Comprehensive Screen of Acidic/Neutral/Basic Drugs from Urine and Plasma using Micro-Prep® HLB Extraction Plate & Analysis on LC-MS/MS

UCT Part Numbers

W96-XTMC-HLB
Micro-Prep®
2 mg, 96 Well Microelution Plate

SLPFPP50ID21-18UM
Selectra® PFPP UHPLC Column
50 X 2.1 mm, 1.8 µm

SLPFPPGDC20-18UMOPT
Selectra® PFPP Guard OT
5 X 2.1 mm, 1.8 µm

SLGRDHLDR-HPOPT
UHPLC Direct Connect Guard Cartridge Holder

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Summary:

Analytical Toxicology involves methods for comprehensive screening of biological matrices for the presence abused drugs. Routine analysis of samples in clinical and forensic settings demands quick and efficient extraction procedures. Smaller sorbent amounts utilized by Solid Phase Extraction (SPE) products allow scaling-down of starting sample size and minimize the total solvent volumes required to wash matrix components and elute the target analytes. 2 mg or less of sorbent particles embedded in a disc membrane allows for sample enrichment and high throughput processing. As compared to lose sorbent, disk format eliminates channeling effects and reduces dead volume. Removal of the evaporation step from the procedure also decreases overall turn-around time.

In this application note, a method for extracting a large drugs of abuse panel from urine and plasma using UCT's Micro-Prep® HLB microelution plate has been described. This plate consists of a highly retentive uncharged hydrophilic and lipophilic sorbent which can effectively retain a range of acids, neutrals and bases via reverse-phase. HPLC separation was carried out using UCT's Selectra® PFPP column prior to detection by LC-MS/MS. The pentafluorophenylpropyl phase can undergo dipole–dipole, and pi–pi interactions, imparting unique selectivity and retention mechanisms to the column that distinguish it from a traditional biphenyl phase. The total run time was 13 minutes at a 0.4 mL/min flow rate.

SPE Procedure:

1) **Sample Preparation**
   To 300 µL 100 mM pH 10.0 Sodium carbonate/bicarbonate buffer, add appropriate amount of internal standard
   Mix/Vortex briefly
   Add 300 µL sample (Urine/Plasma)
   Vortex & Centrifuge the samples for 10 minutes at 3000 rpm

2) **Condition (Optional)**
   1 x 100 µL CH_3OH
   1 x 100 µL 100 mM pH 10.0 Sodium carbonate/bicarbonate buffer

3) **Apply sample**
   Load 400 µL sample onto the microelution plate.

4) **Wash column**
   1 x 100 µL 5% CH_3OH
   Apply full pressure for 30 seconds

5) **Elute**
   1 x 50 µL 2% Formic acid in CH_3OH

6) **Post elution (Optional)**
   Evaporate & Reconstitute in mobile phase
   OR
   Add 50 µL DI H_2O

Notes:

A. Sample-to-buffer dilution ratio depends on two factors;

- State of the biological matrix – Dirtier samples may require greater dilution to avoid clogging of the disc.
- Amount of the organic solvent in the standard – Spiking small sample volumes with stock solution prepared in methanol could cause breakthrough of the analytes retained hydrophobically. Dilution reduces the strength of the organic solvent. While targeting the reverse phase interaction, the sample should be composed of no more than 10%-15% methanol.

B. Prior to adding matrix, spike the standards in the dilution buffer to avoid precipitation.

C. Loading volume: 100 µL to 400 µL

D. 50 µL H_2O (w/o 2% formic acid) is added to the elution solvent due to the following reasons;

- Prevent evaporation of the elution solvent and avert irregular increase in the concentration of the drugs.
- Adequate volume would be suitable for autosamplers with limited needle depth. Favorable for sample re-injection.
- H_2O consisting of Formic acid is used to neutralize NH_4OH.
- To match the mobile phase of the LC system.

