Automated Dispersive Pipette Extraction for Sensitive Analysis of Alcohol Metabolites in Common Forensic Matrices

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HIGHLIGHTS: Automated, fast (10 min), sensitive

SAX - XTR

INTRODUCTION

Various extraction methods for alcohol metabolites in urine have been published, including the use of anion exchange in traditional solid phase extraction. This application demonstrates an automated, fast, and highly sensitive extraction method for alcohol metabolites, ethyl sulfate (EtS) and ethyl glucuronide (EtG), in urine and oral fluid. The method uses only 50 µL of matrix for minimal sample volume, a four-fold total dilution factor, and extracts 96 samples in 10 minutes.

MATERIALS AND METHODS

The method was performed on a MICROLAB® NIMBUS96 automated liquid handler (ALH) from Hamilton Robotics with XTR tips containing strong anion exchange (SAX) sorbent from DPX Technologies (Columbia, SC). The sample is composed of 50 µL of matrix fortified at various concentrations, 10 µL of internal standard (Cerilliant, Round Rock, TX), and 450 µL of acetonitrile. Well plates for conditioning, wash, and elution steps were pre-aliquoted for the extraction. Internal standards used were ethyl-D5 sulfate sodium and ethyl-D5-ß-D-glucuronide. Quantisal was used for oral fluid calibrators and QC's, while donor human urine was used for urine calibrators. Quality control samples were obtained from various sources. Oral fluid quality control samples were made in-house by a separate scientist. The high-level urine quality control was obtained from UTAK (Valencia, CA) while the two other quality control standards were diluted from the high level with blank human urine to two additional quality control levels. The protocol followed bind, wash, and elute steps as shown in **Figure 1** and described in **Table 1**.

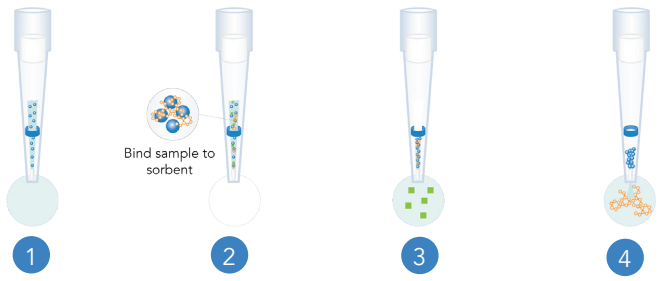


Figure 1. *Extraction of EtS and EtG using the method outlined in **Table 1**.*

Table 1. *Dispersive automated extraction method for EtS and EtG from urine and oral fluid.*

1 CONDITION	Condition XTR tips by aspirating and dispensing 750 µL of 50/50 methanol/acetonitrile to prepare resin for binding.
2 BIND	XTR tips aspirate and dispense 500 µL sample solution 5x to maximize binding.
3 WASH	Aspirate and dispense wash solution, 750 µL of 50/50 methanol/acetonitrile 2x, to remove matrix interferents.
4 ELUTE	Elute analytes of interest by aspirating and dispensing 500 µL of 2% hydrochloric acid in acetonitrile 5x.

Analysis was performed on a Shimadzu LC40 paired with a SCIEX 6500+ mass spectrometer. The analytical LC column was a Restek Ultra Biphenyl, 3 µm, 100 x 2.1 mm HPLC Column (PN 9109312). The choice of this biphenyl column allows for optimal separation of EtS and EtG. Injection volume in this case was 5 µL, and mobile phases were 0.1% formic acid in water and 100% acetonitrile. This method was evaluated for linear dynamic range, extraction recovery, matrix effects, and limits of detection and quantification. Linearity was assessed by analyzing oral fluid and urine at seven concentration points ranging from 25-1500 ng/mL of both EtS and EtG, covering common cutoffs associated with both matrices.

