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APPLICATIONS

Solid Phase Extraction Method Development for Bioanalytical Therapeutic Peptides Using Strata®-X Peptide Screening Microelution SPE 96-Well Plates

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Introduction

While there are many ways to extract peptides from biological matrices, solid phase extraction (SPE) is the only one that can specifically target peptides of interest amongst other endogenous components, while providing a platform that is well-suited for high-throughput DMPK analysis. In particular, Ion-Exchange (IEX) SPE is the ideal chemical selection for peptide extraction and its strong interaction offers high recovery along with a significant reduction in matrix effects relative to other techniques such as protein precipitation or even reversed phase SPE.

However, being able to accurately predict how a peptide will interact with a solid phase extraction chemistry is difficult to determine largely due to the number of interactions taking place between the peptide of interest and stationary phase chemistry.

In this technical note, we will explore how a peptide screening method can be used with a specialized Method Development plate (Strata-X Peptide Screening Microelution Plate) containing a polymeric strong anion-exchange (Strata-X-A) and a polymeric weak cation-exchange phase (Strata-X-CW) to determine which one is the most appropriate choice for a wide range of chemically diverse peptides.

In addition, peptides are notoriously difficult to re-solubilize after a dry down step that is performed with traditional SPE. Using the microelution format allows the drying down to be eliminated while providing maximum recovery while maintaining appropriate sensitivity, which makes it the ideal format for the preparation of peptide samples.

Materials and Methods

SPE Protocol

96-Well Plate: Strata-X Peptide Screening Microelution (Strata-X-CW, Strata-X-A)
Part No.: KS0-9528
Condition: 200 µL Methanol
Equilibrate: 200 µL Water
Load: 400 µL Pre-treated sample (1:1 Plasma/4% Phosphoric acid)
Wash 1: 200 µL 5 % Ammonium hydroxide in Water
Wash 2: 200 µL 20 % Acetonitrile in Water
Elution: 2x 25 µL TFA/Acetonitrile/Water(1:74:25) directly transferred to vial and injected (no dilution)

LC Conditions

Column: Luna® Omega 3 µm PS C18 100 Å
Dimension: 50 x 2.1 mm
Part No.: 00B-4758-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Acetonitrile
Gradient:

Time (min)	% B
0	15
2.5	80
2.51	95
3	95
3.01	95
6	15

Flow Rate: 0.4 mL/min
Injection Volume: 10 µL
Instrument: MS/MS (SCIEX API 4000™)

Table 1.
API 4000 Parameters for Analysis

MS/MS Parameters	
Collision Gas (CAD):	6
Curtain Gas (CUR):	12
Ion Spray Voltage (IS)	4000
Temperature (TEM):	600

Table 2.
Q1 and Q3 Mass Fragments, Declustering Potential, Collision Energy, Collision Cell Exit Potential, and Retention Time Values for peptides

Peptide	Q1 Mass (Da)	Q3 Mass	DP	CE	CXP	RT
BNP	578.3	699.4	67	28	19	0.41
Vasopressin (1)	542.9	120.4	70	54	10	0.44
Vasopressin (2)	542.9	328.5	68	28	8	0.44
Angiotensin II D5	526.6	784.5	66	29	22	0.53
Angiotensin II (1)	523.9	784.3	66	29	10	0.56
Angiotensin II (2)	523.9	262.7	66	31	10	0.56
Neurotensin (1)	558.6	643.8	82	32	17	0.65
Neurotensin (2)	558.6	579	78	31	14	0.65
Angiotensin I (1)	433	109.8	46	32	10	0.70
Angiotensin I (2)	433	269	46	17	10	0.70
Octreotide-d5	513.15	504	69	22	14	1.35
Octreotide(1)	510.5	120.1	52	38	10	1.40
Octreotide (2)	510.5	158.8	32	48	12	1.40
Desmopressin (1)	535.1	328.3	72	22	8	1.44
Desmopressin (2)	535.1	521.5	74	23	13	1.44
Goserelin (1)	635.7	607.4	40	26	15	1.58
Goserelin (2)	635.7	607.4	40	26	15	1.58
Teriparatide (1)	687.5	787.4	90	29	11	1.59
Teriparatide (2)	824.7	984.3	125	38	15	1.59
Teriparatide-13C	688.5	788.2	68	28	22	1.59
Somatostatin (1)	546.9	538	53	22	13	1.61
Somatostatin (2)	546.9	538	53	22	13	1.61
Somatostatin-d5	548.6	539.2	55	23	13	1.62
Bivalirudin	1090.8	650.4	114	60	10	1.70
Enfuvirtide (1)	1124	1343.1	72	27	21	2.26
Enfuvirtide (2)	1124	1381.8	65	42	10	2.26

