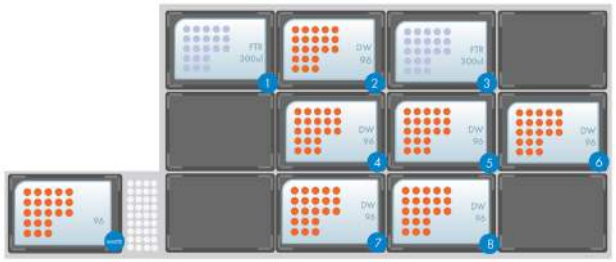
APPLICATION NOTE

# A sensitive non-evaporative extraction of drugs of abuse in urine with HLB dispersive pipette extraction.

## INTRODUCTION

This new approach to the traditional solid phase extraction of therapeutic and abused drugs cuts down on sample volume, standard costs, and time. A XTR tip with 3 mg of Supel™ Swift HLB is utilized to extract 39 compounds across a large range of polarities. A non-evaporative and highly sensitive extraction method for drug monitoring in urine is presented herein. The method provides an overall 10x dilution factor. A small urine volume of 50 µL minimizes the usage of beta-glucuronidase and internal standard. 96 samples are extracted in 15 minutes.



| Location | Description |
|---|---|
| 1 | Transfer tips |
| 2 | ACN Reservoir |
| 3 | µXTR HLB DPX Tips |
| 4 | MeOH Reservoir |
| 5 | Water Reservoir |
| 6 | Sample Plate |
| 7 | Elution Plate |
| 8 | Wash Plate |

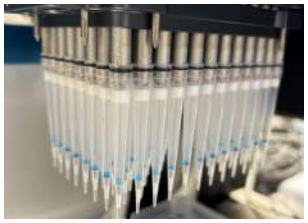


**Figure 1.** *The deck layout used for the Hamilton NIMBUS 96 automated liquid handler (ALH) and a photograph of 96 HLB DPX Tips.*

## MATERIAL AND METHODS

The DPX tips contain 3 mg of Supel™ Swift HLB sorbent (**Figure 2**) (MilliporeSigma (Burlington, MA)) and the automated liquid handling (ALH) platform is a Hamilton MICROLAB® NIMBUS96 (Figure 1). The method does not require solvent evaporation unlike the majority of SPE methods currently used. The sample is composed of 50 µL of urine fortified at various concentrations, 20 µL of B-One (Kura Biotech (Chile)), 100 µL of water, and 30 µL of internal standard (Cerilliant (Round Rock, TX)). Well plates for conditioning, wash, and elution steps are pre-aliquoted. The DPX method begins with a two-step condition -the ALH picks up the DPX tips and aspirates/ dispenses 250 µL of 100% methanol and subsequently 250 µL of 100% water. The DPX tips are moved to the sample plate for binding by aspirating/dispensing the sample six times. The DPX tips are moved to aspirate/dispense 250 µL of water twice to wash off interferents. The DPX tips are eluted by aspirating/dispensing 100 µL of 50/50 methanol/acetonitrile five times.

## ANALYSIS

Analysis is performed on a Shimadzu LC40 paired with a SCIEX 6500+ mass spectrometer. The analytical LC column is a Restek Force Biphenyl, 3 µm, 50 x 2.1 mm LC Column (PN 9629352) paired with a Restek Force Biphenyl, 5 x 2.1 mm EXP Guard Cartridge (PN 962950252). Injection volume is 5 µL, and mobile phases are 0.1% formic acid in water and 100% methanol. This method is evaluated for linear dynamic range, extraction recovery, matrix effects, and limits of detection and quantification (**Figure 3 & Table 1**).Linearity is assessed by analyzing urine samples at 8 concentration points ranging from 1-500 ng/mL with n=3 at each calibration point replicated on three different days (**Table 1**).

