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

UCT Part Numbers

W96-XTMC-MMCX
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® MMCX microelution plate has been described. The mixed-mode cation exchange chemistry allows extraction of polar and non-polar analytes from aqueous samples. 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 6.0 Phosphate 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 6.0 Phosphate buffer

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

4) **Wash column**
   1 x 100 µL 100 mM acetic acid
   1 x 100 µL 40% CH_3OH
   Apply full pressure for 30 seconds

5) **Elute**
   1 x 50 µL 2% NH_4OH in CH_3OH

6) **Post elution**
   Evaporate & Reconstitute in mobile phase
   OR
   Add 50 µL 2% Formic acid in 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

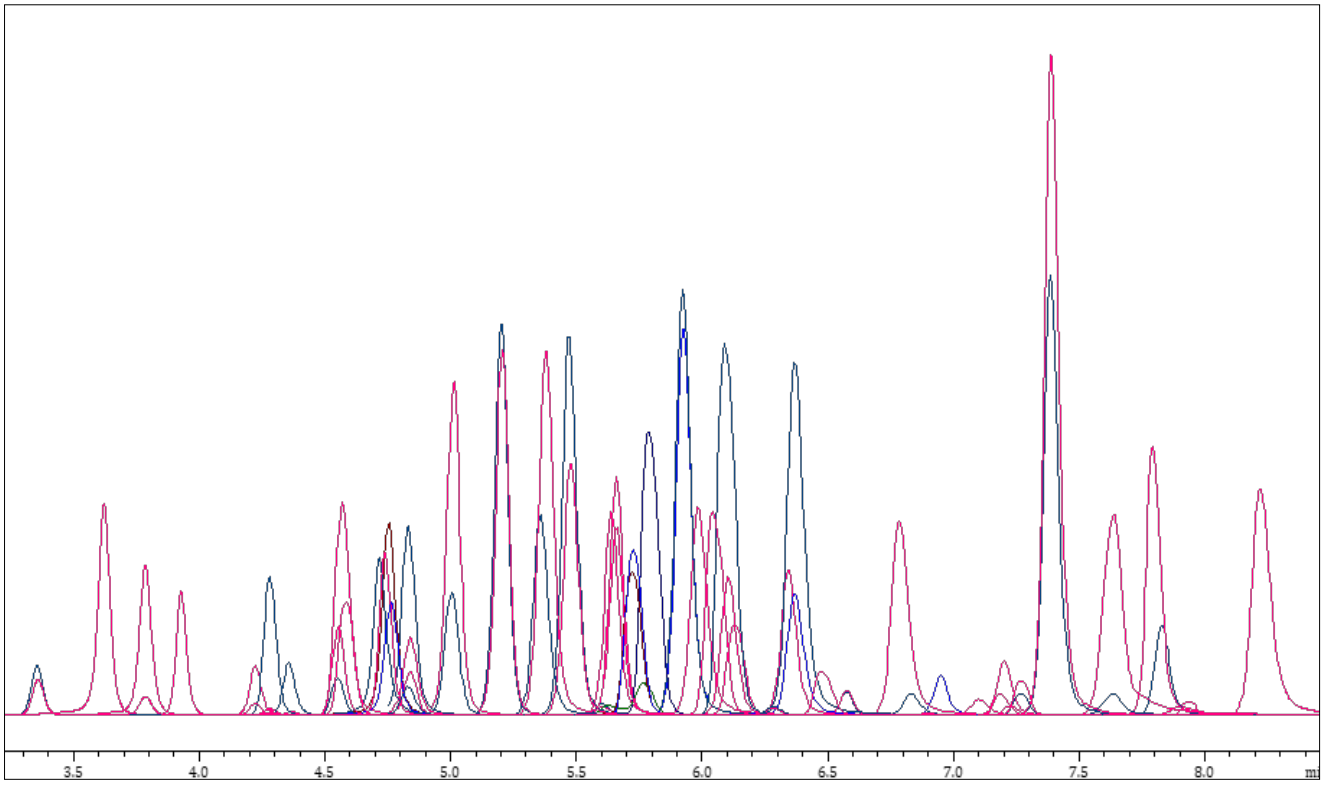

Figure 1: Chromatogram of 50 ng/mL Extracted QC sample

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Representative Calibration Curves:

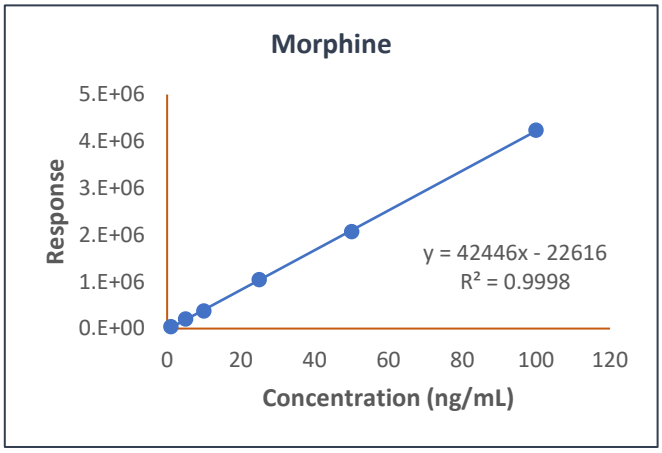
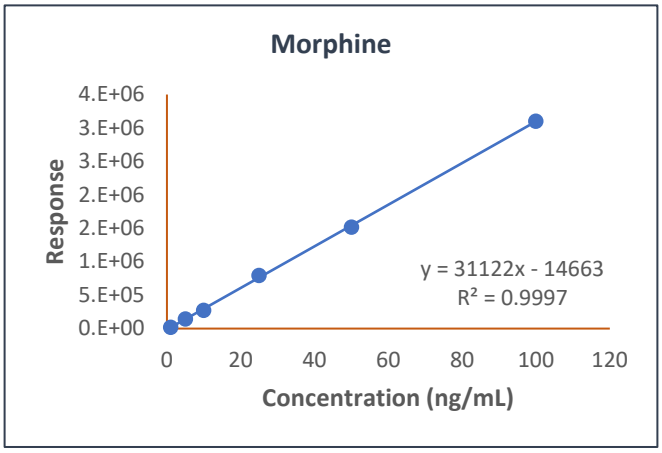
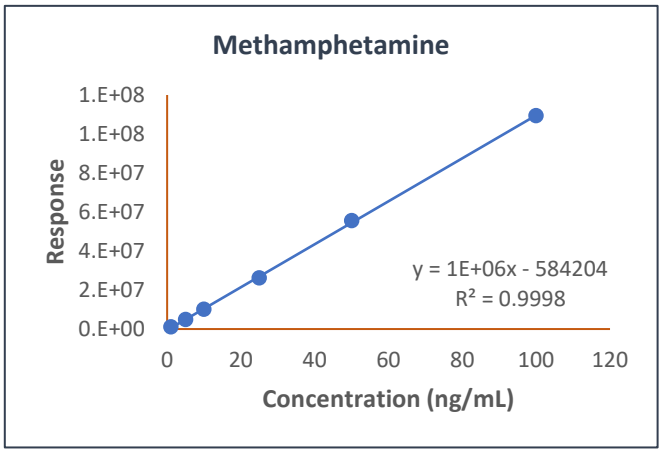
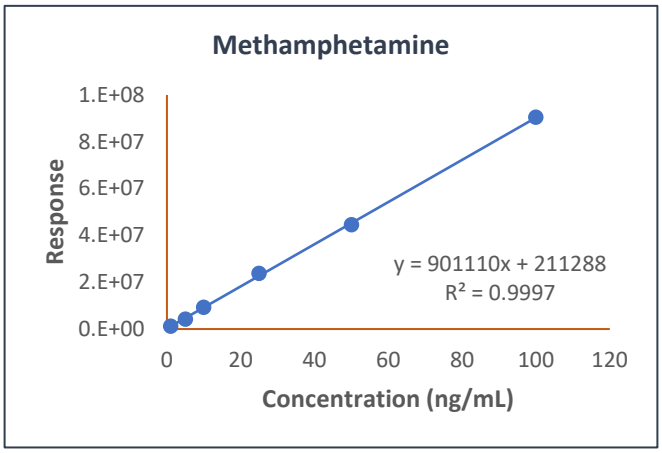
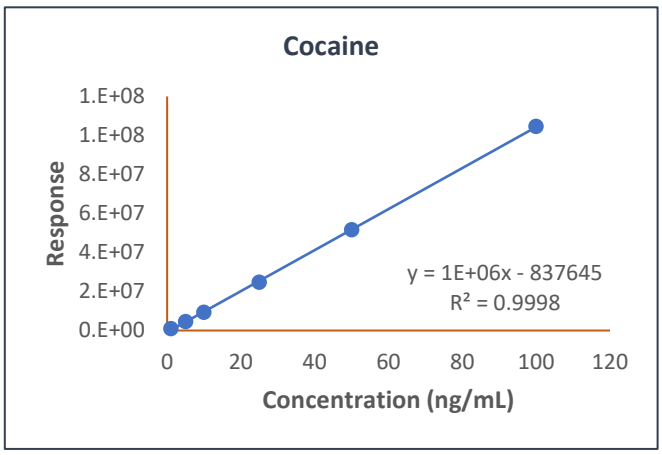
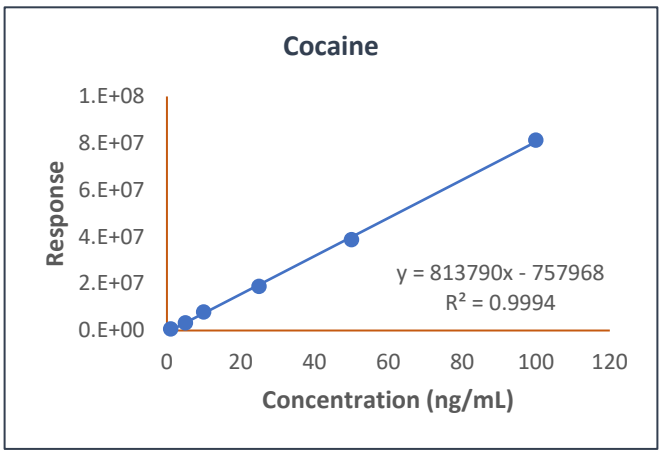
URINE	PLASMA
	
