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

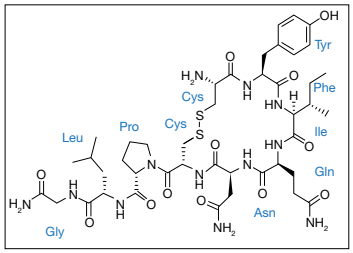
Introduction

Oxytocin [OT] is a nonapeptide typically associated with labor and birth, lactation, and postpartum bonding. Studies also show that OT has anti-stress-like effects such as reduction of blood pressure and cortisol levels[1]. Arginine-vasopressin [AVP] is another peptide that is structurally similar to OT that is associated with vasoconstriction and water retention.

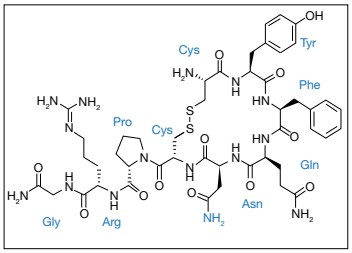
Both OT and AVP are unique in that they are secreted by the posterior pituitary gland and can be released directly into the brain[2]. These so-called neurohypophysial peptide hormones may mediate normal and abnormal behavior. Interest has recently piqued since studies have linked autism to polymorphisms in genes associated with receptors in the vasopressin-oxytocin pathway[3]. It has also been postulated that OT and AVP be used as targets for novel approaches to clinical intervention[4].

Normal plasma levels of AVP are between 0.4-5.2 pg/mL[5] and are 14.4–245.7 pg/mL for OT[6]. Immunoassay, nano-LC systems, and 2D-LC/MS/MS methods are commonly used to reach such low detection levels; 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.

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

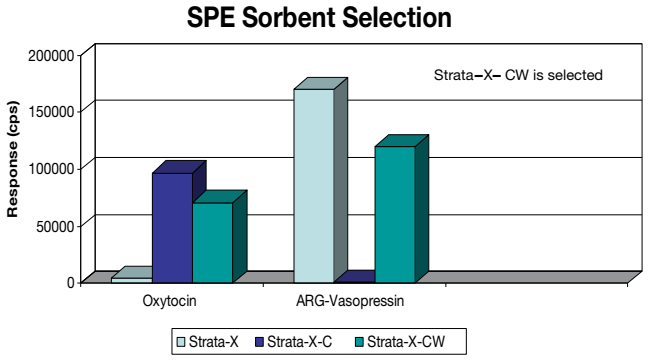


Figure 2. Response of OT and AVP of 2nd Wash Optimization using Strata-X-CW

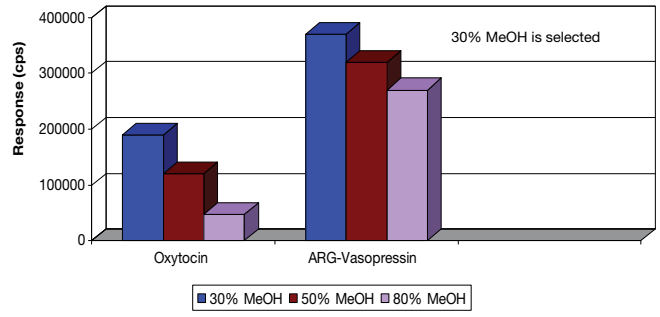
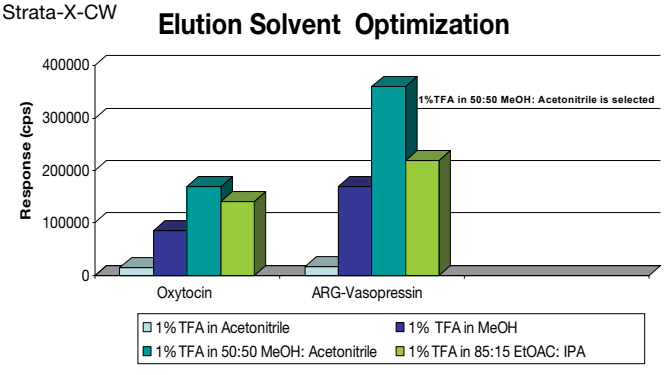


Figure 3. Response of OT and AVP Under Various Elution Strengths using Strata-X-CW



Optimized SPE Method

Cartridge:	Strata-X-CW, 30 mg/well 96-well plate
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: MeOH (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

APPLICATIONS

LC/MS/MS Conditions

LC/MS/MS was performed using a Luna 3 µm PFP(2) 50 x 2.0 mm HPLC column (p/n 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.

