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Analytical method for the analysis of cocaine biomarkers in wastewater

Deliverable D3.2

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EUROPE-WIDE SEWAGE ANALYSIS TO MONITOR EMERGING DRUG PROBLEMS

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Executive Summary

This deliverable consists of the analytical method which was developed for the analysis of crack cocaine biomarkers in wastewater samples.

Materials and reagents

Reference standards of anhydroecgonine and anhydroecgonine methyl ester and their deuterated standards were purchased from Lipomed AG (Lipomed, Arlesheim, Switserland). Acetonitrile, ammonium hydroxide and methanol (ultra-gradient HPLC grade) were obtained from Boom B.V. (Meppel, the Netherlands). Formic acid and hydrochloric acid were purchased from Sigma-Aldrich (Steinheim, Germany). Stock solutions of the reference standards, including internal standards, were prepared at a concentration of 3.5 mg/L in acetonitrile. Individual stock solutions were stored at -20 ˚C. Working solutions containing all individual standards were freshly prepared in acetonitrile with 5% ultrapure water (18.2 MΩ/cm, ELGA LabWater, Lane End, UK) (35 µg/L) each time a new set of samples was processed and analyzed.

Sample preparation

For solid-phase extraction, 50 mL of each sample was transferred in a precleaned HDPE bottle. Internal standard work solution was added to each sample to reach a concentration of 100 ng/L in wastewater and the sample was adjusted to pH = 2.0 with HCl. Samples were horizontally shaken for 5 min at 120 rpm and filtered through a 0.20 µm filter. Samples were then extracted with Oasis MCX cartridges (3 mL, 60 mg, Waters, USA). Cartridges were washed 6 mL of methanol, followed by 3 mL of ultrapure water and 3 mL of acidified ultrapure water (pH = 2.0). Samples were then gently loaded onto the cartridges. Subsequently, the cartridges were washed with 6 mL of acidified ultrapure water (pH = 2.0) and dried under vacuum for 1 h. Thereafter, the cartridges were eluted with 6 mL of MeOH with 2% ammonium hydroxide. Eluates were collected in glass tubes and evaporated to dryness under a gentle stream of nitrogen at 40 °C. Eluates were then reconstituted in 500 µL in acetonitrile with 5% ultrapure water and vortexed for 5 seconds. The extract was filtered through a 0.45 µm filter and transferred in 1.8 mL vials with inserts for analysis.

Analytical method

A Tribrid Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an electrospray ionization (ESI) source was interfaced to a Vanquish HPLC system (Thermo Fisher Scientific, Bremen, Germany). Every batch run mass calibration was performed using a Pierce ESI positive ion calibration solution. The ion transfer tube temperature and the vaporizer temperature were set to 300 ˚C and 350 ˚C respectively. The sheath, auxiliary and sweep gas were maintained at arbitrary units of 45, 5 and 5 respectively. The source voltage was set to 3000 V in positive mode. The RF lens was set to 60% and the scan range was set in the range of 100-400 *m/z*. The Orbitrap resolution was set to 120,000 FWHM and the quadruple isolation was used for acquisition with a 5 ppm mass window. Data-dependent acquisition was performed with a High Collision Dissociation (HCD) of 30%.

For the chromatographic separation an Agilent Zorbax HILIC plus (150 mm x 2.1 mm, 1.8 µm) connected to a krudkatcher ULTRA HPLC In-line Filter, 0.5 µm was used. The column temperature was maintained at 25 ˚C. Mobile phase A consisted of 95% ultrapure water and 5% acetonitrile (v/v) with 5 mM ammonium formate at a pH = 3. Mobile phase B consisted of 95% acetonitrile and 5% ultrapure water (v/v) with 5 mM ammonium formate at a pH = 3. A linear gradient from 100% B to 20% B in 15 min was used. Next, B was held at 20% for 5 min. Then %B was increased to 100% in 1 min and after this the column was equilibrated at 100% B for 6 min which results in a total run time of 27 min. The flow rate was 0.300 mL/min and 50 µL of sample was injected onto the LC column.