	
	

Figure 2: calibration curve examples (1, 5, 10, 25, 50 & 100 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 (%)
Morphine	100%	5%	-2%	101%	3%	-3%
Oxymorphone	92%	1%	-7%	100%	3%	-6%
Hydromorphone	100%	5%	4%	104%	2%	3%
Atenolol	99%	5%	-4%	99%	5%	-7%
7-Aminoclonazepam	91%	11%	-1%	88%	2%	-9%
Norcodeine	99%	4%	2%	103%	0%	1%
BE	91%	4%	-3%	96%	4%	-6%
Codeine	93%	17%	5%	99%	7%	-8%
6-MAM	97%	3%	-5%	102%	3%	-2%
Naltrexone	105%	2%	6%	104%	0%	5%
Oxycodone	97%	2%	-2%	103%	2%	-1%
Hydrocodone	100%	1%	4%	105%	1%	3%
Amphetamine	97%	1%	1%	106%	1%	8%
Lorazepam	101%	1%	6%	104%	2%	9%
Alphahydroxy alprazolam	97%	5%	8%	96%	2%	10%
MDA	96%	4%	-2%	100%	2%	6%
Oxazepam	99%	2%	3%	100%	1%	3%
Methamphetamine	99%	2%	0%	101%	2%	-3%
MDMA	98%	2%	2%	101%	2%	-1%
Levorphanol	98%	12%	7%	99%	0%	2%
Dextorphan	101%	8%	-8%	99%	3%	1%
Temazepam	100%	4%	-1%	98%	1%	-2%
Clonazepam	98%	3%	1%	102%	1%	4%
Alprazolam	100%	1%	7%	98%	2%	1%
Nordiazepam	95%	3%	2%	92%	2%	0%
Norketamine	102%	4%	-6%	103%	3%	1%
Phentermine	96%	2%	-4%	101%	1%	-1%
Diazepam	94%	8%	6%	91%	2%	-3%
MDEA	98%	1%	0%	98%	2%	-3%
Ketamine	98%	2%	-5%	100%	1%	-6%
Tramadol	98%	1%	-5%	99%	2%	-7%
Normeperidine	97%	1%	-6%	99%	2%	-8%
Methylphenidate	100%	5%	-10%	99%	1%	-2%
Meperidine	100%	1%	0%	103%	2%	-1%
Cocaine	98%	3%	-4%	99%	2%	-10%
Norbuprenorphine	100%	9%	2%	98%	2%	5%
COOH-THC	77%	6%	1%	76%	4%	1%
MDPV	93%	4%	-5%	99%	1%	-11%
Midazolam	98%	2%	3%	94%	2%	-5%
Cocaethylene	103%	5%	-1%	101%	2%	-11%
Zolpidem	94%	4%	-7%	101%	2%	0%
Buprenorphine	90%	2%	-14%	91%	1%	-9%
Bupivacaine	92%	5%	-11%	100%	3%	-7%
Fentanyl	91%	1%	-10%	96%	1%	-10%
Nortriptyline	83%	3%	-10%	89%	2%	-7%
PCP	92%	1%	-12%	97%	1%	-9%
Imipramine	88%	2%	-7%	90%	0%	-12%
Amitriptyline	87%	2%	-4%	89%	0%	-7%
EDDP	77%	8%	-21%	85%	3%	-7%
Methadone	93%	6%	-2%	94%	1%	-10%

