K10 Detection of Benzoylecgonine in Urine Using the V-Flex System

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The goal of this presentation is to provide results of a validation study regarding the analysis of benzoylecgonine in urine using an automated solid-phase extraction system (V-Flex).

This presentation will impact the forensic community and/or humanity by evaluating an automated solid-phase extraction system, which provides rapid throughput for an increased sample load with minimal manual labor.

Solid-phase extraction (SPE) is a widely accepted isolation technique utilized for the analysis of drugs and drug metabolites in urine. BioIntegrated Solutions (Palatine, IL) is currently developing the V-Flex, an automated SPE system. This study evaluates the use of this system for the analysis of benzoylecgonine (cocaine metabolite) in urine.

Benzoylecgonine and its deuterated analog (internal standard), $d_3$benzoylecgonine (Cerilliant Corporation, Round Rock, TX), were extracted from urine utilizing the V-Flex automated solid-phase extraction system. Prior to extraction, urine specimens were diluted in 0.1 M phosphate buffer (pH 6.0), alkalinized with 1 N NaOH, centrifuged, transferred to a clean glass culture tube, and submitted to the V-Flex system. With minimal manual intervention, the automated system conditioned the SPE copolymeric bonded phase cartridges (United Chemical Technologies, Inc., Bristol, PA), transferred specimens, performed washes, and eluted the desired compounds with ethyl acetate/methanol/ammonium hydroxide (68/28/4) elution solvent. The extracts were dried under a stream of nitrogen at 50°C, derivatized with N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSFTA), and analyzed with an Agilent 5890 Series II Gas Chromatograph (GC) system equipped with a 5972 Series Mass Selective Detector (MSD) (Little Falls, DE). The GC was fitted with a Restek Rtx-5 capillary column (30 m x 0.25 mm x 0.10 µm) (Bellefonte, PA) with ultra-high-purity helium as the carrier gas at a constant flow rate of 1.0 mL/min. Automated injections were made in splitless mode. The mass spectra were obtained in selected ion monitoring mode by monitoring *m/z* 282.2, 346.2, and 403.2 for benzoylecgonine and *m/z* 349.2 and 406.2 for deuterated benzoylecgonine.

The automated SPE protocol was compared to a manual SPE method employed in the laboratory. Minor differences in the manual method include solvent volumes, an additional wash step with acetonitrile, and the elution solvent utilized was methylene chloride/isopropanol/ammonium hydroxide (78/20/2). Finally, the manual method employed a five-point calibration curve.

Validation studies utilizing one-point calibration at 150 ng/mL and control concentrations of 120, 180, and 500 ng/mL demonstrated intraassay and inter-assay % CV values that were less than 3%, and intra-assay and inter-assay % accuracy values within 11%. The range of linearity was 75-750 ng/mL. Analysis of authentic urine specimens by the automated SPE and manual SPE operating procedures produced excellent correlation. Initial studies have demonstrated a correlation coefficient of 0.95.

In conclusion, automated SPE using the V-Flex system is an efficient method for the analysis of benzoylecgonine in urine.

Benzoylecgonine, Automated Solid-Phase Extraction, GC-MS Analysis

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