APPLICATIONS

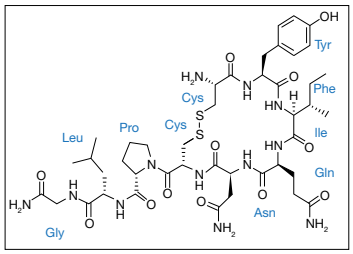
LC-MS/MS Analysis Oxytocin (OT) and ARG-Vasopressin (AVP) in Human Plasma/ Serum using Strata®-X-CW Solid Phase Extraction (SPE) and a Luna® PFP(2) HPLC Column

Xianrong (Jenny) Wei, Erica Pike, Brian Rivera and Sean Orlowicz
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

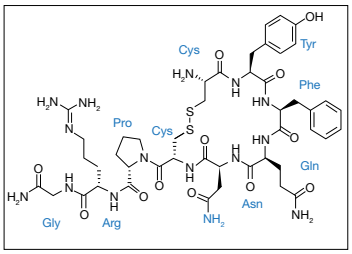
Introduction

Immunoassay, nano-LC systems, and 2D-LC-MS/MS methods are commonly used to reach such low detection levels for Oxytocin (OT) and Arginine-vasopressin (AVP); however, these methodologies also have limitations such as assay reproducibility issues, facility instrument limitations, and high method development and validation costs.

In this study, we provide an accurate and reproducible method to analyze both OT and AVP in one injection.



Oxytocin



ARG-Vasopressin

Materials and Methods

Reagents and Chemicals

Oxytocin, vasopressin, and octreotide standards were purchased from Sigma-Aldrich.

Experimental Conditions

Solid Phase Extraction Method Development

300 µL of plasma/serum was treated with 300 µL 4 % phosphoric acid and 50 µL of internal standard (500 ng/mL of Octreotide) in water and mixed. Strata-X, Strata-X-C, and Strata-X-CW were evaluated to determine the optimal solid phase extraction sorbent (**Figure 1**). For the final selected sorbent, Strata-X-CW, wash and elution solvents were optimized (**Figures 2** and **3**). Although Strata-X performed well for AVP, only Strata-X-CW was able to provide acceptable recoveries for both OT and AVP.

LC-MS/MS Conditions

LC-MS/MS was performed using a Luna 3 µm PFP(2) 50 x 2.0 mm HPLC column (00B-4447-B0) on an Agilent® 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 400 bar, equipped with a binary pump, autosampler and interfaced with an API 5000™ triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA). The ionization source was electrospray ionization (ESI) analyzed in positive ion mode. Ionization source parameters and LC running conditions are indicated.

Figure 1. Response of OT and AVP using Various SPE Sorbents

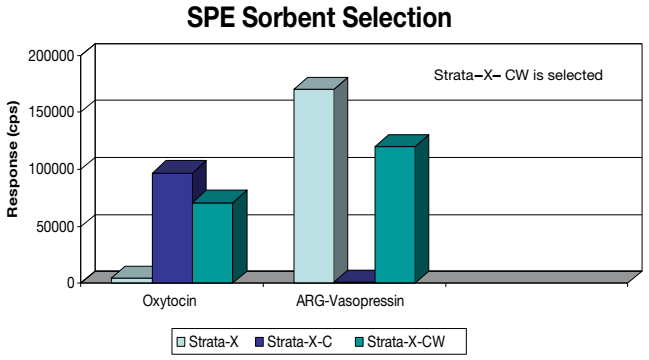


Figure 2. Response of OT and AVP of 2nd Wash Optimization using Strata-X-CW

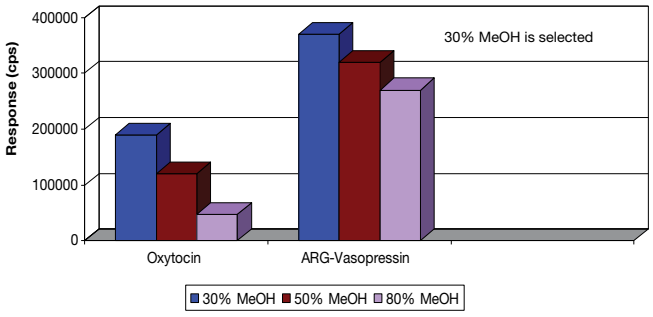
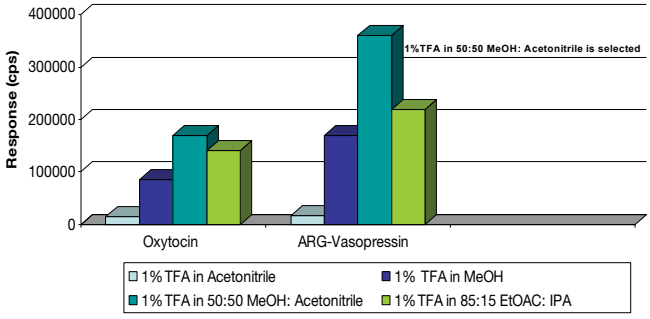


