



K20 Simultaneous Quantification of Amphetamines, Ketamine, and Opiates in Urine Using SPE and LC/MS/MS

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After attending this presentation, attendees will learn of a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) technique for analyzing amphetamine, methamphetamine, MDA, MDMA, morphine, 6-acetylmorphine, codeine, 6-acetylcodeine, ketamine, and norketamine in urine.

This presentation will impact the forensic science community by developing a simple, accurate, and fast analytical method of LC/MS/MS capable of quantifying ten analytes in urine that are abused drugs.

Heroin, methamphetamine, and ketamine have historically been the most commonly abused drugs in Taiwan and are routinely monitored in the laboratory by Gas Chromatography/Mass Spectrometry (GC/MS) methods. The purpose of this study was to evaluate whether the LC/MS/MS-based approach can be more effectively applied to the simultaneous quantitation of amphetamine (AM), methamphetamine (MA), MDA, MDMA, morphine (MOR), codeine (COD), 6-acetylmorphine (6-AM), 6-acetylcodeine (6-AC), ketamine (K), and norketamine (NK) in postmortem urine specimens.

Samples (1mL) were extracted via solid-phase extraction, evaporated, and reconstituted in the mobile phase for injection onto the LC/MS/MS system. Deuterated analogues of the analytes of interest were used as internal standards. Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100mm \times 2.1mm i.d., 1.8- μ m particle) analytical column at 50°C. The mobile phase consisted of 0.1% formic acid (v/v) in water (A) and methanol (B) at a flow rate of 0.32mL/min. The initial gradient composition (A/B 90:10, v/v) was held for 1.5 min, then decreased to 0% A in 8.5 min and held for 2 min, then increased to 90% A in 1 min and held for 2 min. MS analysis was performed by an electrospray ionization in positive-ion Multiple Reaction Monitoring mode (MRM) with optimized collision energy for the precursor ion selected, monitoring two transitions for each analyte.

Validation was performed by extracting drug-free urine fortified with 50 – 1000ng/mL of the 10 analytes, yielding the following results: (1) average extraction recovery (n=5) was >80%, except for MDMA (70%) and MOR (74%); (2) inter-day and intra-day precision ranges (%CV) were 1.59 – 9.13% and 0.57 – 3.89%; (3) calibration linearity (r^2), detection limit, and quantitation limit were >0.997, 1ng/mL and 5ng/mL for all analytes, respectively; and, (4) matrix effects: ion suppression was lower than 20% for all analytes; it was compensated by using deuterated internal standard. Compared with traditional GC/MS methods, the conclusion arose that this relatively simple protocol can be used for routine and reliable identification and quantitation of AM, MA, MDA, MDMA, MOR, COD, 6-AM, 6-AC, K, and NK in urine. This method was successfully applied to the analysis of postmortem and antemortem specimens from forensic cases.

Drugs of Abuse, Urine, LC/MS/MS