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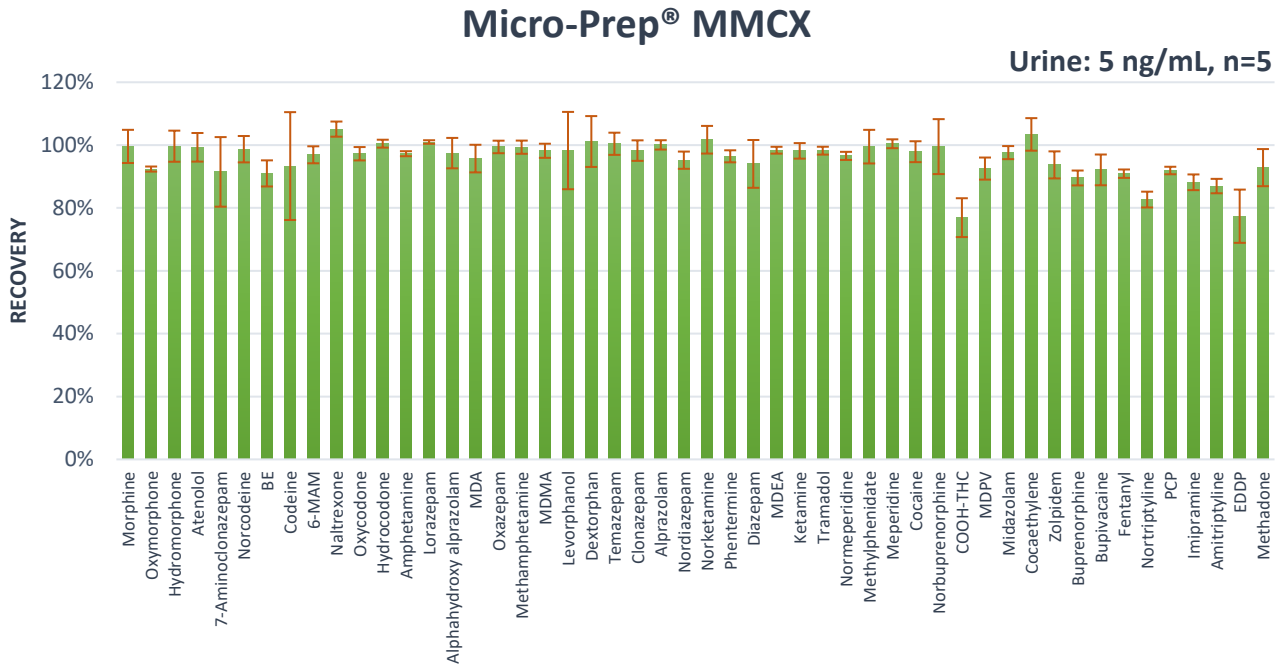

Figure 3: Recovery data of 5 ng/mL urine quality control replicates extracted with Micro-Prep® MMCX

