Electronic supplementary material to the manuscript

Gas Chromatography – High-Temperature Proton-Transfer Reaction Mass Spectrometry as Novel Tool for Forensic Drug Testing

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S1. Suspect screening

S1.1. GC-electron ionization-MS analysis

The GC-MS system consisted of a 6890N GC device with a 5973 inert mass-selective detector (Agilent Technologies, Santa Clara, CA, USA). A 30 m × 0.25 mm, 0.25-µm DB-XLB column (J&W Scientific) was used for chromatographic separation. Carrier gas was helium with a flow rate of 1.0 mL/min. The injection volume was 1.0 µL (splitless). The injection temperature was 250 °C. The temperature program was as follows: 50 °C, hold 1 min; increase to 150 °C with 25 °C/min, to 320 °C with 10 °C/min, hold for 8 min and to 330 °C in 20 °C/min, hold for 7.5 min. MS was performed in electron ionization mode (70 eV) scanning *m/z* 50-600. Mass spectral data were recorded on a personal computer with the HP MS ChemStation software G1034C version D01.00 (Agilent Technologies). The "Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants, and Their Metabolites, 5th Edition" authored by Hans H. Maurer, Karl Pfleger and Armin A. Weber (John Wiley & Sons, Hoboken, NJ, USA) was used for compound identification.

S1.2. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

Plasma samples were prepared for LC-MS/MS analysis with protein precipitation. 250 µL of plasma were mixed with 750 µL acetonitrile. The mixture was vortexed for 1 minute. After a five-minute sonification step, the sample was centrifuged for 5 minutes at 8000 x g. The supernatant was transferred to a clean 1.5 mL glass vial and evaporated to dryness at room temperature with employing a nitrogen stream. The residue was reconstituted in 50 µL water/MeOH (v/v; 1/1) and submitted to LC-MS/MS analysis.

For determining the limits of identification of 106 compounds (Supplementary Material Tables S1), blank plasma samples were donated with the corresponding reference standards at 1.0 ng/mL, 2.5 ng/mL, 5.0 ng/mL, 10 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL and 250 ng/mL.

Non-targeted LC-MS/MS analysis with subsequent compound identification by suspect screening in tandem mass spectral libraries was accomplished as described in [14-17].

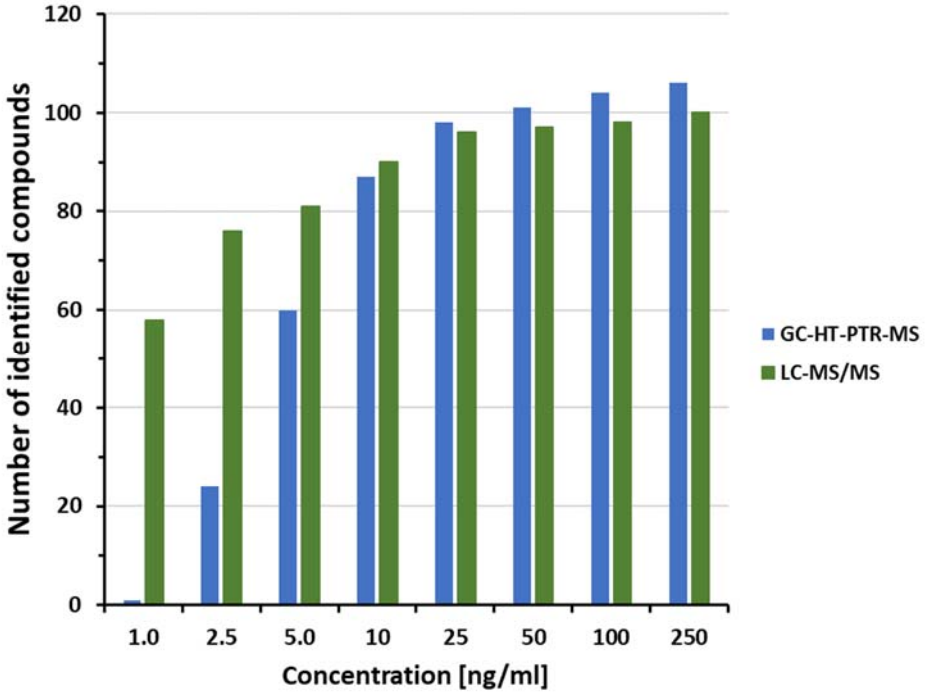

Figure S1. Comparison of the detection capabilities obtained for 106 reference compounds with GC-HT-PTR-MS and LC-MS/MS.