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Results and Discussion

This panel of peptides was specifically chosen to demonstrate the wide applicability of the proposed Strata®-X peptide screening method and 96-well SPE plate. The peptides in this suite range from just over 1000 Da up to 4400 Da and span a wide range of isoelectric points (pI) from about 4 (Bivalirudin) up to 12 with BNP. The Mass Spectrometry conditions can be found in **Table 1** and **Table 2**. The recovery values and relative standard deviations provided in **Table 3** show greater than 70 % recovery for nine out of the twelve peptides and a % RSD of less than 12 for all cases, with no method modification. This data provides vital information as to which sorbent is the correct phase for each peptide (Strata-X-CW vs. Strata-X-A) and illustrates how the proposed methodology can be implemented to a wide range of novel peptides not specifically mentioned in this work.

Figure 1.
Representative Extracted Chromatogram on Strata-X-CW

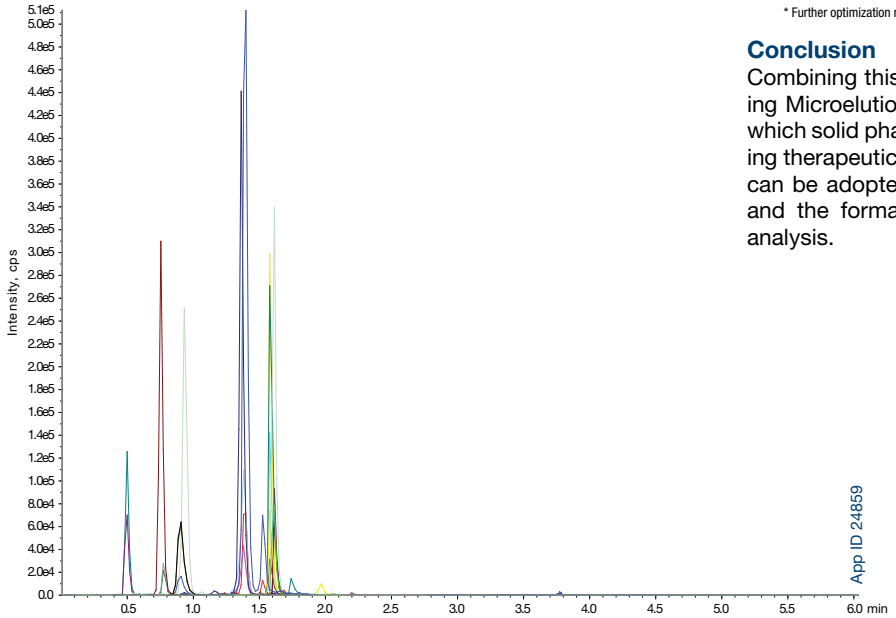

Figure 2.
Representative Extracted Chromatogram on Strata-X-A

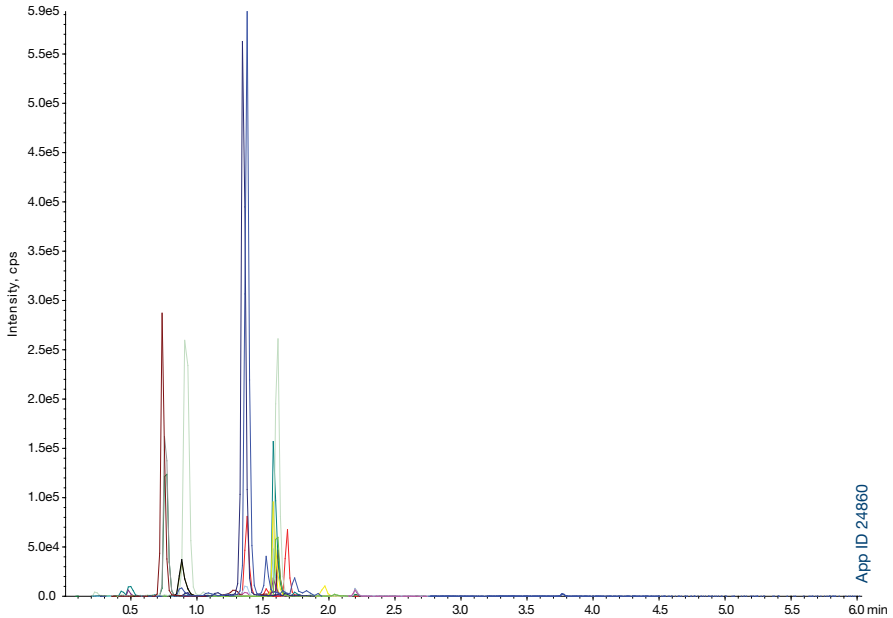

Table 3.
Absolute Recovery Values and Relative Standard Deviations

	Strata-X Sorbent	Average % Recovery	Average % RSD (n=4)
Desmopressin	X-CW	92	4
Vasopressin	X-CW	92	11
Octreotide	X-CW	88	4
Bivalirudin	X-A	70	10
BNP*	X-CW	49	12
Goserelin	X-CW	91	5
Enfuviritde*	X-A	32	10
Angiotensin I	X-A	92	7
Angiotensin II	X-A	75	4
Neurotensin	X-CW	71	8
Somatostatin	X-CW	81	6
Teriparatide*	X-A	46	7

* Further optimization needs to be performed to increase recovery

Conclusion