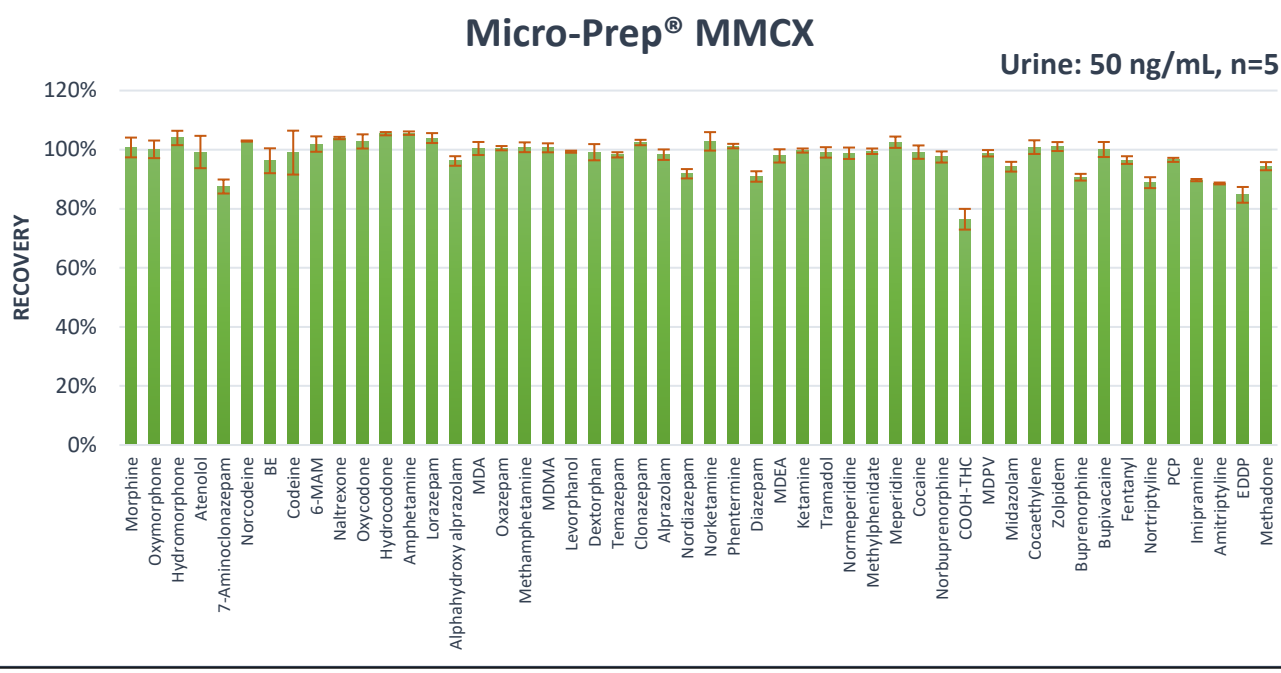

Figure 4: Recovery data of 50 ng/mL urine quality control replicates extracted with Micro-Prep® MMCX

