

K77 The Quantification of 4-Fluoroamphetamine, 4-Fluoromethamphetamine, 4-Chloroamphetamine, and 4-Chloromethamphetamine in Postmortem Blood and Urine by Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS)

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Learning Overview: The goal of this presentation is to provide insights of a highly sensitive UHPLC-MS/MS approach for the analysis of four New Psychoactive Substances (NPS)—4-fluoroamphetamine, 4-fluoromethamphetamine, 4-chloroamphetamine, and 4-chloromethamphetamine—in blood and urine specimens following a liquid-liquid extraction sample preparation protocol.

Impact on the Forensic Science Community: This presentation will impact the forensic science community through the development and validation of the UHPLC-MS/MS method to improve the identification and quantification of certain NPS in postmortem specimens.

4-fluoroamphetamine, 4-fluoromethamphetamine, 4-chloroamphetamine, and 4-chloromethamphetamine are substituted phenethylamine-type synthetic NPS, with amphetamine- and methamphetamine-like structural feature. Reportedly, 4-fluoroamphetamine and 4-fluoromethamphetamine produce mainly sympathomimetic effects and exhibit entactogenic properties, while 4-chloroamphetamine and 4-chloromethamphetamine stimulate the central nervous system, with a longer half-life than their fluoro-counterparts. These phenethylamine-type NPS have gained popularity on the illegal market and have been detected in specimens from many death cases in Taiwan during the past six years.

Blood or urine (0.5mL) were mixed with sodium hydroxide and extracted with ethyl acetate. The extracts were evaporated and reconstituted in the mobile phase (initial gradient composition) for injection onto the UHPLC-MS/MS system. Deuterated analogs of the analytes were used as internal standards. Chromatographic separation was achieved using an Agilent® ZORBAX® SB-Aq (100mm × 2.1mm i.d., 1.8-µm particle) analytical column at 50°C. The mobile phase included 0.1% formic acid (v/v) in water (A) and methanol (B), with a flow rate of 0.32mL/min. The initial gradient composition (A/B 90:10, v/v) was decreased to 0% A in 6min; then increased to 90% A in 1min. Parameters for mass spectrometric analysis included: (1) Agilent® Jet Stream Technology electrospray ionization in positive-ion Multiple Reaction Monitoring (MRM) mode, (2) optimized collision energy levels for selected precursor ions, and (3) monitoring two transitions for analytes and internal standards.

Validity of the methodology was assessed using drug-free blood and urine that were fortified with 100ng/mL–2000ng/mL of the analytes. The following analytical parameters were obtained: (1) average extraction recovery, derived from five different sources of blood and urine, was higher than 75%; (2) matrix effect (ion suppression) was observed, except for blood samples of 4-chloroamphetamine at the 100ng/mL and 250ng/mL concentration levels, but was adequately compensated for by using respective deuterated internal standards; (3) intra-/inter-day precision (%CV) and accuracy ranges for blood were 0.6%–7.0%/3.7%–10.2% and 85%–109%/93%–105%, while the corresponding ranges for urine were 0.8%–5.1%/3.5%–17.7% and 86%–110%/92%–106%; (4) calibration linearity (r^2) for all analytes were >0.997; and (5) the limits of detection for 4-fluoroamphetamine, 4-fluoromethamphetamine, 4-chloroamphetamine, and 4-chloromethamphetamine in urine and blood were 2.5ng/mL, 0.5ng/mL, 1ng/mL, 5ng/mL and 0.5ng/mL, 0.5ng/mL, 2.5ng/mL, 5ng/mL, respectively.

In conclusion, this relatively simple protocol was found effective and reliable for routine identification and quantification of these NPS in blood and urine. This method was applied to the analysis of a limited number of postmortem specimens (one blood and four urine) from forensic cases during the April-July 2018 period. 4-chloroamphetamine and 4-chloromethamphetamine were found in the blood and the urine specimens from the same case, with 233ng/mL and 11,151ng/mL for blood and 807ng/mL and 185,095ng/mL for urine. 4-fluoroamphetamine was found in the other three urine specimens with the concentrations ranging from 183ng/mL to 2,526ng/mL.

Phenylethylamine Derivatives, Postmortem, UHPLC-MS/MS