Semi-automated Protein Precipitation Method Using Tip-on-Tip Technology and the Integra ASSIST PLUS for Analysis of Benzodiazepines in Biological Fluids

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HIGHLIGHTS: Replaces manual vortex and centrifugation | WAX - XTR | ToT

INTRODUCTION AND OBJECTIVE

- Introduce innovative, automated INTip™ solution as an alternative to centrifugation utilizing a patent-pending Tip-on-Tip (ToT) technology
- Low Porosity Filtration Tips and an Integra ASSIST PLUS minimize laborious manual steps such as vortex mixing and centrifugation
- Extend the use of the Integra for the extraction method that Orange County Crime Lab (OCCL) uses to analyze benzodiazepines. The extraction enables the OCCL to quantitate 23 benzodiazepines, z-drugs and antihistamines, and qualitatively detect 7 metabolites in urine, blood and tissue specimens.

METHODS

Table 1. Current manual protein precipitation procedure using vortex and centrifugation

Protein Precipitation	• Manually pipette 250 µL sample, 50 µL internal standards, 750 µL acetonitrile (ACN) • Vortex and Centrifuge • Manually decant supernatant to new test tube
Sample Cleanup	• Aspirate/dispense supernatant 3x using WAX-XTR tips
Prep for injection	• Manually transfer 50 µL of eluant, add 800 µL initial mobile phase into LC vials

Table 2. Tip-on-Tip protein precipitation procedure and sample cleanup using the Integra ASSIST PLUS

Protein Precipitation	• Manually pipette 250 µL sample/ standard in test tube. Add 50 µL of internal standard • Add 750 µL of cold ACN • Mix sample and ACN using wide bore tips - crash proteins • Attach wide bore tips to Low Porosity Filtration tips and dispense supernatant into new test tubes
Sample Cleanup	• Aspirate/dispense supernatant 3x using WAX-XTR tips
Prep for injection	• Manually transfer 50 µL of eluant, add 800 µL initial mobile phase into LC vials