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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 (%)
Morphine	86%	4%	-12%	85%	0%	-14%
Oxymorphone	81%	5%	-18%	82%	0%	-19%
Hydromorphone	81%	1%	-18%	91%	3%	-8%
Atenolol	86%	4%	-18%	85%	2%	-20%
7-Aminoclonazepam	94%	19%	-9%	80%	4%	-12%
Norcodeine	77%	0%	-14%	88%	4%	-15%
BE	65%	10%	-11%	57%	6%	-10%
Codeine	93%	10%	-10%	89%	4%	-2%
6-MAM	85%	3%	-18%	88%	4%	-19%
Naltrexone	88%	7%	-23%	89%	1%	-19%
Oxycodone	89%	6%	-11%	88%	4%	-14%
Hydrocodone	96%	3%	-1%	101%	8%	-6%
Amphetamine	75%	5%	-29%	86%	2%	-15%
Lorazepam	88%	5%	-6%	91%	2%	-13%
Alphahydroxy alprazolam	82%	8%	-9%	85%	1%	-9%
MDA	83%	3%	-10%	85%	2%	-10%
Oxazepam	87%	6%	-18%	88%	3%	-17%
Methamphetamine	83%	3%	-17%	84%	3%	-18%
MDMA	85%	5%	3%	88%	1%	1%
Levorphanol	81%	10%	-22%	88%	3%	-12%
Dextorphan	86%	17%	-27%	85%	4%	-20%
Temazepam	84%	5%	-20%	85%	3%	-12%
Clonazepam	87%	8%	-20%	90%	1%	-14%
Alprazolam	87%	2%	-9%	92%	3%	-4%
Nordiazepam	86%	5%	0%	86%	2%	-5%
Norketamine	92%	3%	-5%	90%	1%	-14%
Phentermine	91%	2%	-19%	87%	2%	-17%
Diazepam	91%	2%	1%	82%	2%	-7%
MDEA	86%	3%	-7%	88%	2%	-10%
Ketamine	91%	1%	-7%	86%	1%	-14%
Tramadol	87%	3%	-17%	87%	2%	-17%
Normeperidine	88%	7%	-27%	89%	0%	-19%
Methylphenidate	90%	6%	7%	93%	3%	-20%
Meperidine	87%	2%	-6%	89%	2%	-12%
Cocaine	79%	7%	-24%	80%	4%	-19%
Norbuprenorphine	92%	15%	-21%	93%	2%	-16%
COOH-THC	81%	1%	-5%	85%	2%	0%
MDPV	89%	1%	-3%	88%	3%	-11%
Midazolam	91%	3%	3%	90%	1%	-2%
Cocaethylene	82%	6%	-14%	85%	3%	-9%
Zolpidem	91%	3%	-5%	91%	3%	-10%
Buprenorphine	78%	7%	-18%	86%	4%	-15%
Bupivacaine	93%	5%	-14%	86%	3%	-20%
Fentanyl	86%	5%	-17%	86%	3%	-17%
Nortriptyline	85%	4%	-2%	85%	4%	-21%
PCP	81%	3%	-16%	83%	2%	-14%
Imipramine	81%	2%	-14%	84%	1%	-19%
Amitriptyline	83%	2%	-3%	88%	1%	-4%
EDDP	91%	3%	14%	79%	5%	-18%
Methadone	81%	1%	0%	84%	2%	10%

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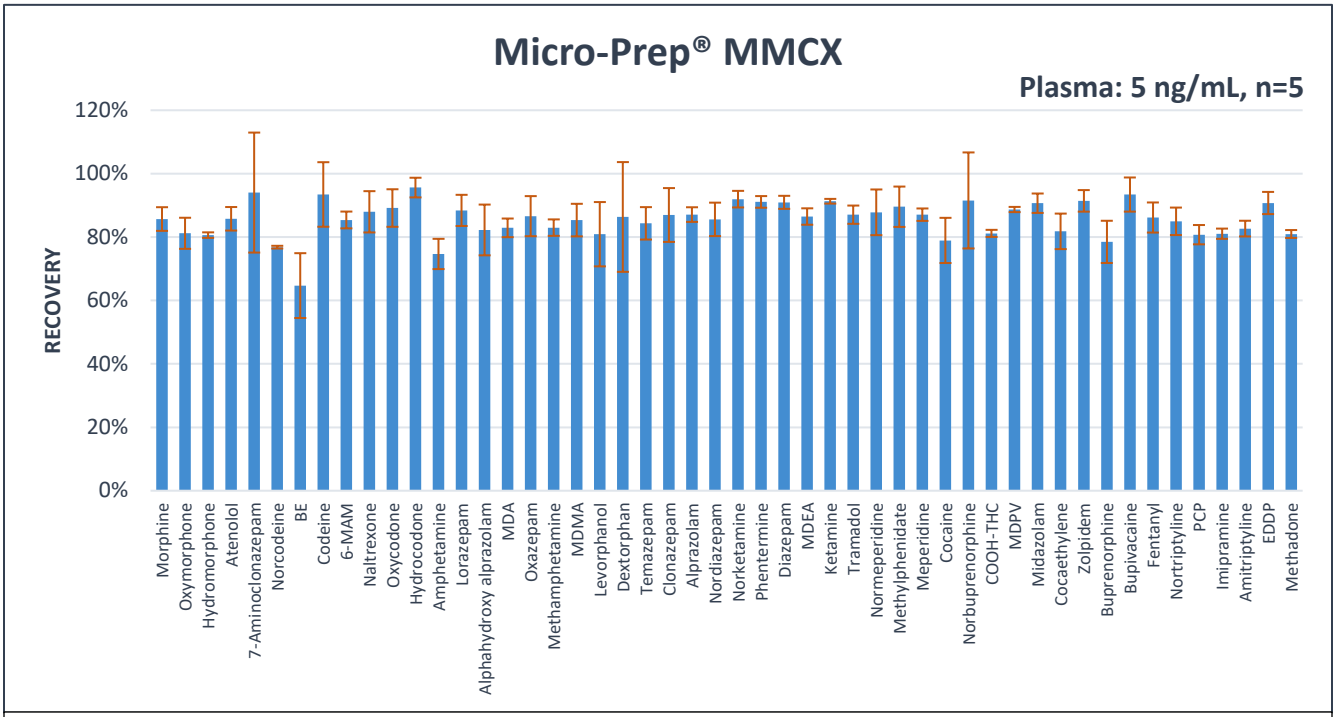