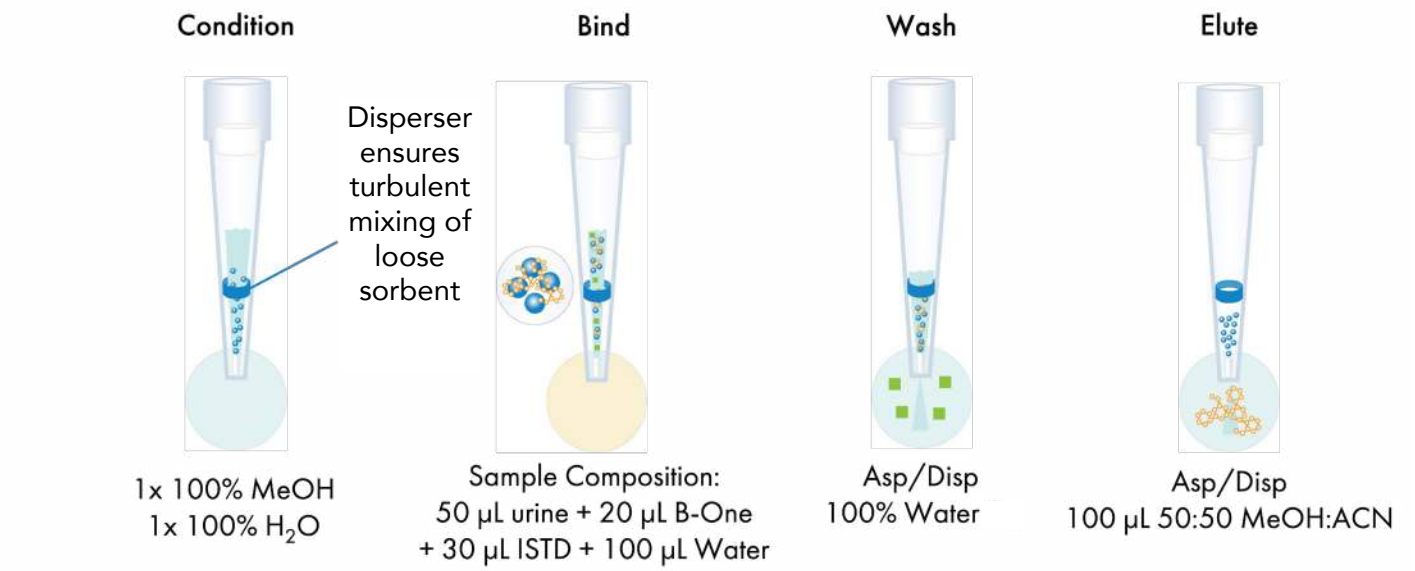



**Figure 2**. *An illustration of the DPX protocol described. The eluate is diluted with 400 µL of $H_2O$ and injected. This limits the percentage of organic to 20% in the final solution while also maintaining a minimal dilution factor.*

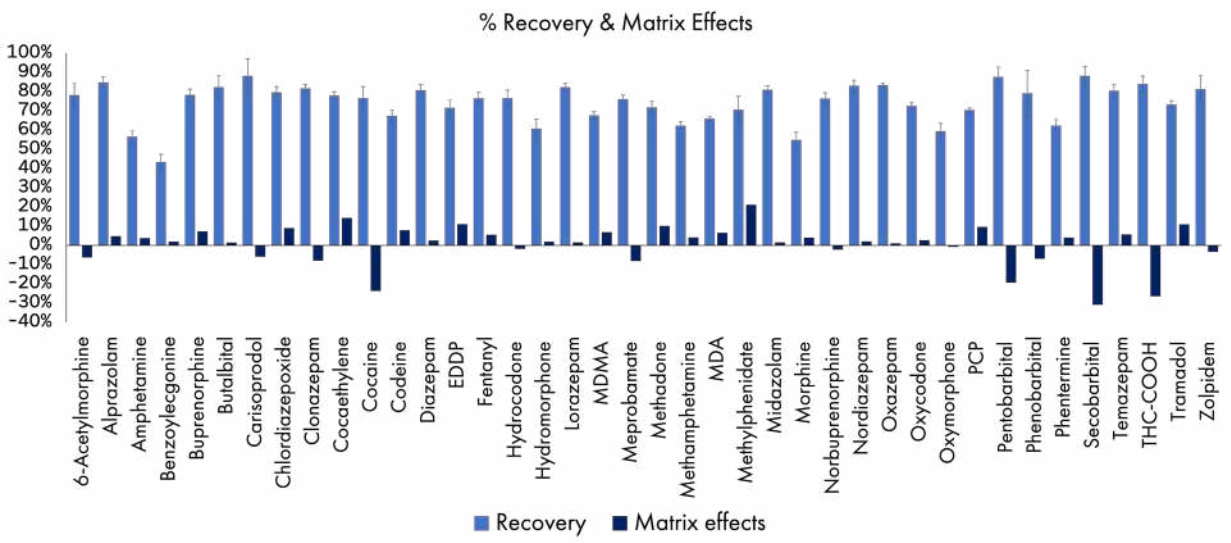



**Figure 3**. *A table displaying the recoveries and matrix effects (ion enhancement/suppression) for each analyte.*

## RESULTS AND DISCUSSION

**Table 1.** The correlation coefficient and precision values from a 3-day study involving triplicate calibration curves ranging from 1 ng/mL to 500 ng/mL and the limit of quantitation (LOQ) based on signal-to-noise (S/N) above 10.