Figure 3. Response of OT and AVP Under Various Elution Strengths using Strata-X-CW



Optimized SPE Method

96-Well Plate:	Strata-X-CW, 30 mg/well
Part No.:	8E-S035-TGB
Condition:	1 mL Methanol
Equilibrate:	1 mL Water
Load:	Pretreated plasma/serum
Wash 1:	1 mL 4 % H_3PO_4
Wash 2:	1 mL Methanol/Water (30:70)
Dry:	3 to 4 minutes under 10" Hg vacuum
Elute:	2x 0.75 mL 1 % TFA in Acetonitrile/Methanol (50:50)
Dry down:	Dry down completely under a stream of nitrogen @ 50 °C
Reconstitute:	100 µL of 1 % TFA in Mobile Phase A/Mobile Phase B (80:20) Mobile Phase A: 0.1 % Formic acid; B: Methanol/Acetonitrile (50:50) with 0.1 % Formic acid

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/LiveChat** to get in touch with one of our Technical Specialists*

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Results and Discussion

Figure 4 and **Figure 5** show extracted ion chromatograms at QCL levels of 0.500 ng/mL. **Figure 6** shows a representative chromatogram at analyte levels of 500 ng/mL, showing that the Luna PFP(2) HPLC column provided good selectivity and sensitivity for both Oxytocin and ARG-Vasopressin in a single injection.

Table 2 shows the accuracy and precision of three QC levels. The results showed that the assay was accurate, precise, and reproducible. Linearity was determined to be acceptable from 0.2 -500 ng/mL (**Figures 7** and **8**).

Ionization Source Parameters:

Gas 1 & Gas 2:	25
CAD:	8
Cur:	25
IS:	5500
Temp:	450
ihe:	ON
DP:	120
EP:	10

Column:	Luna® 3 µm PFP(2)
Dimensions:	50 x 2.0 mm
Part No.:	00B-4447-B0
Mobile Phase:	A: 0.1 % Formic acid
	B: Methanol/Acetonitrile (50:50) with 0.1 % Formic acid

Gradient:

Time (min)	B (%)
0	10
0.2	10
0.5	30
3.5	30
3.51	70
4	70
4.01	10
5	10

Flow Rate:	0.45 mL/min
Injection:	10 µL
Temperature:	40 °C
Detection:	SCIEX® API 5000™

Table 1. MRM Transitions for OT and AVP

Analyte Peak Name	Q1 (Da)	Q3 (Da)	Dwell (msec)	CE	CXP
Oxytocin 1	1007.9	723.4	75	39	15
Oxytocin 2	504.8	86.2	75	35	15
Vasopressin 1	542.5	328.6	75	28	15
Vasopressin 2	542.5	120.1	75	28	15
Octreotide (IS)	510.5	120.1	75	30	13

Q1. Quantitation mass transitions

Q2. Confirmation mass transitions

Conclusion

In this study, we combined OT and AVP and analyzed them within a single method. To maximize assay recoveries, the 2nd strong wash and elution solvents have been optimized. We also provided stable MRM transitions for both OT and AVP, the assay dynamic range is 0.2 - 500 ng/mL for both compounds with precision less than 13.9 % and accuracy less than 7.4 %. This method would provide a good starting point for reference labs as an alternative approach to immunoassay. Although our resulting method was accurate at concentration ranges of 0.2 - 500 ng/mL, the LLOQ level could be decreased significantly if coupled with a nano-LC system or HRMS.

