvent and also allowed us to elute in only 50 µL of elution solvent **(Table 1)**. By reducing the elution solvent, we were able to produce a concentrated sample without the need to dry down and reconstitute.

After extraction, 10 µL of each of the two eluents was analyzed by LC/MS/MS to quantify the amount of peptide present in each eluent. The eluent from the 10 mg 96-well SPE plate which required a dry down step resulted in a recovery of 50 % with a % CV of 10.5 while the eluent from the microelution 96-well plate (which was not dried down) resulted in a recovery of 95 % with a % CV of 5.7 **(Figure 1)**. These results indicate that peptide was lost in the collection plate during the dry down and reconstitution steps which is most likely due to the fact that peptides can have a strong affinity for plastic and glassware. The microelution 96-well plate, however, did not require a dry down and reconstitution step and resulted in excellent recoveries that were also reproducible, making the microelution 96-well plate format an ideal solution for the extraction and concentration of therapeutic peptides. In addition to preventing analyte loss, eliminating the dry down step by using the microelution 96-well plate also saved approximately 30-60 minutes per 96-well plate, instantly increasing throughput.

Conclusions

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. Skipping this step prevented analyte loss, almost doubling our recoveries, and also saved approximately 30-60 minutes. The microelution format not only promotes high recoveries of compounds that have an affinity for plastic and glassware, it is also an excellent solution for compounds that are thermolabile and are prone to loss when subjected to dry down steps.

References

i Tips and Tricks of the Trade Hidden Peptide Losses. Buckingham S.. Lab Times. Issue 7, 2011.

ii CNS Delivery and Pharmacokinetic Evaluations of DALDA Analgesics Peptide Analog Administered in Nano-Sized Oil-in-Water Emulsion Formation. Shah L., Gattacceca F. and Amiji MM. Pharm Res (2014) 31:1315-1324.

iii Analgesic Efficacy and Safety of DALDA Peptide Analog Delivery to the Brain using Oil-in-Water Nanoemulsion Formation. Shah L., Kulkarni P., Ferris C., and Amiji MM. Pharm Res. (2014) 31:2724-2734.

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‡
Phases	50 x 2.1	100 x 2.1	3/pk
C8	00B-4608-AN	00D-4608-AN	AJ0-8784
			for 2.1 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
C8	00B-4608-E0	00D-4608-E0	00F-4608-E0	00G-4608-E0	AJ0-8770
					for 4.6 mm ID

2.6 µm Minibore Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
C8	00A-4497-AN	00B-4497-AN	00C-4497-AN	00D-4497-AN	00F-4497-AN	AJ0-8784
						for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	00F-4497-Y0	AJ0-8777
						for 3.0 mm ID

2.6 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
C8	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AJ0-8770
					for 4.6 mm ID

1.7 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C8	00A-4499-AN	00B-4499-AN	00D-4499-AN	00F-4499-AN	AJ0-8784
					for 2.1 mm ID

1.7 µm MidBore Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	30 x 3.0	50 x 3.0	100 x 3.0	3/pk
C8	00A-4499-Y0	00B-4499-Y0	00D-4499-Y0	AJ0-8777
				for 3.0 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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