Detection of Benzoylecgonine in Urine using the V-Flex System

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INTRODUCTION

Solid-phase extraction (SPE) is a widely accepted isolation and purification technique in the field of forensic toxicology for the analysis of drugs and drug metabolites in blood and urine. BioIntegrated Solutions, Inc. (Palatine, IL) is currently developing the V-Flex, an automated SPE system (**see Figure 1**). The vertical system allows SPE in microplates and columns with the ability to provide complete automation of processing for 2 microplates or 96 SPE columns. Other capabilities of the system include: (1) solvent compatibility with organic/inorganic solutions and bulk dispensing capability (8 channels); (2) closed system with venting for harmful vapors of solvents; and (3) user-friendly software to enable rapid development of analytical protocols.

This study evaluated the V-Flex automated SPE system for the analysis of benzoylecgonine (cocaine metabolite) in urine.

Figure 1: The V-Flex, a vertical automated solid-phase extraction system.

MATERIALS AND METHODS

Benzoylecgonine and its deuterated analog 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 clean glass culture tubes, 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 gentle stream of nitrogen at 50°C, derivatized with N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA), and analyzed by gas chromatography-mass spectrometry (GC-MS).

An Agilent (Wilmington, DE) 5890 Series II Gas Chromatographic system (GC) equipped with a 5972 Series Mass Selective Detector (MS) was utilized. The GC was fitted with a deactivated cross-linked methyl siloxane capillary column (Rtx-5; 30 m x 0.25 mm x 0.10 μm) from Restek (Bellefonte, PA) with ultra-high-purity helium as the carrier gas (constant flow rate, 1.0 mL/min). Automated injections were made in splitless mode. The GC parameters were as follows: injection port, 250°C; detector, 280°C; initial column temperature, 150°C; hold time, 0.50 min; temperature ramp, 25°C/min to 320°C and held for 3.0 min. The total run time was 10.3 min. The mass spectra were obtained in Selected Ion Monitoring (SIM) 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 conventional SPE method employed in our laboratory. Minor differences in the conventional 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 conventional method employed a five-point calibration curve with a linear range of 50-1000 ng/mL.

RESULTS

Validation studies utilizing one-point calibration at 150 ng/mL and control concentrations of 120, 180 and 500 ng/mL demonstrated intra-assay and inter-assay % CV values that were less than 3%, and intra-assay and inter-assay % accuracy values within 11% (**see Table 1**). The range of linearity was 75-750 ng/mL, which was determined by analyzing calibrators within the given range.

Analysis of authentic urine specimens by the automated SPE and conventional SPE operating procedures produced excellent correlation (**see Figure 2**). Only positive urine specimens were tested, but 3 of the 19 specimens were beyond the linear range of the calibration curve. The results of the other 16 specimens were highly correlated with an R^2 value of 0.99. However, 1 of the 16 quantitated below 150 ng/mL for the conventional SPE method, but quantitated above the cut-off by the V-Flex method.

	Conc. (ng/mL)	Day	N	Mean (ng/mL)	Precision (%CV)	Accuracy (%)
Intra-assay	120	1	6	125	2.76	104
		2	6	124	0.81	103
		3	6	126	1.70	105
	180	1	6	187	1.05	104
		2	6	183	0.26	102
		3	6	185	2.04	103
	500	1	6	465	1.26	93.0
		2	6	448	0.88	89.6
		3	6	454	1.94	90.8
Inter-assay	120	–	18	125	1.86	104
	180	-	18	185	1.52	103
	500	–	18	456	2.06	91.2

Table 1: Validation data for the benzoylecgonine assay.

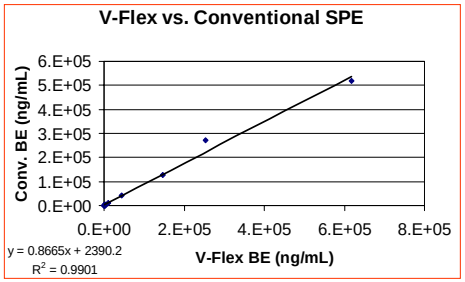

Figure 2: Comparison of results from authentic specimens from the V-Flex compared to a conventional SPE method.

CONCLUSION

Automated SPE using the V-Flex system is an efficient method for the analysis of benzoylecgonine in urine. This automation process decreases manual labor, which minimizes analyst exposure to hazardous materials such as solvents and biological specimens. It has multi-level vacuum control capability with the ability to control the pressure in real-time down to 0.1 mm Hg. This helps to ensure similar conditions for a range of batch sizes (1-96 cartridges). The instrument utilizes disposable tips for sample transfer, which prevents cross-contamination and carryover.

The V-Flex system has the potential to increase laboratory productivity, especially with a large batch size. It was designed with a sophisticated liquid handling system for bulk dispensing, solvent compatibility, real-time vacuum control and integrated gripper to enable unattended operations. The ability to perform multiple elutions with the integrated gripper is unique to the V-Flex instrument for multi-analyte analysis from the same specimen.

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