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LC-MS/MS Parameters:

System: Shimadzu LC30AD w/ MS-8050
UHPLC Column: Selectra® PFPP (50 X 2.1 mm, 1.8 µm)
Guard Column: Selectra® PFPP (5 X 2.1 mm, 1.8 µm)
Column Temperature: 40°C
Column Flow Rate: 0.4 mL/min
Injection Volume: 5 µL
Auto-sampler temperature: 10°C

Gradient Program:

Time (min)	% Mobile Phase A 5 mM Amm. Formate + 0.1% Formic Acid in Water	% Mobile Phase B 5 mM Amm. Formate + 0.1% Formic Acid in Methanol
0	100	0
8	0	100
9	0	100
9.01	100	0
13.00	100	0

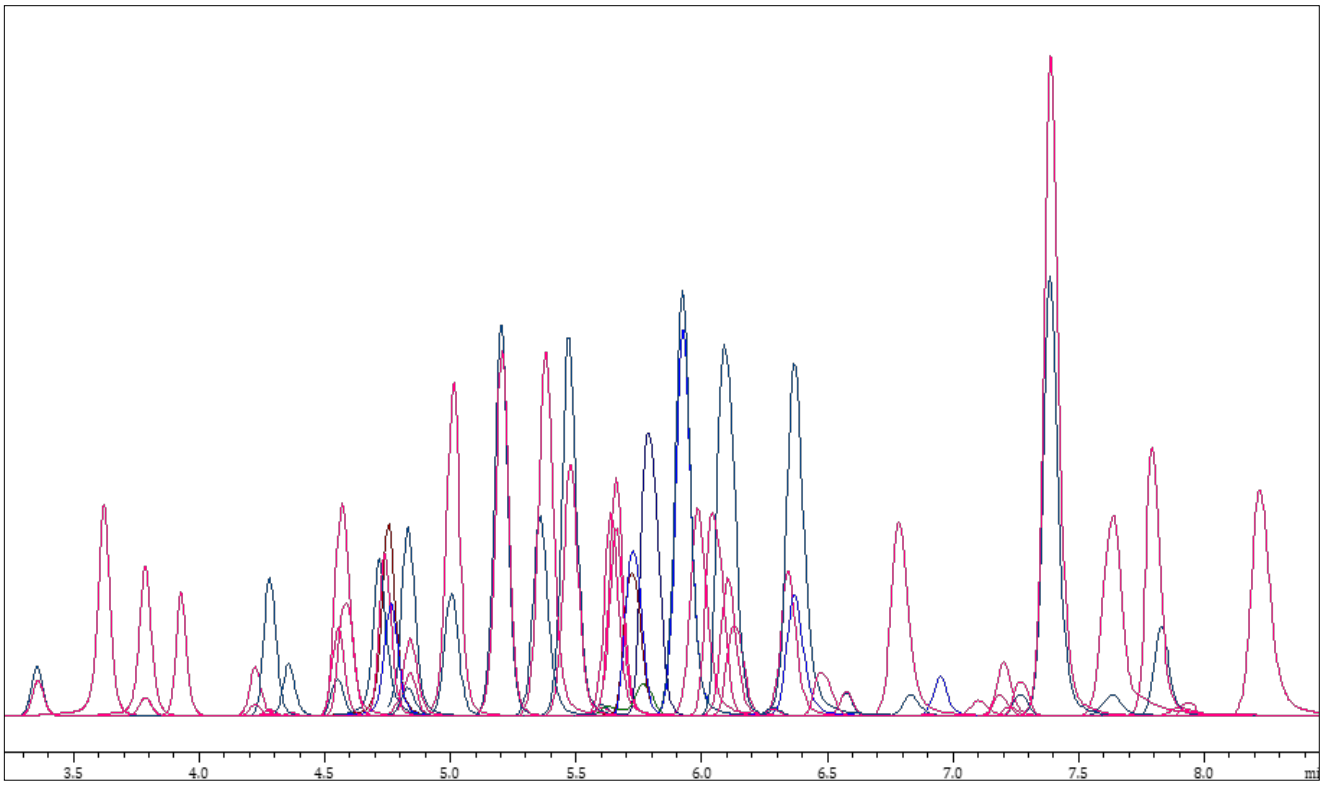

Figure 1: Chromatogram of 50 ng/mL Extracted QC sample

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Representative Calibration Curves:

