

Toxicology Section – 2010

K26 Sample Collection Tips for Automated and Comprehensive Drug Analysis in Biological Specimens Using LC/MS/MS

William E. Brewer, PhD*, University of South Carolina, Department of Chemistry and Biochemistry, 631 Sumter Street, Columbia, SC 29208; and Fred D. Foster, BS, GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090

After attending this presentation, attendees will learn how to automate sample preparation for biological specimens using minimal manual labor. Most importantly, the method is comprehensive for basic, acidic, and neutral drugs.

This presentation will impact the forensic science community by demonstrating how this automated method permits the possibility of forensic labs to improve chain-of-custody of samples, and increase confidence of results due to automation and tandem mass spectrometry.

The SC-Tips for biological fluids are pipette tips that contain a screen, an absorbent material, and a cap. The screen is used to contain the absorbent material and to filter subsequent solutions during automated extractions. The absorbent material serves two major purposes, to absorb and to remove sample matrix components including salts and proteins. The cap comprises a thin membrane to contain the biological samples, has grooves for robotic transportation, and an entry for a syringe needle. A multi-purpose sampler (MPS) is used to process extractions directly from the SC-Tips into LC vials and perform automated injections into the LC/MS/MS instrumentation.

The automated liquid extraction process takes approximately 1 minute to perform. By using an automated dry down station, the total extraction time is less than 6 minutes, which is less than the chromatographic analysis time. Therefore, the extraction of one sample is being performed during the chromatographic analysis of the previous sample, so high throughput is achieved one sample at a time.

For analysis of urine specimens, the samples are first pre-treated with enzymatic hydrolysis. Afterwards, 0.5 mL of acetonitrile (with spiked internal standards) and 0.26 mL of the hydrolyzed urine solution (0.2 mL equivalent of urine) are transferred to the top of the SC-Tip, and then the cap is added to close the tip. The tip is then placed on the sample tray of the MPS for robotic liquid handling. There is no other manual labor for this sample preparation. Oral fluid is prepared similarly to urine except hydrolysis pretreatment is not required.

For blood specimens, 0.2 mL is transferred onto the absorbent material of the SC-Tip, then 0.5 mL of acetonitrile with spiked internal standard is dispensed into the SC-Tip either manually (before adding the cap) or robotically (after adding the cap). The use of the absorbent material and acetonitrile combine to precipitate proteins, remove salts, and provide a clean extract with reduced ion suppression.

High recoveries are shown for the analysis of opiates and opioids, benzodiazepines, barbiturates, stimulants (amphetamines and cocaine), analgesics (propoxyphene, tramadol), hallucinogens (PCP, THC), and muscle relaxants (carisoprodol, meprobamate). The comprehensive nature of this extraction is exemplified by simultaneous extraction of over 40 pain management drugs in a single specimen.

Duplicate or triplicate analyses of specimens can be readily performed by using 2 to 3 SC-Tips per sample without significantly

subsequently elution time can be used to determine the identity of the increasing labor, providing better quality and confidence of results. This may be relevant to forensic toxicological specimens, where the analytical quantitative results include the error associated with the analysis. Furthermore, the use of bar code labels on the SC-Tips ensures sample integrity and minimizes possible mishandling errors and chain-of- custody issues.

Recoveries and %RSDs for over 60 drugs are shown, with most recoveries and %RSDs being greater than 70% and less than 10%, respectively.

Sample Preparation, LC/MS/MS, Automation