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
Flow Rate:	0.45 mL/min

Gradient:

Time (min)	B (%)
0.00	10
0.20	10
0.50	30
3.50	30
3.51	70
4.00	70
4.01	10
5.00	10

Injection:	10 µL
Temperature:	40 °C
Detection:	AB 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. Quatitation mass transitions

Q2. Confirmation mass transitions

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**).

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

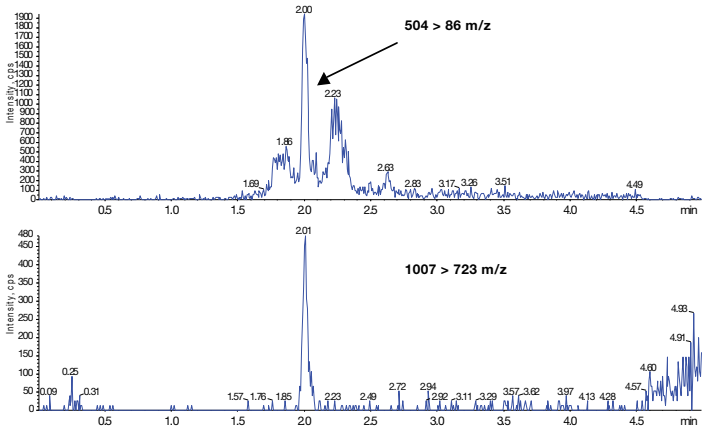


Figure 5. ARG-Vasopressin Extracted Ion Chromatograms, QCL (0.500 ng/mL)