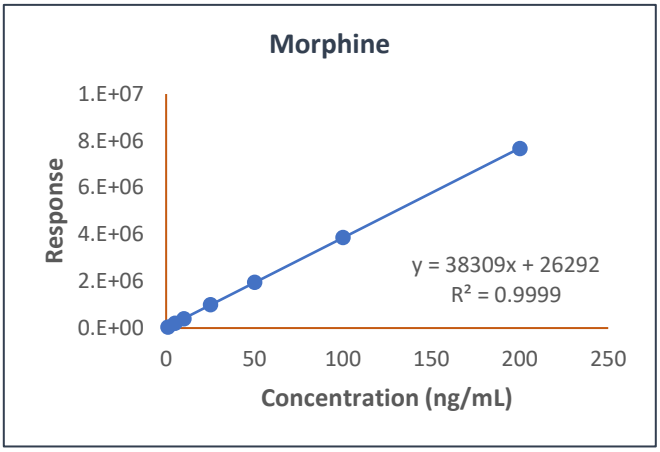
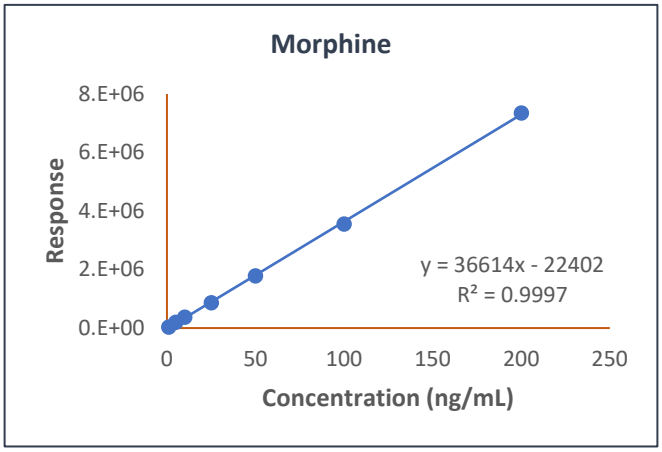
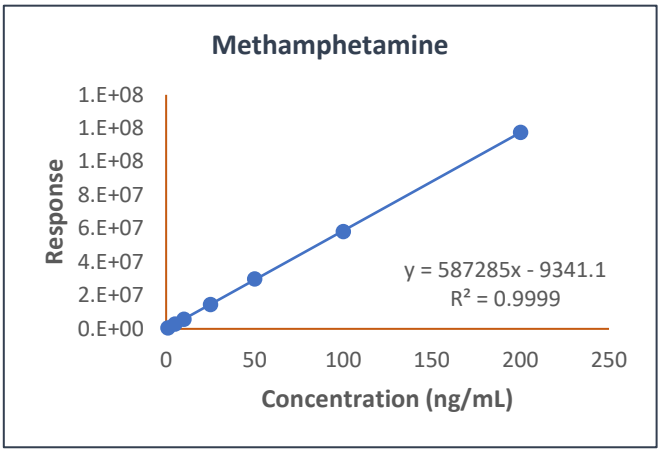
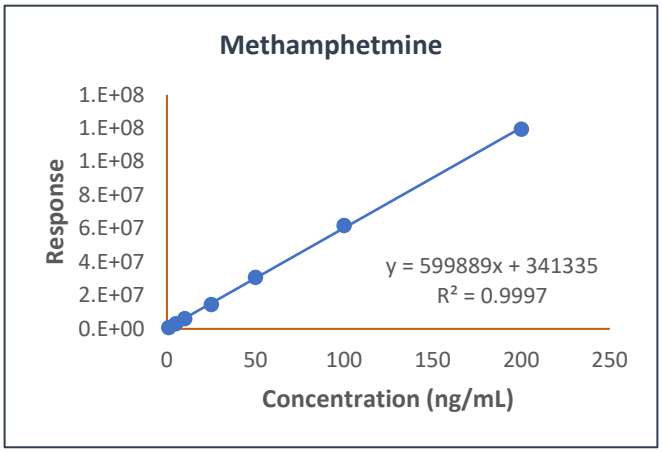
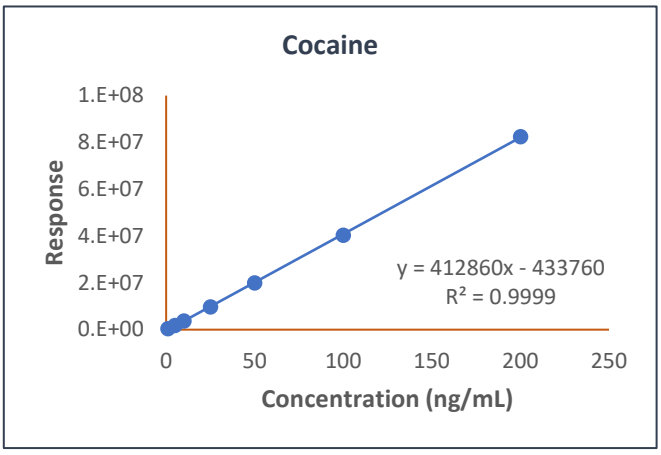
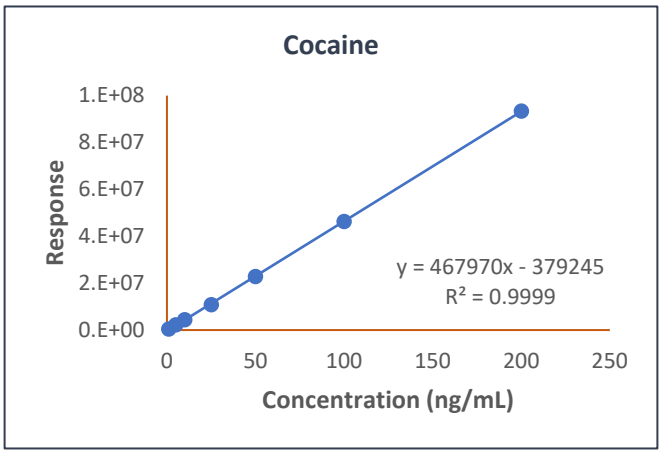
URINE	PLASMA
	