S2. Quantitative analysis

S2.1. Cocaine

1.0 mL plasma sample was mixed with 25 µL internal standard solution (cocaine-D3, 0.5 µg/mL), 3 mL water, and 2 mL 0.1 M phosphate buffer (pH 6.0), centrifuged for 5 minutes at 8000 x g and processed by solid-phase extraction employing SPE-ED Scan ABN columns (200 mg/3 ml, Applied Separations, Allentown, PA, USA). The cartridges were equilibrated with 2 mL methanol, 2 mL water and 2 mL 0.1 M phosphate buffer (pH 6.0). After sample application, the cartridges were washed with 2 mL water and 2 mL 0.01 M aqueous hydrochloric acid solution. The cartridges were dried for 20 minutes. Elution was accomplished with 2.0 ml of a mixture of dichloromethane, propan-2-ol and ammonium hydroxide (80/20/2, v/v). The obtained eluate was evaporated to dryness at 60 °C employing a nitrogen stream. The residue was reconstituted in 50 µL 2,2,3,3,3-pentafluoro-1-propanol and 75 µL pentafluoropropionic anhydride. The mixture was kept at 60 °C for 45 minutes for derivatization. The solution was evaporated at 60 °C to dryness and reconstituted in 50 µL acetone. The solution was submitted to GC-MS analysis (see S2.5). The ions targeted in selected ion monitoring mode are summarized in Table S3. Blank samples spiked with known concentrations of the reference compounds served as calibration standards. The calibration range was 2.5-25 ng/mL.

S2.2. Δ9-Tetrahydrocannabinol (THC)

1.0 mL plasma sample was mixed with 20 µL internal standard solution (THC-D3, 0.4 µg/mL) and 2 mL acetonitrile for protein precipitation. The sample was vortexed for 20 seconds and centrifuged for 5 minutes at 8000 x g. The supernatant was separated, spiked with 5 mL 0.1 M acetic acid solution, and submitted to solid-phase extraction employing SPE-ED Scan ABN THC columns (200 mg/3 ml, Applied Separations). The cartridges were equilibrated with 2 mL methanol and 1 mL water. After sample application, the cartridges were washed with 3 mL water and dried for 20 minutes. 1.5 mL acetone were used for elution. The eluate was evaporated to dryness and reconstituted in 0.3 mL dimethyl sulfoxide/tetramethylammonium hydroxide. The solution was incubated for 2 minutes prior to the addition of 50 µL iodomethane. The reaction mixture was kept at room temperature for 15 minutes before the addition of 700 µL 0.1 M HCl solution. The sample was then further processed by liquid-liquid extraction with 2 times 2 mL hexane. The organic layer was then evaporated to dryness and reconstituted in 50 µL heptane and submitted for GC-MS (see S2.5). The ions targeted in selected ion monitoring mode are summarized in Table S3. Blank samples spiked with known concentrations of the reference compounds served as calibration standards. The calibration range was 1-10 ng/mL.

S2.3. Amphetamines

1.0 mL plasma sample was mixed with 15 µL internal standard solution (amphetamine-D8, methamphetamine-D8, MDA-D5, MDMA-D5, 5 µg/mL each) and 3 mL water, centrifuged for 5 minutes at 8000 x g and processed by solid-phase extraction employing SPE-ED Scan ABN columns (200 mg/3

ml, Applied Separations). The cartridges were equilibrated with 2 mL methanol, 2 mL water and 1 mL 1 M acetic acid solution. After sample application, the cartridges were washed with 2 mL water, 1 mL 1 M acetic acid solution and 2 mL methanol/water (v:v; 1:10) and dried for 20 minutes. Elution was accomplished with 2.0 ml of a mixture of dichloromethane, propan-2-ol and ammonium hydroxide (80/20/2, v/v). The obtained eluate was evaporated to dryness at 60 °C employing a nitrogen stream. The residue was reconstituted in 75 µL pentafluoropropionic anhydride and kept at 60 °C for 60 minutes for derivatization. The solution was evaporated at 60 °C employing a nitrogen stream and reconstituted in 50 µL ethyl acetate. The solution was submitted to GC-MS analysis (see S2.5). The ions targeted in selected ion monitoring mode are summarized in Table S3. Blank samples spiked with known concentrations of the reference compounds served as calibration standards. The calibration ranges were 16-150 ng/mL.