Combining this SPE procedure with the Strata-X Peptide Screening Microelution Plate provides an effective means for identifying which solid phase extraction chemistry is most suitable for extracting therapeutic peptides from plasma. It is shown that this method can be adopted for a wide range of chemically diverse peptides and the format provides an ideal platform for high-throughput analysis.

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Ordering Information

Strata®-X Microelution 96-Well Plates

96-Well Plates (ea)	
Phase	**2 mg**
Strata X-AW	8M-S038-4GA
Strata X-A	8M-S123-4GA
Strata-X	8M-S100-4GA
Strata-X-C	8M-S029-4GA
Strata-X-CW	8M-S035-4GA

Strata-X Microelution Method Development 96-Well Plates

Part No.	Description	Unit
KS0-9528	**Strata-X Peptide Screening** Strata-X-CW 2 mg/well (6 rows) Strata-X-A 2 mg/well (6 rows)	ea
KS0-9529	**Strata-X Method Development** Strata-X-C 2 mg/well (3 rows) Strata-X-AW 2 mg/well (3 rows) Strata-X-CW 2 mg/well (3 rows) Strata-X-A 2 mg/well (3 rows)	ea

Collection Plates (deep well, polypropylene)

Part Number	Description	Unit
AH0-7192	350 µL/well, Square	50/pk
AH0-7193	1 mL/well, Square	50/pk
AH0-7279	1 mL/well, Round/Round Bottom, 7 mm	50/pk
AH0-7194	2 mL/well, Square	50/pk
AH0-8635	2 mL/well, Square/Round-Conical Bottom	50/pk
AH0-8636	2 mL/well, Round/Round Bottom, 8 mm	50/pk

Sealing Mats (silicone)

Part Number	Description	Unit
AH0-8633	Pierceable, 96-Round Well 8 mm	50/pk

96-Well Plate Vacuum Manifold

Part No.	Description	Unit
AH0-8950	96-Well Plate Manifold, Universal w/vacuum gauge	ea

Luna® Omega LC Columns

3 µm Minibore Columns (mm)					SecurityGuard™ Cartridges*
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**4 x 2.0 (10/pk)**
PS C18	00A-4758-AN	00B-4758-AN	00D-4758-AN	00F-4758-AN	AJ0-7605
				for ID:	2.0 - 3.0 mm

* SecurityGuard Standard Analytical Cartridges require holder, Part No.: KJ0-4282

guarantee

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