

## **Fast, simple, and validated LC-HR-MS/MS assay for identification and quantification of drugs in human blood plasma often requested in context of brain death diagnosis**

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### **Abstract**

**Aims:** In the presented study, an LC-HR-MS/MS approach based on protein precipitation was developed for fast screening, identification, and quantification for 12 drugs often requested in the context of brain death diagnosis. The procedure was validated and tested for its applicability.

**Methods:** Blood plasma samples (250  $\mu$ L) were precipitated with 750  $\mu$ L of a ZnSO<sub>4</sub>/water/methanol solution. Codeine-d<sub>6</sub> (final concentration 10  $\mu$ g/L) was added as internal standard. Separation, identification, and quantification were performed with a Thermo-Fisher (TF) Accela LC system (Accucore RPMS or PhenylHexyl column, 150 x 2.1 mm, 2.6  $\mu$ m, each) coupled to a TF Q Exactive high resolution mass spectrometer (ESI+ mode). The method was validated with respect to selectivity, ion suppression/enhancement of co-eluting analytes, recovery, matrix effects, process efficiency, accuracy, and precision, stabilities, and limits of quantification and detection. For the accuracy and precision studies, a three-point calibration was performed.

**Results and Discussion:** During validation, no selectivity problems and matrix effects could be detected. The calibration range ( $\mu$ g/L) was 30-600 (alfentanil), 100-2000 (diazepam), 100-500 (etomidate), 3-300 (fentanyl), 1000-6000 (ketamine), 25-100 (midazolam), 10-100 (morphine), 100-2000 (nordazepam), 3-14 (piritramide), and 0.5-20 (sufentanil). The lower limit of quantification was set at the lowest calibrator concentration and this concentration corresponded at least to the lowest therapeutic concentrations of the corresponding drugs. The validation criteria were fulfilled for all compounds. Three-point calibration was shown to be suitable for all analytes.

**Conclusion:** The presented LC-HR-MS/MS approach was suitable for fast screening, identification, and quantification of drugs often requested in the context of brain death diagnosis.

**Original publication in preparation.**