	
	

Figure 2: Calibration Curve Examples (5, 10, 25, 50, 100, 150 & 200 ng/mL)

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Results:

Urine extraction						
Analyte	5 ng/mL (n=5)			50 ng/mL (n=5)		
	Recovery	Rel. Std Dev	Matrix Effects	Recovery	Rel. Std Dev	Matrix Effects
EME	93%	2%	-3%	95%	2%	-1%
Morphine	102%	1%	2%	102%	2%	0%
Oxymorphone	100%	2%	0%	97%	2%	0%
Hydromorphone	99%	1%	-1%	98%	1%	1%
Atenolol	102%	3%	7%	100%	2%	2%
Meprobamate	98%	5%	-6%	102%	1%	-1%
Norcodeine	100%	5%	-2%	99%	2%	-1%
BE	111%	4%	-2%	102%	1%	-2%
Codeine	89%	6%	-8%	97%	3%	-3%
6-MAM	97%	3%	-2%	96%	1%	-2%
Naltrexone	96%	2%	-4%	100%	2%	0%
Oxycodone	101%	1%	-6%	99%	1%	-7%
Hydrocodone	95%	5%	0%	100%	1%	-3%
Amphetamine	88%	2%	-4%	86%	2%	-2%
Lorazepam	98%	5%	-19%	100%	3%	-12%
Alphahydroxy alprazolam	96%	6%	0%	88%	2%	0%
MDA	90%	2%	-5%	90%	2%	-3%
Oxazepam	97%	2%	-16%	98%	1%	-10%
Methamphetamine	98%	2%	-2%	99%	1%	-5%
MDMA	98%	4%	2%	101%	1%	0%
Levorphanol	101%	6%	4%	102%	2%	2%
Dextorphan	93%	7%	2%	99%	4%	2%
Temazepam	94%	2%	-5%	97%	1%	-2%
Clonazepam	92%	5%	-2%	95%	2%	3%
Alprazolam	102%	6%	23%	96%	3%	20%
Nordiazepam	87%	5%	9%	96%	1%	8%
Norketamine	100%	2%	2%	99%	1%	-2%
Phentermine	100%	2%	-1%	100%	1%	2%
MDEA	102%	3%	14%	96%	2%	3%
Ketamine	101%	3%	3%	101%	1%	1%
Tramadol	97%	2%	-1%	99%	1%	-2%
Normeperidine	74%	8%	1%	77%	1%	-1%
Methylphenidate	73%	10%	0%	81%	3%	1%
Meperidine	98%	3%	1%	99%	1%	-1%
Cocaine	81%	1%	-5%	88%	1%	-3%
COOH-THC	87%	7%	-24%	86%	3%	-6%
MDPV	92%	4%	1%	93%	1%	1%
Cocaethylene	82%	5%	-4%	90%	3%	-5%
Zolpidem	88%	5%	-3%	95%	2%	0%
Bupivacaine	89%	2%	-1%	94%	2%	-1%
Fentanyl	73%	2%	-4%	82%	2%	-3%
Nortriptyline	64%	7%	2%	80%	3%	-2%
PCP	81%	5%	-8%	90%	2%	-3%
Imipramine	59%	6%	-11%	75%	2%	-5%
Amitriptyline	52%	5%	-6%	68%	2%	-1%
EDDP	54%	3%	-16%	80%	3%	1%
Methadone	64%	3%	-8%	81%	2%	-1%