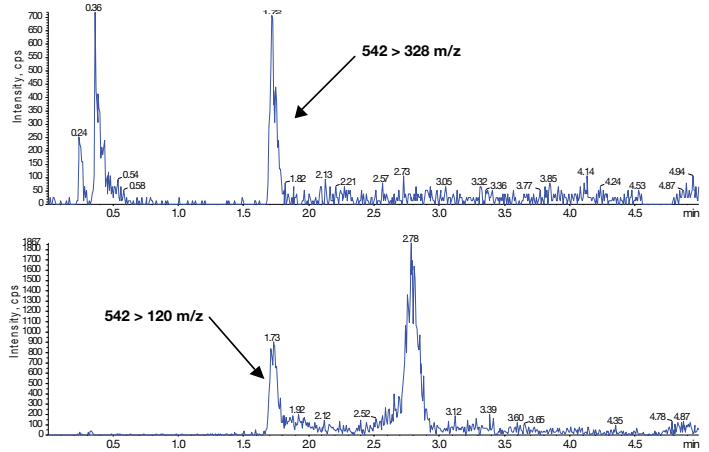


Figure 6. Representative Chromatogram of ARG-Vasopressin and Oxytocin

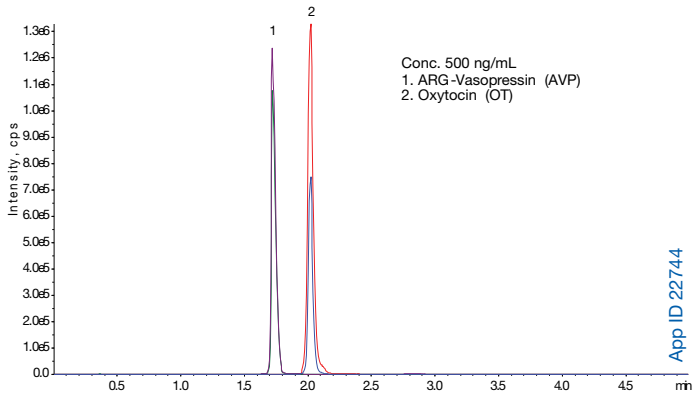


Figure 7. Representative Curve of Oxytocin

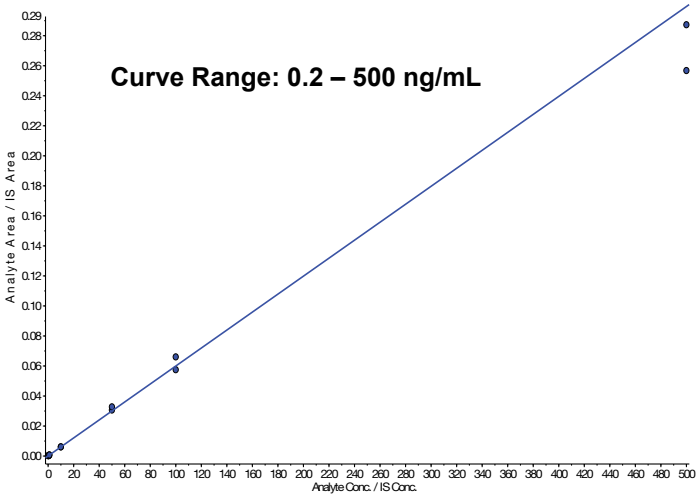


Figure 8. Representative Curve of ARG-Vasopressin

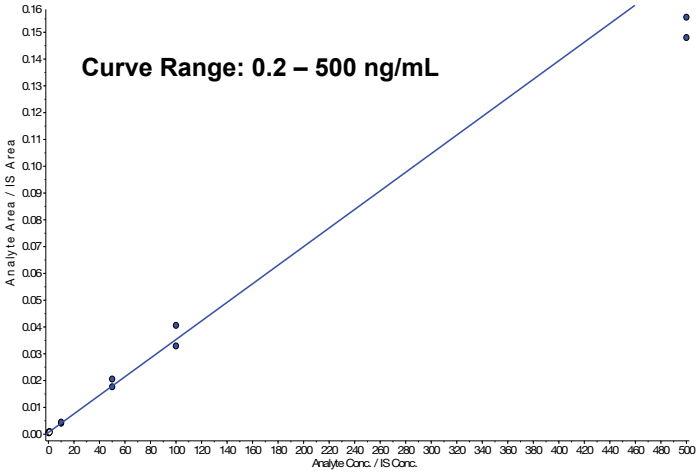


Table 2. Accuracy and Precision

	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

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.

References

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APPLICATIONS

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			
	500 mg	8B-S035-HDG	12 mL (20/box)
	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

Luna® PFP(2) HLC Column Ordering Information

3 µm Microbore and 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 Narrow Bore and 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 for ID: 2.0-3.0 mm	AJ0-8327 3.2-8.0 mm

5 µm Microbore and 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 Narrow Bore and 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 for ID: 2.0-3.0 mm	AJ0-8327 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

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@phenomenex.com

Austria
t: 01-319-1301
f: 01-319-1300
anfrage@phenomenex.com

Belgium
t: 02 503 4015 (French)
t: 02 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: (800) 543-3681
f: (310) 328-7768
info@phenomenex.com

Denmark
t: 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: 09 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: 01 30 09 21 10
f: 01 30 09 21 11
franceinfo@phenomenex.com

Germany
t: 06021-58830-0
f: 06021-58830-11
anfrage@phenomenex.com

India
t: 040-3012 2400
f: 040-3012 2411
indiainfo@phenomenex.com

Ireland
t: 01 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: 051 6327511
f: 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 001-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: 030-2418700
f: 030-2383749
nlinfo@phenomenex.com

New Zealand
t: 09-4780951
f: 09-4780952
nzinfo@phenomenex.com

Norway
t: 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: (800) 541-HPLC
f: (310) 328-7768
info@phenomenex.com

Sweden
t: 08 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: 01625-501367
f: 01625-501796
ukinfo@phenomenex.com

United States
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

All other countries: Corporate Office USA
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

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