S2.4. Opiates

1.0 mL plasma sample was mixed with 20 µL internal standard solution (EDDP-D3, 5 µg/mL; methadone, 10 µg/mL; dihydrocodeine-D6, 10 µg/mL; morphine-D3, 5 µg/mL) and 5 mL water, centrifuged for 5 minutes at 8000 x g and processed by solid-phase extraction employing SPE-ED Scan ABN columns (200 mg/3 ml, Applied Separations). The cartridges were equilibrated with 2 mL methanol and 1 mL 0.005 M hydrochloric acid solution. After sample application, the cartridges were washed with 2 mL water and 1 mL 0.005 M hydrochloric acid solution and dried for 20 minutes. Elution was accomplished with 2.0 ml of a mixture of dichloromethane, propan-2-ol and ammonium hydroxide (80/20/2, v/v). The obtained eluate was evaporated to dryness at 60 °C employing a nitrogen stream. The residue was reconstituted in 60 µL pentafluoropropionic anhydride and kept at 60 °C for 60 minutes for derivatization. The solution was evaporated at 60 °C, the residue was reconstituted in 50 µL acetone. The solution was submitted to GC-MS analysis (see S2.5). The ions targeted in selected ion monitoring mode are summarized in Table S3. Blank samples spiked with known concentrations of the reference compounds served as calibration standards. The calibration ranges were 1.25-21 ng/mL for EDDP, 32-420 ng/mL for methadone, 12.5-210 ng/mL for morphine, and 25-420 ng/mL for dihydrocodeine.

S2.5. GC-electron ionization-MS analysis

The GC-MS system consisted of a 6890N GC device with a 5973 inert mass-selective detector (Agilent Technologies, Santa Clara, CA, USA). A 30 m × 0.25 mm, 0.25-µm DB-XLB column (J&W Scientific) was used for chromatographic separation. Carrier gas was helium with a flow rate of 1.0 mL/min. The injection volume was 1.0 µL (splitless). The injection temperature was 250 °C. The temperature program was as follows: 50 °C, hold 1 min; increase to 150 °C with 25 °C/min, to 320 °C with 10 °C/min, hold for 8 min and to 330 °C in 20 °C/min, hold for 7.5 min. MS was performed in electron ionization mode (70 eV). Mass spectral data were recorded on a personal computer with the HP MS ChemStation software G1034C version D01.00 (Agilent Technologies).

Table S3. Overview of quantifier and qualifier ions used for quantitative drug analysis with GC-electron ionization-MS.

Analyte	***Quantifier m/z***	***Qualifier 1 m/z***	***Qualifier 2 m/z***	***Qualifier 3 m/z***
Cocaine	182	303	82	
Cocaine-D3	185	306	85	
THC ME	328	285	245	
THC-D3 ME	331	288	248	
Amphetamine-D8 PFP	193	126	96	69
Amphetamine PFP	190	91	118	65
Methamphetamine-D8 PFP	211	163	123	92
Methamphetamine PFP	204	160	118	91
MDA-D5 PFP	167	330	194	136
MDA PFP	162	325	190	135
MDMA-D5 PFP	208	344	163	136
MDMA PFP	204	339	162	135
EDDP-D3	279	203	265	280
EDDP	277	200	262	276
Methadon-D9	78	303	226	
Methadon	72	309	223	
Dihydrocodeine-D6 PFP	306	290	393	453
Dihydrocodeine PFP	300	284	390	447
Morphine-D3 2PFP	580	417	433	364
Morphine 2PFP	577	414	430	361