RESULTS

Linear correlation coefficients for EtS and EtG were 0.99 and above in urine and oral fluid. In comparison to a centrifugation method with the same dilution factor, DPX XTR tips provided almost identical sensitivity of the analytes of interest in urine, further exhibiting the benefits of a DPX SPE method for this application. The common contaminant associated with EtS is removed by greater than 70%, allowing for seamless integration of the two normally coeluting peaks and increased sensitivity in the quantification and qualification ions of EtS. The relevant cutoff values for EtS (25-100 ng/mL) and EtG (100-500 ng/mL) in urine and oral fluid are achieved with signal-to-noise above 10, further demonstrating the removal of matrix interferents.

Table 2. *Oral fluid and urine accuracy and precision studies performed over 3 days with QC values covering common cutoffs. In oral fluid, EtS and EtG values were the same: QC 1 = 30 ng/mL EtS and EtG; QC 2 = 90 ng/mL EtS and EtG. For EtS in urine: QC 1 = 205 ng/mL, QC 2 = 410 ng/mL, and QC 3 = 820 ng/mL. For EtG in urine: QC 1 = 400 ng/mL, QC 2 = 800 ng/mL, and QC 3 = 1600 ng/mL.*

ORAL FLUID			
EtS	Intra-day Precision (%)	Inter-day Precision (%)	Accuracy (%)
QC 1 (30 ng/mL)	10.5	3.3	109.5
QC 2 (90 ng/mL)	6.7	6.2	110.9
EtG	Intra-day Precision (%)	Inter-day Precision (%)	Accuracy (%)
QC 1 (30 ng/mL)	9.8	8.0	105.7
QC 2 (90 ng/mL)	9.1	5.0	106.8
URINE			
EtS	Intra-day Precision (%)	Inter-day Precision (%)	Accuracy (%)
QC 1 (205 ng/mL)	5.8	3.9	102.1
QC 2 (410 ng/mL)	7.7	2.4	102.8
QC 3 (820 ng/mL)	2.2	1.1	106.2
EtG	Intra-day Precision (%)	Inter-day Precision (%)	Accuracy (%)
QC 1 (400 ng/mL)	4.7	5.9	91.1
QC 2 (800 ng/mL)	5.7	3.7	92.8
QC 3 (1600 ng/mL)	4.2	8.0	96.8

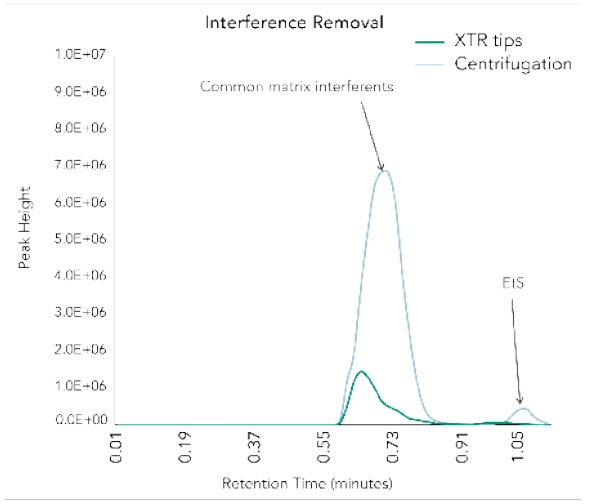


Figure 2. *Chromatography of EtS and its common interferent in urine shown comparing a centrifugation method (light blue) against the fully-automated dispersive pipette extraction method using XTR tips (green). More than 70% of interferent is gone with the use of XTR tips.*

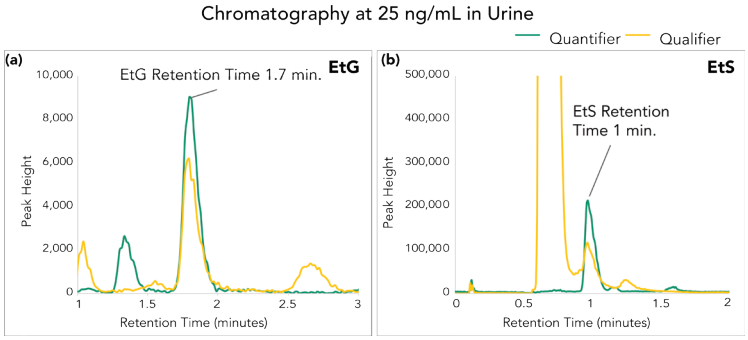


Figure 3. *The chromatography of EtG (a) and EtS (b) in urine at 25 ng/mL. This displays the sensitivity and reduced baseline produced by using this cleanup method with XTR tips.*