Figure 4. Oxytocin Extracted Ion Chromatograms, QCL (0.500 ng/mL)

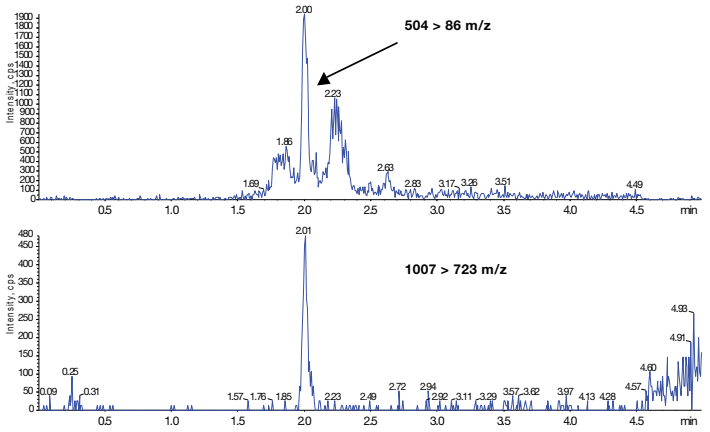

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Figure 5. ARG-Vasopressin Extracted Ion Chromatograms, QCL (0.500 ng/mL)

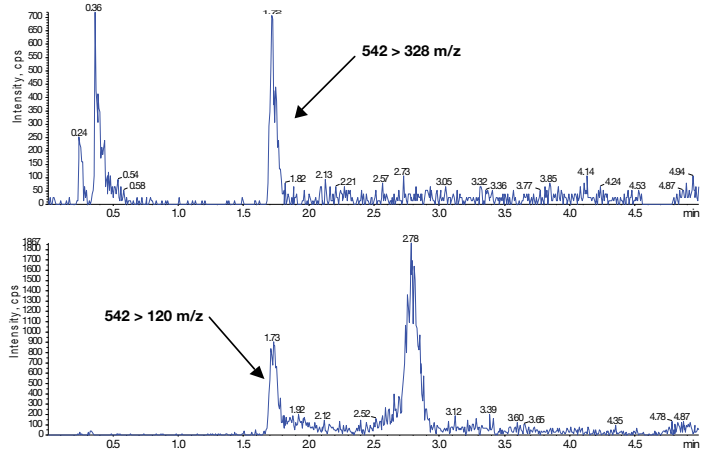

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Figure 6. Representative Chromatogram of ARG-Vasopressin and Oxytocin

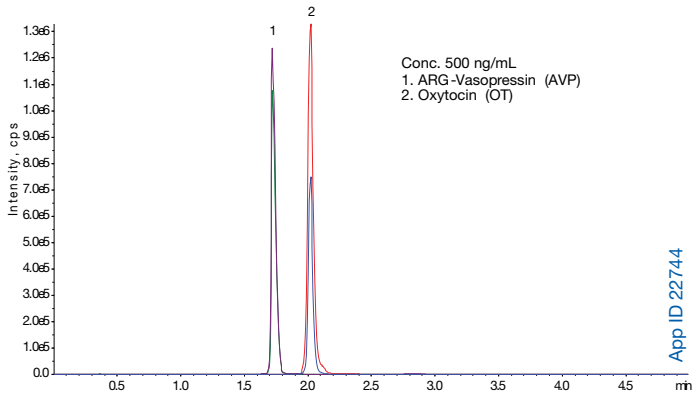

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Figure 7. Representative Curve of Oxytocin

