

## Supplementary data for:

### **Proteomics tools reveal startlingly high amounts of oxytocin in plasma and serum**

Ole Kristian Brandtzaeg<sup>a</sup>, Elin Johnsen<sup>a</sup>, Hanne Roberg-Larsen<sup>a</sup>, Knut Fredrik Seip<sup>b</sup>, Evan L. MacLean<sup>c,d</sup>,  
Laurence R. Gesquiere<sup>e</sup>, Siri Leknes<sup>f,g</sup>, Elsa Lundanes<sup>a</sup> and Steven Ray Wilson<sup>a\*</sup>

<sup>a</sup>Department of Chemistry, University of Oslo, Post Box 1033, Blindern, NO-0315 Oslo, Norway

<sup>b</sup>School of Pharmacy, University of Oslo, PO Box 1068, Blindern, NO-0316, Oslo, Norway

<sup>c</sup>Department of Evolutionary Anthropology, Duke University, Durham, NC, 27708, USA

<sup>d</sup>School of Anthropology, University of Arizona, Tucson, AZ, 85721, USA

<sup>e</sup>Department of Biology, Duke University, Durham, NC, 27708, USA

<sup>f</sup>Department of Psychology, University of Oslo, PO Box 1094, Blindern, NO-0317, Oslo Norway

<sup>g</sup>The Intervention Centre, Oslo University Hospital, PO Box 4950, Nydalen, NO-0424 Oslo, Norway

\*Corresponding author. Tel.: +47 970 10 953.

E-mail address: [stevenw@kjemi.uio.no](mailto:stevenw@kjemi.uio.no) (S.R. Wilson).

