# Measurement of ultra-trace level of intact oxytocin in plasma using SALLE combined with nano-LC-MS

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**UPLC-QTOF**

The detection of model compounds were performed using Agilent 1290 Series UPLC system with Agilent Poroshell 120 SB-C4 column (150 × 3.0 mm, 1.8 μm ). A mobile phase consisting of solvent A 0.5% formic acid and solvent B acetonitrile was applied at a flow rate of 0.3mL/min. Gradient elution was 0-5 min, 5-65% B; 5-8 min, 65-100% B; 8–13 min, 100% B; 13-15min, 5% B. Each sample volume injected was 5μl. Mass spectrometry was carried out by using an Agilent 6520 Q-TOF MS (Agilent Corporation, CA, USA) equipped with an electrospray ionization (ESI) interface. The optimum operating parameters was as follows: drying gas (N2) flow rate of 9.0 L/min; drying gas temperature of 350℃; voltage of capillary of 4000 V; skimmer of 65 V; fragmentor voltage of 180V. Full-scan data acquisition in positive ion mode was operated from 450～550 with accurate mass of 5 ppm and acquisition rate of 3 spectra per second. The divalent ions of OT (Calc. m/z: 504.2271) and stable isotope-labeled OT (as IS, Calc. m/z: 507.2325) were detected by extracted ion chromatograms (EIC). Data were handled on the MassHunter WorkStation Data Acquisition software (Version B.02.01) and Quantitative Analysis software (Version B.04.00).

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**Protein precipitation (PPT) method**

Protein precipitation was performed by adding ice-cold AcN and whirl mixing 30s, then countrified 15min at 15,000g. The supernatant was evaporated to dryness.

**SDS-PAGE method**

Each sample lyophilized was dissolved in 1x SDS-PAGE sample buffer (2ml 0.5mol/L Tris-HCl (pH 6.8), 1.6ml glycerol, 3.2ml 10% SDS, 0.8ml 2-mercaptoethanol, 0.4ml 1.0% bromophenol blue) and incubated at 90℃ for 7 min. 5ul each sample or 3ul standard polypeptide marker was applied to a 15% commercial polyacrylamide gels (Willget biotech Co., Ltd in shanghai, China.). The gel was run at a constant voltage of 120V for 1h in a Mini Protein Tetra System (BioRad) and then stained by 0.1% silver nitrate solution.