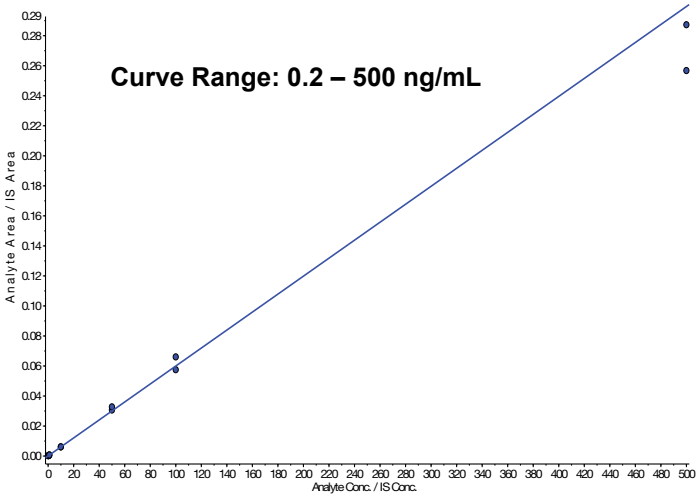

Figure 8. Representative Curve of ARG-Vasopressin

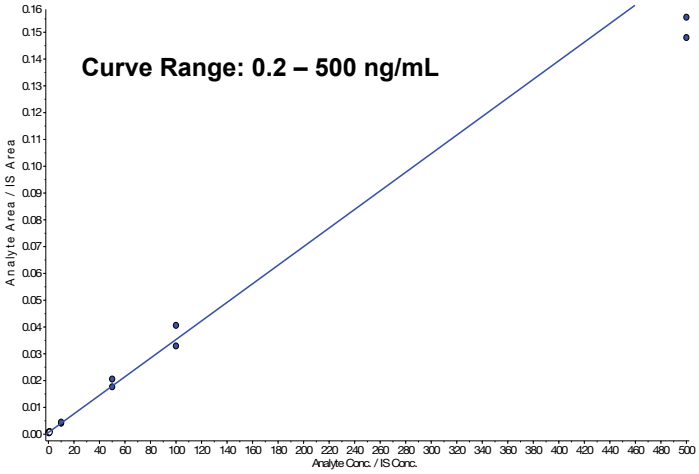

Table 2. Accuracy and Precision

	QCL	QCM	QCH
Nominal Conc. (ng/mL)	**0.5**	**10**	**400**
	Oxytocin (OT)		
Mean Conc. Fund (ng/mL)	0.521	10.1	382
STDV	0.025	0.597	25.8
CV %	4.72	5.94	6.76
Accuracy (%)	104	101	95.5
	ARG-Vasopressin (AVP)		
Mean Conc. Fund (ng/mL)	0.473	10.2	370
STDV	0.666	0.367	23.3
CV %	13.9	3.59	6.29
Accuracy (%)	94.5	102	92.6

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Strata®-X-CW SPE Ordering Information

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S035-TAK**	1 mL (100/box)
	30 mg	8B-S035-TBJ	3 mL (50/box)
	60 mg	8B-S035-UBJ**	3 mL (50/box)
	100 mg	8B-S035-ECH	6 mL (30/box)
	200 mg	8B-S035-FBJ	3 mL (50/box)
	200 mg	8B-S035-FCH	6 mL (30/box)
	500 mg	8B-S035-HBJ	3 mL (50/box)
	500 mg	8B-S035-HCH	6 mL (30/box)
Giga™ Tube			
	1 g	8B-S035-JDG	12 mL (20/box)
	1 g	8B-S035-JEG	20 mL (20/box)
	2 g	8B-S035-KEG	20 mL (20/box)
	5 g	8B-S035-LFF	60 mL (16/box)
96-Well Plate			
	10 mg	8E-S035-AGB	2 Plates/Box
	30 mg	8E-S035-TGB	2 Plates/Box
	60 mg	8E-S035-UGB	2 Plates/Box
96-Well Microelution Plate			
	2 mg	8M-S035-4GA	ea

**Tab-less tubes available. Contact Phenomenex for details.

Luna® PFP(2) HPLC Column Ordering Information

3 µm Minibore Columns (mm)

Phases	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	SecurityGuard™ Cartridges (mm) 4 x 2.0*
PFP(2)	00A-4447-B0	00B-4447-B0	00D-4447-B0	00F-4447-B0	AJ0-8326
					for ID: 2.0-3.0 mm

3 µm MidBore™ Analytical Columns (mm)

Phases	50 x 3.0	150 x 3.0	50 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard Cartridges (mm) 4 x 2.0*	4 x 3.0*
PFP(2)	00B-4447-Y0	00F-4447-Y0	00B-4447-E0	00D-4447-E0	00F-4447-E0	AJ0-8326	AJ0-8327
						for ID: 2.0-3.0 mm	3.2-8.0 mm

5 µm Minibore Columns (mm)

Phases	30 x 2.0	50 x 2.0	150 x 2.0	SecurityGuard Cartridges (mm) 4 x 2.0*
PFP(2)	00A-4448-B0	00B-4448-B0	00F-4448-B0	AJ0-8326
				for ID: 2.0-3.0 mm

5 µm MidBore™ Analytical Columns (mm)

Phases	50 x 3.0	150 x 3.0	50 x 4.6	SecurityGuard Cartridges (mm) 4 x 2.0*	4 x 3.0*
PFP(2)	00B-4448-Y0	00F-4448-Y0	00B-4448-E0	AJ0-8326	AJ0-8327
				for ID: 2.0-3.0 mm	3.2-8.0 mm

5 µm Analytical Columns (mm)

Phases	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard Cartridges (mm) 4 x 3.0*
PFP(2)	00D-4448-E0	00F-4448-E0	00G-4448-E0	AJ0-8327
				for ID: 3.2-8.0 mm

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

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t: +61 (0)2-9428-6444
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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: +48 22 104 21 72
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
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All other countries/regions Corporate Office USA
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