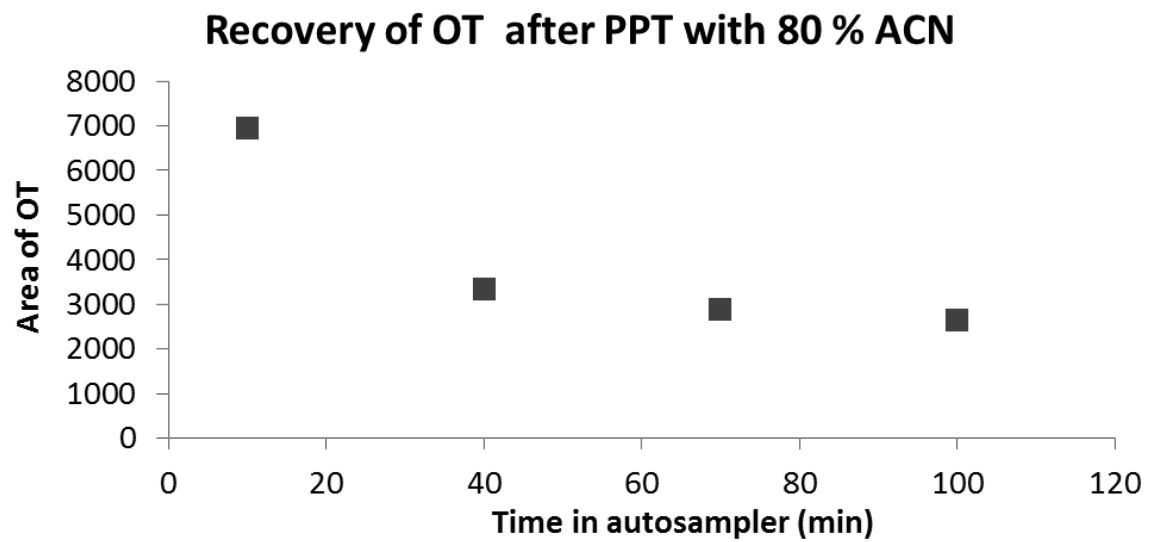
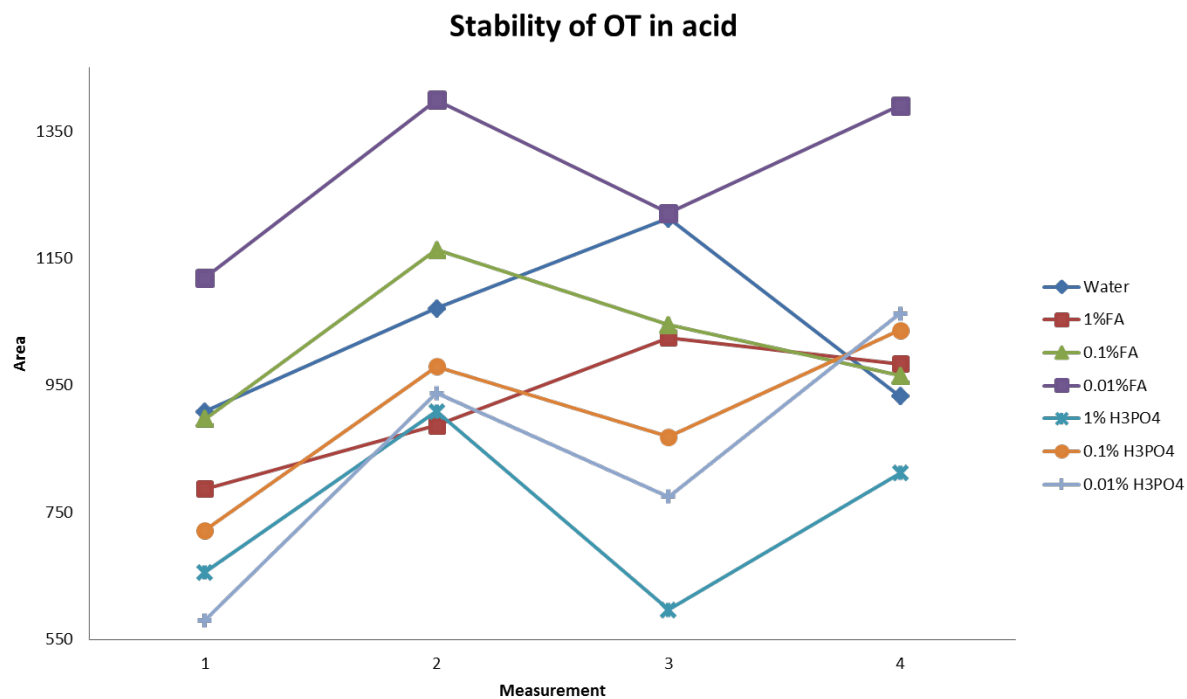
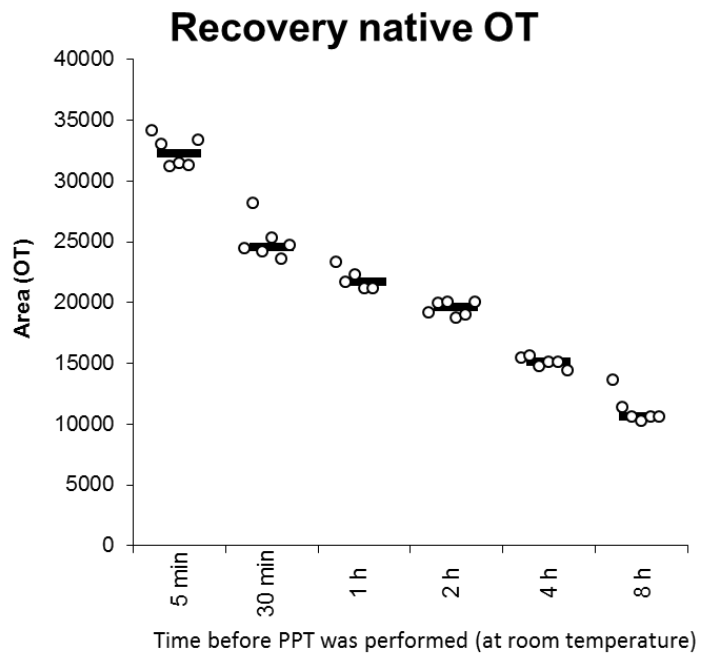