WORKFLOW USING INTEGRA ASSIST PLUS

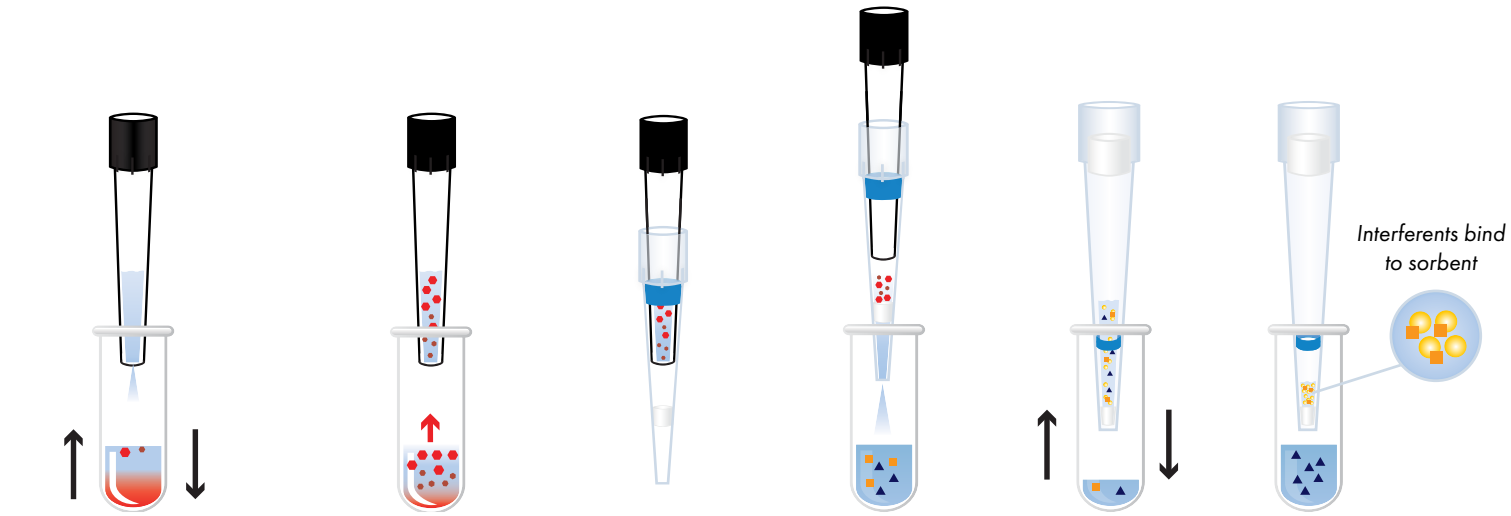

Figure 1. Schematic represents workflow for ToT Filtration used for protein precipitation and WAX-XTR cleanup method.

RESULTS

- **Casework** - Percent differences of DPX Tip-on-Tip method versus manual method for AM/PM blood, urine, liver, brain and gastric casework ranged from -15.5% to 15.4%.
- **Reproducibility** - Concentrated liver homogenate had within-run precision of 2.5% and 7.9% for alprazolam and hydroxyzine, respectively.
- **Linearity** - R^2 was greater than 0.99 for all drugs.
- **Accuracy**- As shown in figure 2, QC's of porcine blood were extracted with the manual and automated DPX extraction methods. Percent differences ranged from -5 to 13%

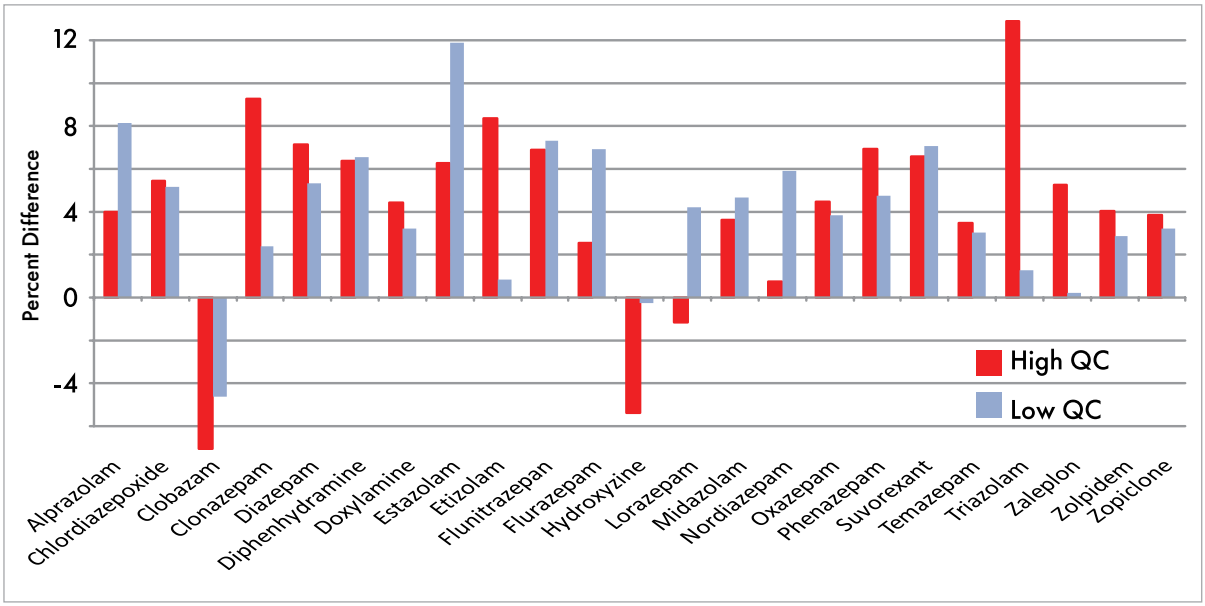

Figure 2. Percent Difference for the High and Low QC between the Manual Extraction and Automated Extraction. QC's are made in porcine blood.

CONCLUSIONS

These initial investigative results demonstrate that protein precipitation can be automated for a SWGTOX validated forensic application of blood, urine and tissues by LC-MS/MS. Adaptation of an automated crash method for forensic laboratories offers several advantages including reducing the hands-on time requirement for the analyst, minimizing potential human errors, and further negating the long-term health effects of repeated pipetting.