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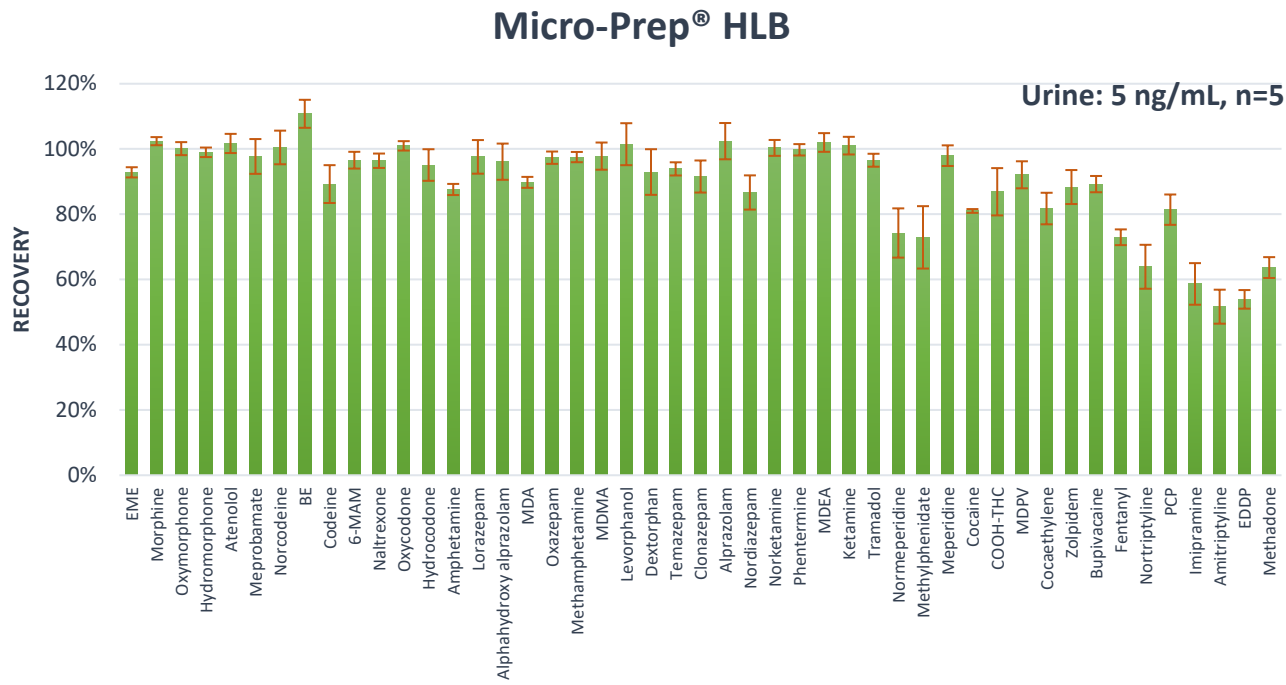

Figure 3: Recovery data of 5 ng/mL urine quality control replicates extracted with Micro-Prep® HLB