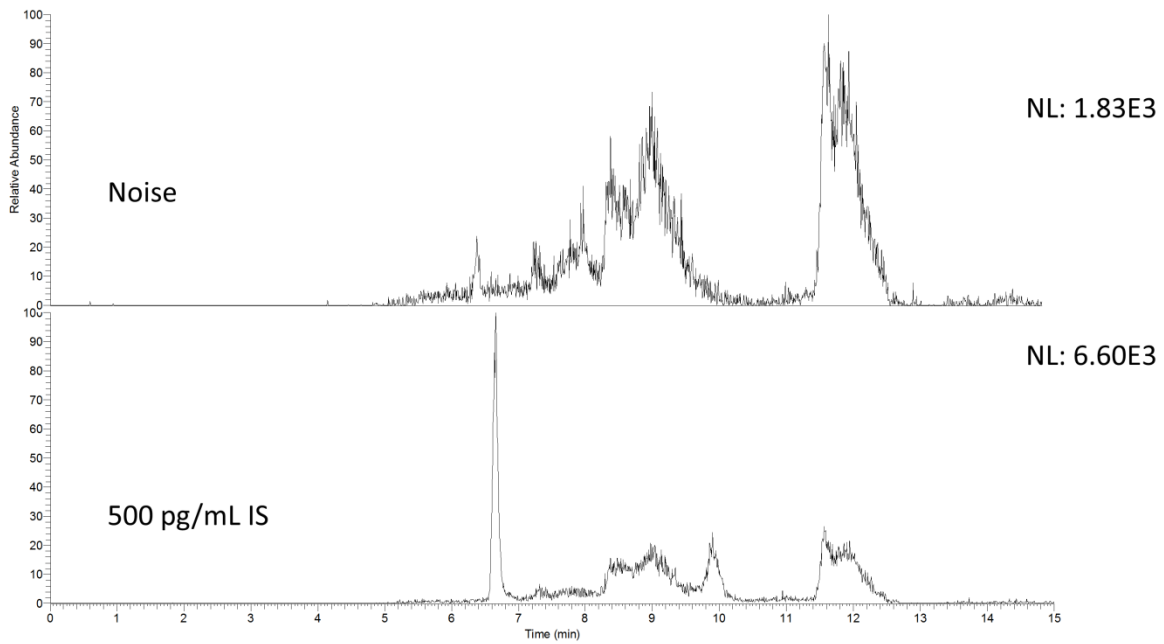
Figure SM 1 Recovery of OT after PPT with 80 % ACN. Plasma was spiked with 500 pg/mL OT and analyzed with the nano-AFFL-SPE-LC-MS/MS system after 10, 40, 70 and 100 minutes.



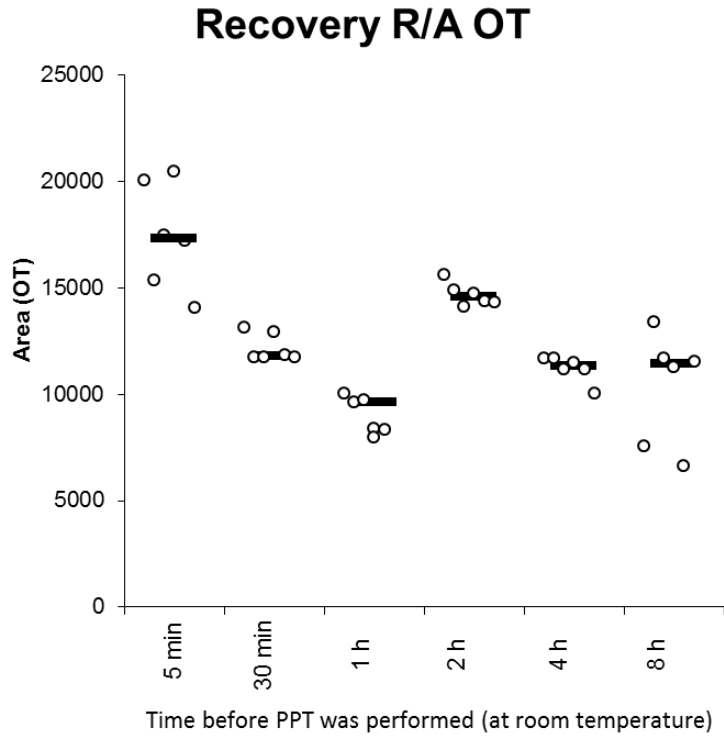
**Figure SM 2: Stability of OT (100 ng/mL) spiked in water, formic acid (0.01-1 %) and H<sub>3</sub>PO<sub>4</sub> (0.01-1 %). Measurement 1 was conducted 10 min after addition of acid, measurement 2 after 2 hours, measurement 3 after 3 hours and measurement 4 after 5 hours.**



