





Development of a quantification method for Vasopressin and Oxytocin using liquid chromatography coupled to tandem mass spectrometry.

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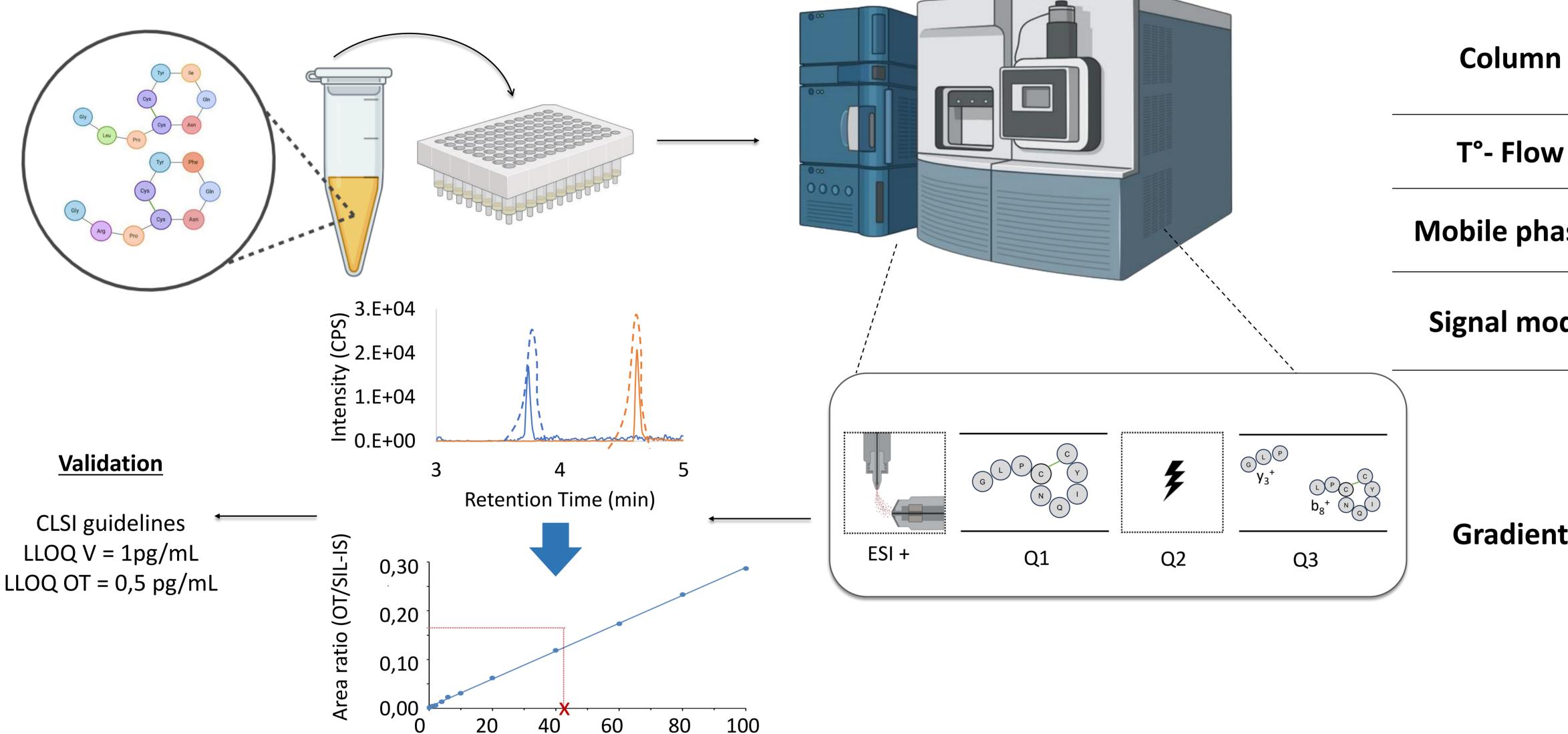
OT/V concentration (pg/mL)

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Introduction

Vasopressin and oxytocin are both neuropeptides, each consisting of 9 amino acids, produced by the hypothalamus and secreted by the posterior pituitary. Their respective roles include the regulation of plasma osmolarity through urinary volume modulation and the regulation of social behaviors or the initiation of uterine contractions. These two hormones are involved in pathologies such as diabetes insipidus for vasopressin while oxytocin is involved in autism, depression, and schizophrenia¹⁻². The implication of these peptides in various disorders suggests that precise quantification could benefit patient care. Currently, quantification of vasopressin and oxytocin is mainly performed through immunological methods but is still uncommon. These techniques face issues with specificity and can vary across laboratories. The development of a quantification method through a gold standard technique, such as liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), is essential for harmonizing biological sample assays and standardizing vasopressin and oxytocin quantification.