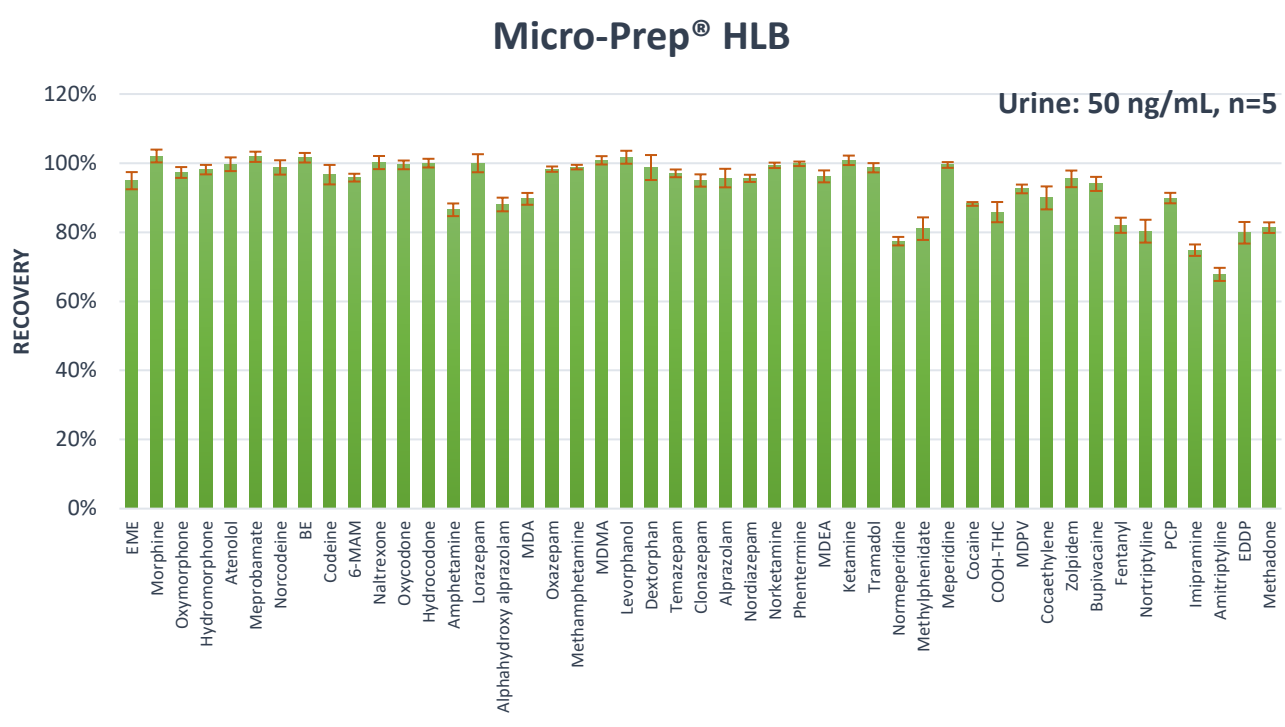

Figure 4: Recovery data of 50 ng/mL urine quality control replicates extracted with Micro-Prep® HLB

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Results:

Plasma extraction						
Analyte	5 ng/mL (n=5)			50 ng/mL (n=5)		
	Recovery	Rel. Std Dev	Matrix Effects	Recovery	Rel. Std Dev	Matrix Effects
EME	92%	6%	-1%	93%	2%	-5%
Morphine	95%	2%	-5%	101%	1%	0%
Oxymorphone	88%	3%	-6%	98%	1%	-1%
Hydromorphone	87%	2%	-9%	95%	1%	-3%
Atenolol	89%	4%	4%	99%	2%	4%
Meprobamate	93%	5%	-15%	103%	2%	-3%
Norcodeine	88%	3%	-6%	96%	2%	-3%
BE	88%	3%	-7%	98%	1%	-3%
Codeine	88%	2%	-37%	98%	3%	4%
6-MAM	83%	2%	-8%	90%	3%	-5%
Naltrexone	81%	3%	-8%	93%	3%	2%
Oxycodone	85%	4%	-2%	94%	1%	-2%
Hydrocodone	89%	3%	-6%	98%	2%	1%
Amphetamine	79%	1%	-26%	78%	2%	-5%
Lorazepam	88%	6%	-4%	98%	3%	-4%
Alphahydroxy alprazolam	90%	8%	-6%	91%	2%	0%
MDA	72%	3%	-6%	86%	2%	1%
Oxazepam	83%	4%	-7%	90%	2%	-5%
Methamphetamine	88%	1%	-12%	96%	2%	-3%
MDMA	90%	2%	-1%	97%	2%	-3%
Levorphanol	84%	3%	-9%	96%	1%	-2%
Dextorphan	87%	6%	1%	98%	2%	-2%
Temazepam	60%	6%	-10%	72%	3%	-3%
Clonazepam	81%	4%	0%	92%	2%	-1%
Alprazolam	88%	3%	-4%	90%	2%	-3%
Nordiazepam	79%	5%	0%	91%	2%	2%
Norketamine	95%	4%	2%	100%	2%	-2%
Phentermine	88%	2%	-7%	93%	1%	-5%
MDEA	90%	2%	1%	97%	1%	-1%
Ketamine	96%	3%	2%	99%	2%	2%
Tramadol	86%	2%	-9%	98%	2%	-3%
Normeperidine	77%	4%	-4%	83%	1%	-5%
Methylphenidate	85%	3%	-2%	82%	2%	0%
Meperidine	92%	4%	-1%	98%	2%	-3%
Cocaine	82%	3%	-14%	88%	1%	-1%
COOH-THC	78%	4%	-30%	78%	2%	-5%
MDPV	89%	4%	-2%	95%	1%	-1%
Cocaethylene	82%	4%	0%	90%	1%	1%
Zolpidem	96%	3%	5%	102%	2%	3%
Bupivacaine	69%	4%	8%	71%	1%	1%
Fentanyl	89%	1%	2%	96%	1%	3%
Nortriptyline	89%	3%	0%	96%	2%	1%
PCP	85%	4%	-4%	95%	1%	-2%
Imipramine	71%	2%	-10%	89%	1%	-1%
Amitriptyline	79%	2%	3%	90%	2%	2%
EDDP	79%	4%	3%	84%	3%	-1%
Methadone	82%	4%	47%	98%	2%	46%

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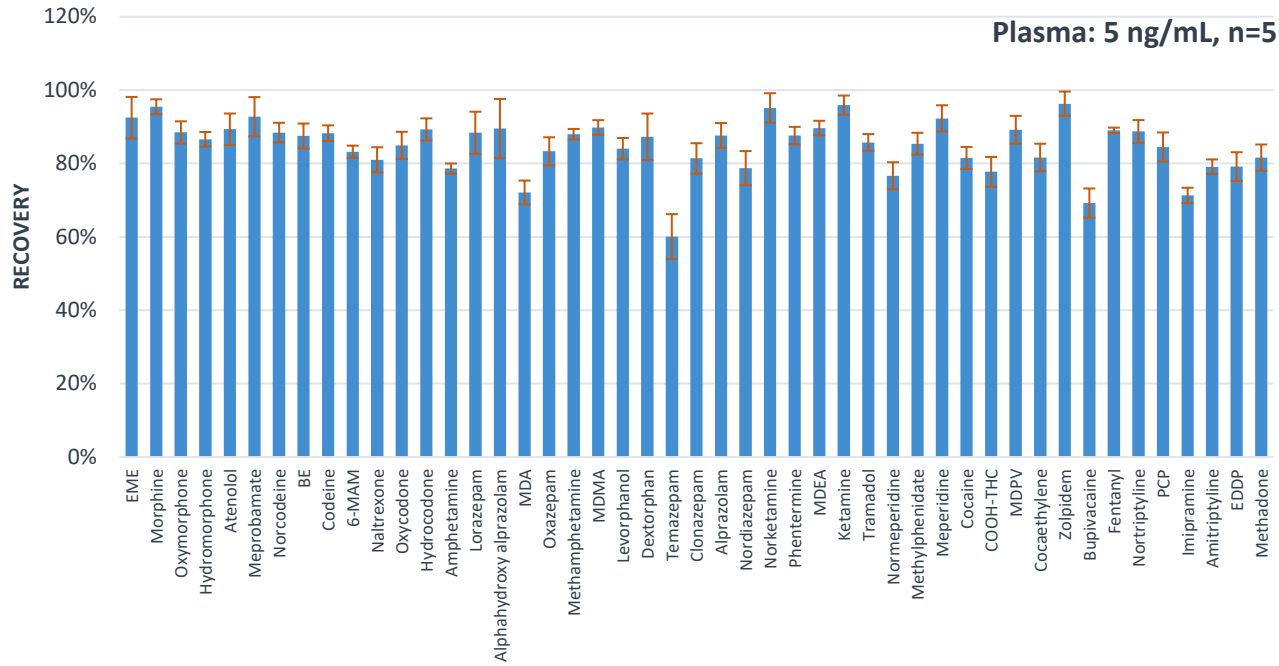