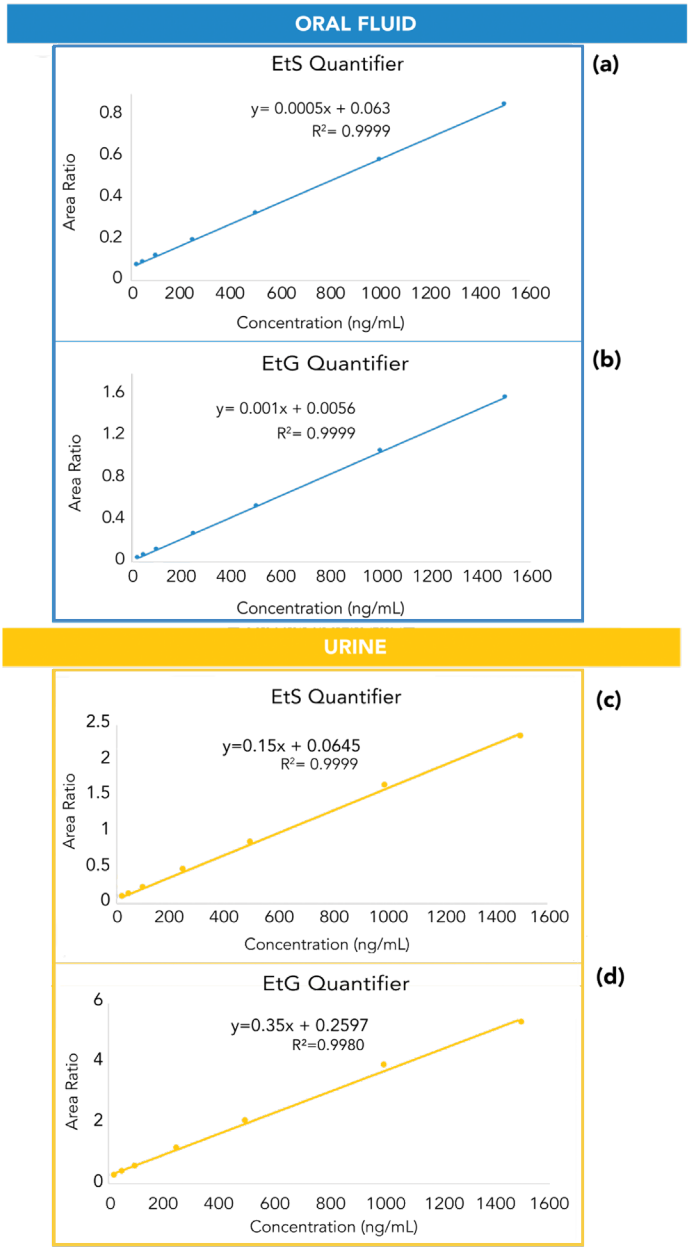


Figure 4. *The linearity of EtS and EtG in oral fluid (a and b) and urine (c and d) at 7 calibration points. Linear dynamic range spanned from 25 ng/mL to 1500 ng/mL for both analytes in both matrices in triplicate across 3-days.*

DISCUSSION

The use of XTR tips with SAX sorbent for the analysis of alcohol metabolites in urine and oral fluid is highly reproducible, sensitive, and aids in instrument cleanliness by removing matrix that can damage analytical systems. This method removes common interferents associated with alcohol metabolites in urine and oral fluid by more than 70% with a four-fold dilution factor and allows for easy downstream analysis.