Materials and Methods

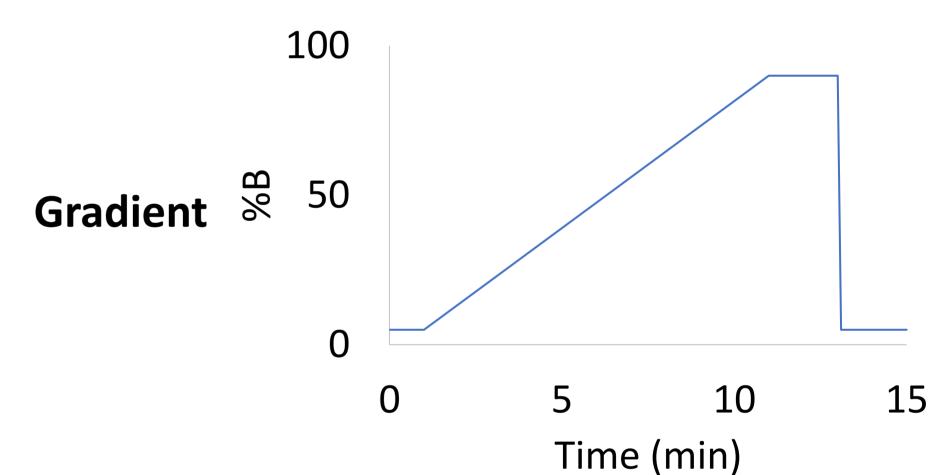


Acquity UPLC BEH C18, 130 Å, 1,7 μm, 2,1 mm x 100 mm, Waters.