Figure 5: Recovery data of 5 ng/mL plasma quality control replicates extracted with Micro-Prep® MMCX

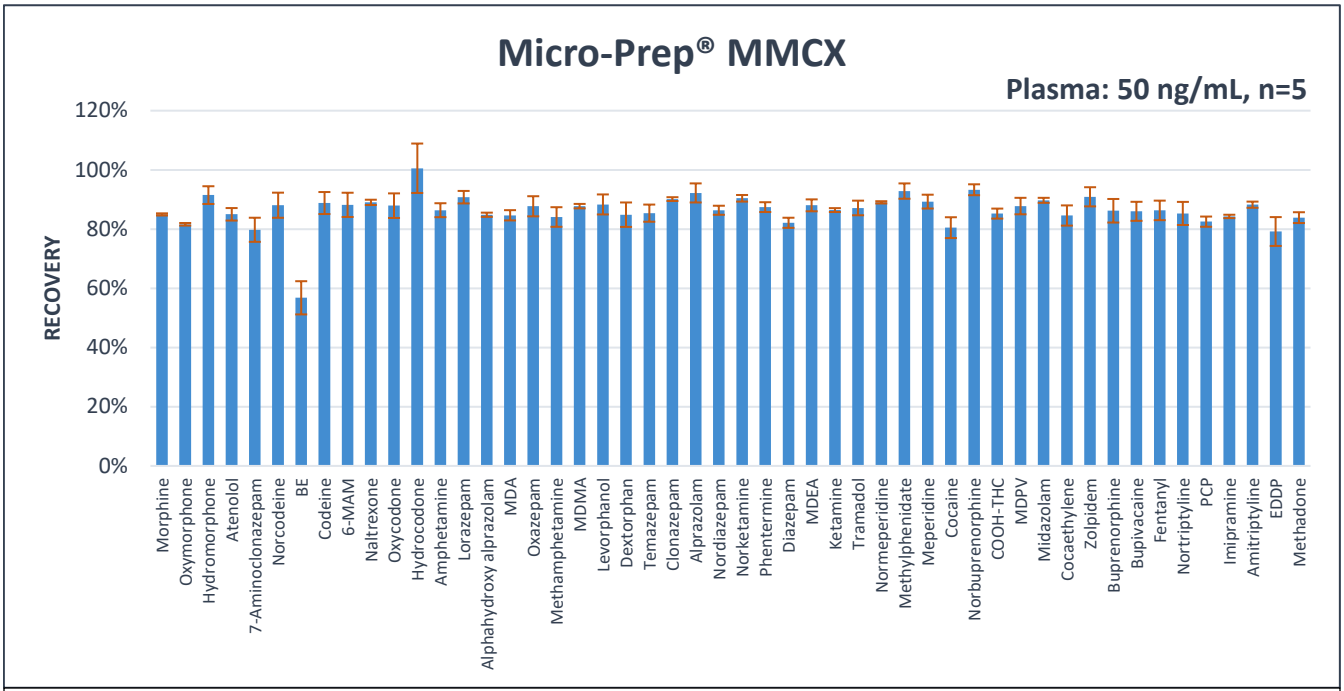

Figure 6: Recovery data of 50 ng/mL urine quality control replicates extracted with Micro-Prep® MMCX

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Results & Discussion:

Urine & Plasma quality control samples extracted with MMCX microelution plate yielded excellent recoveries for most of the analytes in a 50 drugs panel confirming the method to be very efficient. 45 drugs spiked at 5 ng/mL and 48 drugs spiked at 50 ng/mL showed more than 80% recovery. The RSD values for both concentrations were <20%.

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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