Quantitative analysis of oxytocin and vasopressin by LC-MS/MS

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Overview

Detection of oxytocin and vasopressin at endogenous plasma concentrations by LC-MS/MS

Introduction

Oxytocin and vasopressin are biologically significant neuropeptides. Some of their functions, such as the role of oxytocin during childbirth, are extensively documented[1]. Other functions, such as their role in social interaction and a potential link with autism spectrum disorders, are being investigated[2].

Current studies involving quantitation of oxytocin and vasopressin in mammalian plasma and cerebral spinal fluid rely on immunoassay techniques. Radioimmunoassay (RIA) and enzyme immunoassay (EIA) give the required sensitivity, but there are concerns that cross-reactivity may affect the results. An LC-MS/MS method would resolve this issue by providing greater specificity. In addition, recoveries and response linearity can be verified by spiking samples with stable isotope labeled analogues.

Oxytocin and vasopressin are present in rhesus monkey plasma at about 200 fg/uL, and in cerebral spinal fluid at 5 to 50 fg/uL.[3] The typical sample size is ≤ 200uL, providing limited opportunities for concentration during sample preparation.

Immunoprecipitation is an elegant method for isolating a target protein from a complex matrix.[4] Briefly, an antibody for the protein is immobilized on an appropriate substrate, the sample is incubated with the substrate allowing the target protein to bind with the antibody, the substrate is washed removing sample contaminants, then the target protein is eluted using a buffer that dissociates the target protein from the antibody.

Sample Preparation

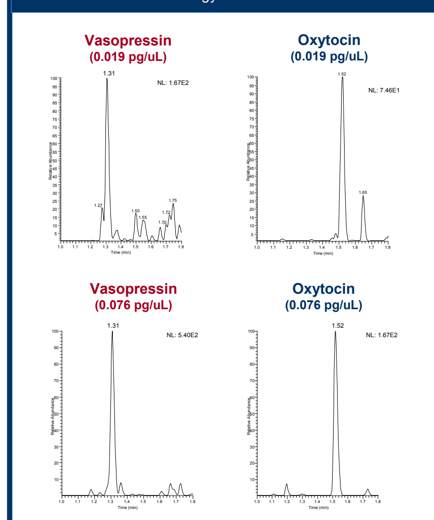
Immunoprecipitaton (IP) was performed with the Seize Primary IP kit from Pierce, following the protocol supplied by the manufacturer. Oxytocin and vasopressin antibodies were purchased from Millipore Corporation. Synthetic oxytocin and vasopressin were obtained from Bachem, Torrance, CA. An initial test of the IP assay for vasopressin isolation and recovery was performed using phosphate buffered saline (PBS) spiked with oxytocin and vasopressin. The incubation time was 6 hours at room temperature. The initial sample volume was 200uL and each elution volume was 100uL at pH 2.8. The elution volumes were analyzed without further modification.

Sample Analysis

Samples to generate the calibration curve were analyzed on an Accela HPLC and Vantage triple quadrupole mass spectrometer from Thermo Fisher Scientific. The column was a 2.1x30mm Agilent Zorbax SB-C18 3.5um cartridge, with a flow rate of 400uL/min. Initial conditions were 98% solvent A (0.1% formic acid in water)/2% solvent B (0.1% formic acid in acetonitrile) ramped to 25:75 in 2 minutes, returned to initial conditions at 3 minutes, and equilibrated for 2 minutes, for a total run time of 5 minutes. The injection volume was 20uL. The most intense transition was chosen for monitoring during sample analysis. Angiotensin from Sigma-Aldrich was spiked at 1 pg/uL before chromatographic analysis as an internal standard.

Samples to test the IP assay were analyzed with the same HPLC method on an LCQ Classic ion trap mass spectrometer. Because this instrument is less sensitive than the Vantage, the spiking level was 100 pg/uL.

FIGURE 1. ESI-LC/MS/MS chromatograms for vasopressin (CYFQNCPRG-NH2) and oxytocin (CYIQNCPLG-NH2). Vasopressin transition: 543.1 → 328.26 m/z at normalized collision energy=18. Oxytocin transition: 1007.5 → 723.3 m/z at normalized collision energy=30.



Results

The limit of detection for vasopressin was 5 fg/uL, and the limit of quantitation was 20 fg/uL. The limit of detection for oxytocin was 20 fg/uL and the limit of quantitation was 50 fg/uL. The calibration curve was linear over three orders of magnitude, from 0.019 to 19.5 pg/uL. Higher calibration levels were not attempted because higher sample concentrations are not expected.

Recovery of vasopressin from the initial IP assay was generally poor (Table 1). The post incubation flow thru was free from the target peptide. Oxytocin was detected in the flow thru as expected. No vasopressin was detected in the first three elution volumes. Vasopressin was detected in the fourth elution volume at about 20% of the spike level. Some vasopressin was detected in the fifth elution volume. It is possible that the target peptide degraded during incubation or that the elution buffer did not completely release the target peptide from the bound antibody.

FIGURE 2. Calibration curves for oxytocin and vasopressin covering a range from 0.019 to 19.5 pg/uL.

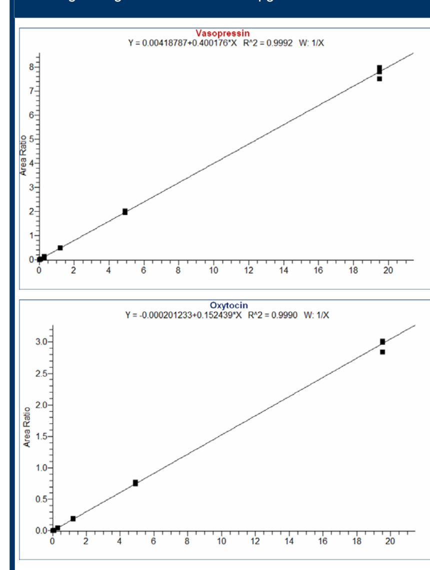


TABLE 1. Recoveries from initial vasopressin immunoprecipitation.

Sample No.	Description	Sample Volume (uL)	Peak area Vasopressin	Peak area Oxytocin	% Recovery
1	Spiking solution	100	831945	899244	NA
2	Post incubation flow through	400	ND	199685	89%
3	Elution volume 3	100	ND	ND	NA
4	Elution volume 4	100	169445	ND	20%
5	Elution volume 5	100	108523	ND	13%

Conclusions

An LC-MS/MS method for quantitation of oxytocin and vasopressin at endogenous plasma concentrations was developed. The limit of detection for vasopressin was 5 fg/uL, and the limit of quantitation was 20 fg/uL. The limit of detection for oxytocin was 20 fg/uL and the limit of quantitation was 50 fg/uL.

Future efforts will focus on sample prep and HPLC:

- Developing a robust sample preparation method. Stable isotope labeled internal standards will facilitate estimates of sample recovery from the processing steps. Solid phase extraction may provide the necessary level of sample cleanup with better recoveries.
- Further improvement of detection limits for analysis of cerebral spinal fluid. Chromatography with narrow bore columns and micro or nanospray ionization should provide an improvement in the lower limit of quantitation.

References

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