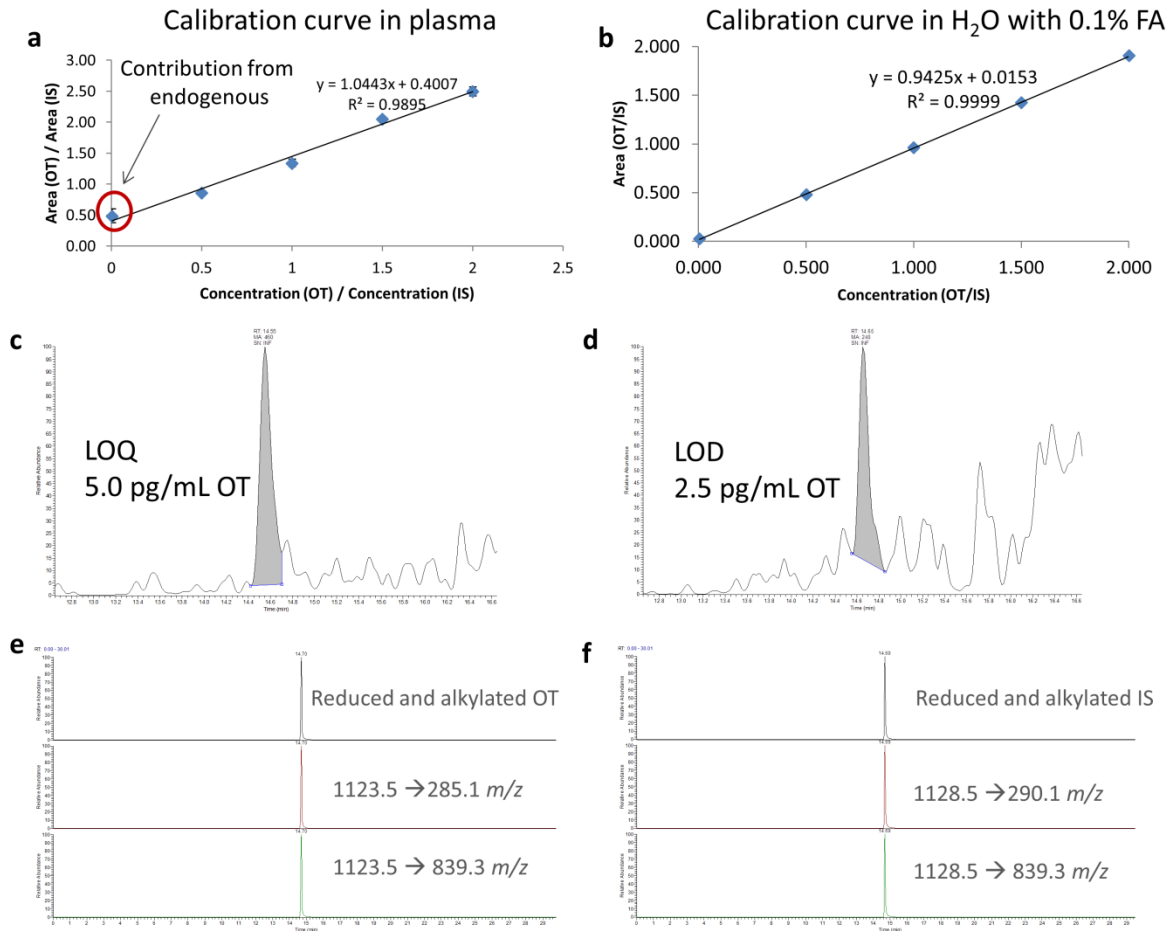
**Figure SM 3 Recovery of native OT spiked into plasma (500 pg/mL) and incubated for 5 min – 6 hours in room temperature before PPT.**



**Figure SM 4 EIC (top, OT,  $m/z$  1007  $\rightarrow$  723, bottom, IS,  $m/z$  1012  $\rightarrow$  723) analyzed on a Bruker easy nLC system without AFFL system (preliminary experiments). Mobile phase A was 0.1 % FA in H<sub>2</sub>O, while mobile phase 2 was 0.1 % FA in ACN. For elution a step gradient was used (0-10 min 0 -50 % B, 10-13 min 50-90 %B and 13-15 min 90 % B). Injection volume was 20  $\mu$ L and flow rate was 800 nL/min**



**Figure SM 5 Recovery of R/A OT spiked into plasma (500 pg/mL) and incubated for 5 min – 6 hours in room temperature before PPT.**



**Figure SM 6 a:** Calibration curve for reduced and alkylated OT in plasma. Pooled human plasma spiked with 5, 500, 1000, 1500 and 2000 pg/mL OT and 1000 pg/mL IS. **b:** Calibration curve for reduced and alkylated OT in water with 0.1% FA. Calibration standards were spiked with 5, 500, 1000, 1500 and 2000 pg/mL OT and 1000 pg/mL IS. **c:** Limit of quantification of 5 pg/mL reduced and alkylated oxytocin in water with 0.1% FA. **d:** Limit of detection was 2.5 pg/mL reduced and alkylated OT in water with 0.1% FA. **e:** Fragment transitions of reduced and alkylated oxytocin. **f:** Fragment transitions of reduced and alkylated IS.