Figure 5: Recovery data of 5 ng/mL plasma quality control replicates extracted with Micro-Prep® HLB

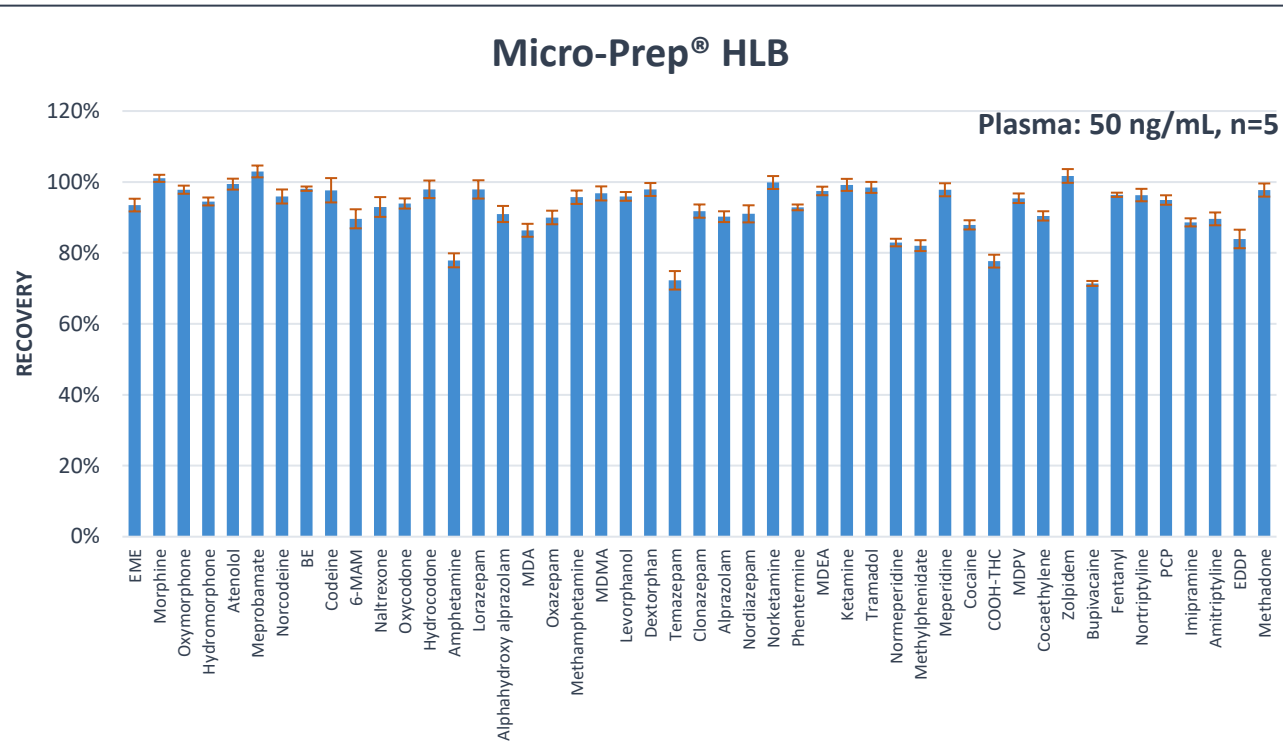

Figure 6: Recovery data of 50 ng/mL urine quality control replicates extracted with Micro-Prep® HLB

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Results & Discussion:

HLB microelution plate utilized to extract urine and plasma quality control samples yielded excellent recoveries for a majority of the analytes in the panel. From a total of 47 drugs, >80% recoveries were achieved for 37 drugs fortified at 5 ng/mL and for 43 drugs spiked at 50 ng/mL. Corresponding RSD values were <10% at both concentration levels.

The use of UCT Selectra® PFPP UHPLC column resulted in excellent peak shape and good linear calibration curves for all the analytes. In addition to using minimal wash and elution solvent volumes, the elimination of the drying step reduced the overall processing time to approximately less than 30 to 40 minutes. The potential for automation and the option to load the collection plate directly on to the autosampler make this extraction technique very convenient for high throughput forensic and clinical labs.

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