



# UNIVERSITY OF FLORIDA

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Arnab Mallik, Ph.D.  
BioIntegrated Solutions, Inc.  
1299 North Arbor Lane  
Palatine, IL 60067

Dear Dr. Mallik

Thank you for the opportunity to evaluate the BIS V Flex system. I've summarized the findings of our studies below.

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## Introduction:

Solid phase extraction (SPE) is a widely employed isolation/purification technique in the field of forensic toxicology for the analysis of drugs and drug metabolites in blood and urine. BioIntegrated Solutions, Inc. is currently developing the V Flex, an automated SPE system. The vertical system allows SPE in both microplates and columns with the ability to provide complete automation to process 2 microplates or 96 1/3 cc columns. Other capabilities of the system include: (1) the solvent compatibility for organic/inorganic solutions with 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 protocols.

This study evaluated the use of the automated system for the analysis of alprazolam (benzodiazepine derivative) in blood, as well as benzoylecgonine (cocaine metabolite) and  $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid (THC-acid) in urine.

## Materials and Methods:

Alprazolam, benzoylecgonine and THC-acid and their deuterated analogs were extracted utilizing the V-Flex automated solid-phase extraction system. Prior to automated SPE extraction, the specimens were aliquoted, pretreated and placed onto 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. The extracts were manually processed further before analysis by an Agilent Gas Chromatograph (GC) system equipped with a Mass Selective Detector (MSD) (Little Falls, DE).

To validate the method, control samples of known concentration were also assayed by the automated V Flex method. In addition, the V Flex protocol was compared with a manual SPE method employed in our laboratory by analyzing authentic (biological) specimens.

- Alprazolam: Ten authentic blood specimens were assayed by manual and automated methods.
- Benzoyllecgonine: Nineteen authentic urine specimens were assayed by manual and automated methods.
- THC-acid: To date, only method development has been completed. Further validation needs to be conducted.

### **Results:**

- Alprazolam: A total of ten blood specimens were tested; two specimens were negative and eight were positive. Of the eight positive specimens, one was beyond the linear range of the calibration curve and another was an outlier. At this point, it is unknown if the error occurred in the manual extraction or V-Flex system. The results of the 6 specimens were highly correlated with an  $r^2$  value of 0.96.
- Benzoyllecgonine: 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. The benzoyllecgonine validation study using the V Flex system has been submitted to the AAFS for presentation at the upcoming 2005 annual meeting.
- THC-acid: A method for the extraction of THC-acid in urine has been developed.

### **Conclusions:**


The V Flex system is effective for the analysis of alprazolam in blood and benzoyllecgonine in urine. This automation process decreases manual labor, which minimizes analyst exposure to hazardous materials such as solvents and biological specimens. The ability to control vacuum in real-time without overshoot, down to 0.1 mm Hg, enables detection of low analyte concentration. The PID controlled vacuum loop ensures identical conditions for sample size of 1-96 columns. The user-friendly software interface allows rapid development of new protocols from scratch, with the multi-level vacuum control capability enabling high level of repeatability.

This system has the potential to increase laboratory productivity, especially with increased batch size, since the V Flex was designed specifically for SPE applications with the flexibility of a sophisticated liquid handling system with 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.

The ability to provide walk-away automation with a high level of repeatability, along with the ability to use both columns and microplates (24/96/384), makes the V Flex system an ideal tool for many toxicologists. The instrument has the capacity to process multiple specimens using disposable tips for sample transfer preventing cross-contamination and carryover. The real-time capability of vacuum control ensures high degree of repeatability as with current manual procedures.

If you have any questions, I can be reached at (352) 265-0680 ext72001.

Sincerely,

  
Bruce A. Goldberger, Ph.D., DABFT  
Professor and Director of Toxicology