| Analyte | LOQ (ng/mL) | $R^2$ | Precision (% RSD) QC 1 10 ng/mL | QC 2 50 ng/mL | QC 3 250 ng/mL |
|---|---|---|---|---|---|
| 6-Acetylmorphine | 5 | 0.999 | 8.5 | 6 | 1.9 |
| Alprazolam | 1 | 0.998 | 5.7 | 5.5 | 0.9 |
| Amphetamine | 1 | 0.999 | 4.1 | 2.8 | 13.8 |
| Benzoylecgonine | 1 | 0.993 | 8.9 | 14.3 | 12.9 |
| Buprenorphine | 1 | 0.998 | 10 | 6.1 | 5 |
| Butalbital | 5 | 0.999 | 8.7 | 4.3 | 3.1 |
| Carisoprodol | 1 | 0.996 | 9.9 | 8.5 | 6.3 |
| Chlordiazepoxide | 1 | 0.996 | 10.5 | 7.3 | 4.5 |
| Clonazepam | 1 | 0.999 | 2.2 | 1.7 | 3.2 |
| Cocaethylene | 1 | 0.997 | 2.9 | 3.9 | 2 |
| Cocaine | 1 | 0.999 | 14 | 3.4 | 4.3 |
| Codeine | 1 | 0.999 | 5 | 4.6 | 1.3 |
| Diazepam | 1 | 0.999 | 2.9 | 1.6 | 2 |
| EDDP | 1 | 1.00 | 1.5 | 2.2 | 1 |
| Fentanyl | 1 | 0.995 | 9 | 4.2 | 2.8 |
| Hydrocodone | 1 | 0.999 | 2.3 | 2.1 | 1.8 |
| Hydromorphone | 1 | 0.999 | 4.3 | 4.5 | 2.8 |
| Lorazepam | 1 | 0.999 | 2.6 | 1.4 | 2.9 |
| MDMA | 1 | 0.999 | 2 | 2.2 | 1.5 |
| Meprobamate | 1 | 0.999 | 1.8 | 2.3 | 0.8 |
| Methadone | 1 | 0.999 | 3.9 | 2.7 | 1.4 |
| Methamphetamine | 1 | 0.997 | 6.5 | 4.5 | 3.7 |
| MDA | 1 | 0.998 | 3.1 | 2 | 2.6 |
| Methylphenidate | 5 | 0.996 | 7.2 | 5.4 | 6.3 |
| Midazolam | 1 | 0.998 | 2.6 | 2.5 | 1.8 |
| Morphine | 1 | 1.00 | 5.3 | 2.7 | 1.8 |
| Norbuprenorphine | 1 | 0.999 | 3.6 | 2.9 | 2.5 |
| Nordiazepam | 1 | 0.999 | 2.2 | 0.9 | 3.4 |
| Oxazepam | 1 | 0.999 | 1.3 | 2.5 | 1.3 |
| Oxycodone | 1 | 0.999 | 3.1 | 3.1 | 2.4 |
| Oxymorphone | 1 | 0.998 | 2.9 | 2.4 | 2.1 |
| PCP | 1 | 1.00 | 2 | 1.5 | 1.5 |
| Pentobarbital | 5 | 0.998 | 10 | 5.1 | 3.5 |
| Phentermine | 1 | 0.998 | 6.5 | 3.6 | 2.6 |
| Secobarbital | 5 | 0.999 | 11.2 | 5 | 1.9 |
| Temazepam | 1 | 0.999 | 2 | 2.5 | 1.1 |
| THC-COOH | 1 | 0.997 | 1.2 | 0.9 | 1.5 |
| Tramadol | 1 | 0.999 | 1.6 | 1.7 | 0.9 |
| Zolpidem | 5 | 0.998 | 4.4 | 7.4 | 3.1 |

*a Analytes LDR ranges from 5 ng lml and $R^2$ follows the same Plot.*
*b Analytes LDR ranges from 10ng lml, and $R^2$ follows the same Plot.*

## CONCLUSION

A total of 39 compounds were analyzed. Linear correlation coefficients for all analytes ranged from 0.995-1.00. Percent recoveries at 15 ng/mL ranged from 70-100% for most analytes measured. Matrix effects were all between -31% to 21%; in this case, a negative value represents ion suppression while positive is ion enhancement. All analytes were quantified below the commonly used confirmation cutoff. HLB is a versatile and robust sorbent for extracting a large range of polarities. The use of DPX XTR HLB Tips for the analysis of drugs of abuse in urine is reproducible, sensitive, and fast. This method provides relevant LOQ's for a high throughput and fast turnaround laboratory. This method provides optimal efficiency for drug monitoring.