50°C - 0,25 mL/min

A: $H_2O + 0.1\%$ FA **Mobile phases** B: ACN + 0.1% FA

Signal mode Electrospray – Positive

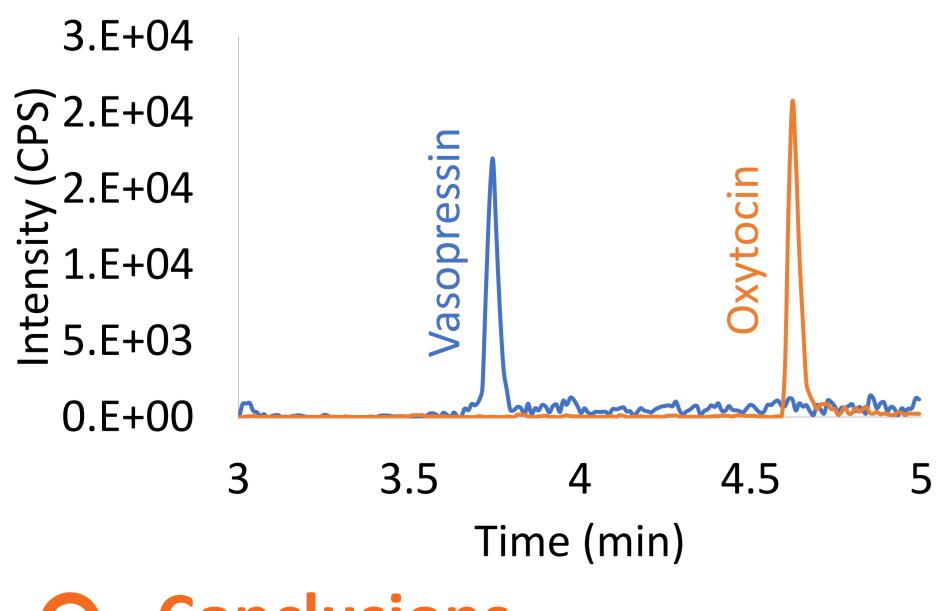


O Results

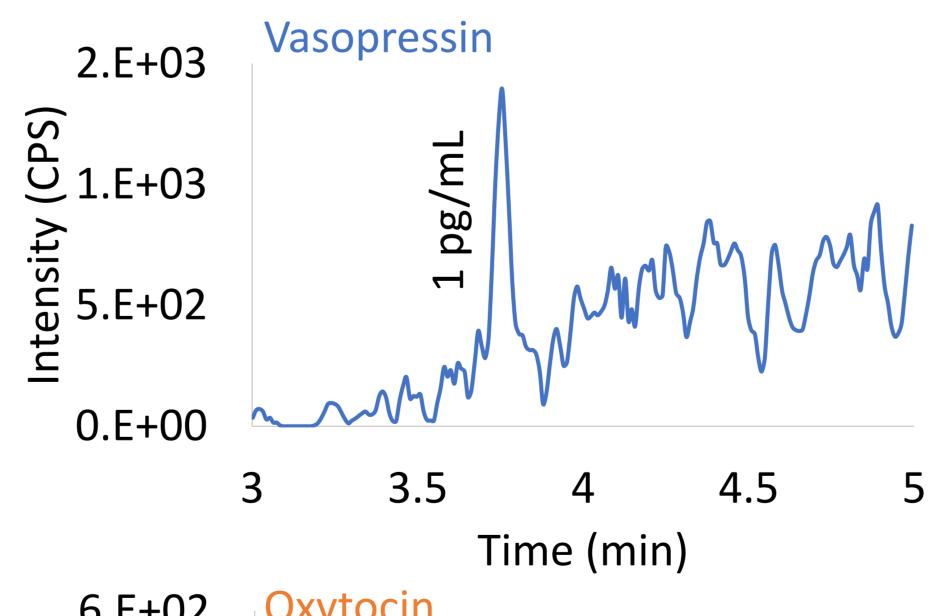
Validation steps

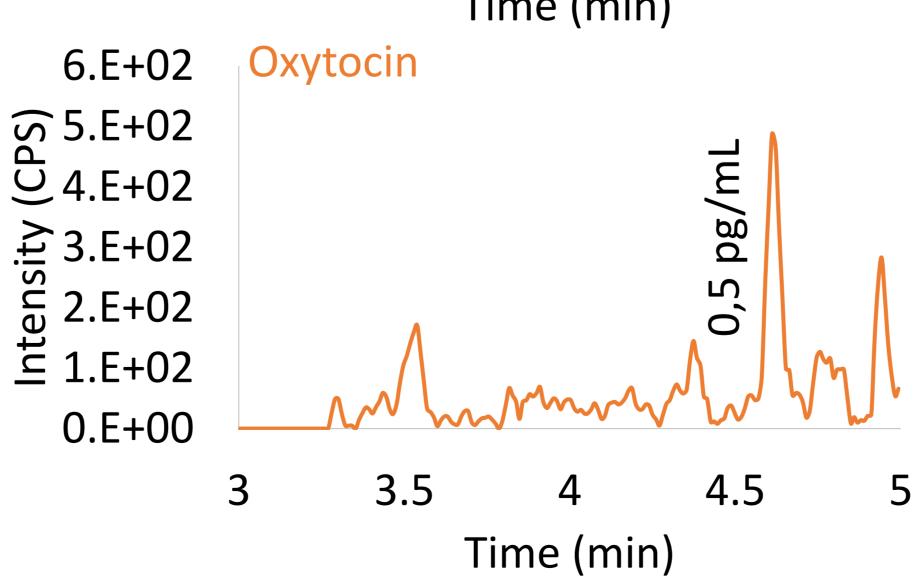
Oxytocin	Vasopressin			
Recovery				
88%	82%			
Matrix effect				
20%	55%			

Chromatographic separation



Lower limit of quantitation





Linearity

vasopressin				
Expected concentration (pg/mL)	Calculated concentration (pg/mL)	Accuracy (%)	CV (%)	
24	53,3	222,2	7,5	
48	92,0	191,6	19,5	
72	298,3	414,4	28,2	
96	262,0	267,4	45,4	
120	534,0	445	14,1	

Oxytocin

Expected concentration (pg/mL)	Calculated concentration (pg/mL)	Accuracy (%)	CV (%)		
24	25,7	107	9		
48	50,3	104	2		
72	82,2	114	6		
96	97,9	102	4		
120	122,1	102	5		

Conclusions

LC, MS and SPE were optimized and led to a LLOQ of 1 pg/mL for Vasopressin and 0,5 pg/mL for Oxytocin.

- Perspectives
- 1. Synthesize the IS of Vasopressin
- 2. Decrease the LLOQ for Vasopressin
- 3. Validation of the method according
 - to CLSI guidelines

- 1. Sabatier, N et al. Biochem. Soc. Trans. 2007, 35 (5), 1247-1251
- 2. Zhang, D et al. J. Pharm. Biomed. Anal. 2014, 99: 67-73