Drugs of Abuse in Urine Extracted with Microelution SPE Technology and Analyzed via LC-MS/MS

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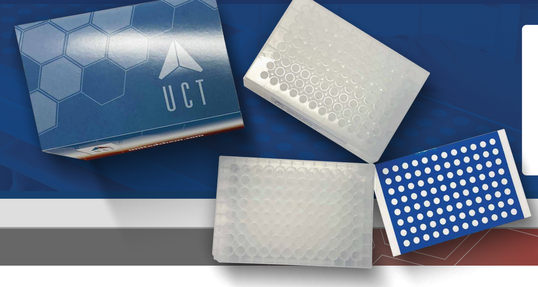


INTRODUCTION

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 poster, methods for extracting a large drugs of abuse panel from urine using UCT's Micro-Prep™ HLB and MMCX microelution plates have been described. HLB consists of a highly retentive uncharged hydrophilic and lipophilic sorbent which can effectively retain a range of acids, neutrals and bases via reverse-phase. The mixed-mode cation exchange chemistry of MMCX 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.

MICROELUTION PLATE GENERAL METHODOLOGY

	HLB W96-XTMC-HLB	MMCX W96-XTMC-MMCX
1 Sample Prep	• 300 µL sample + ISTD • 300 µL 100 mM pH 10.0 Sodium carb/bicarbonate buffer	• 300 µL sample + ISTD • 300 µL 100 mM pH 6.0 Phosphate buffer
2 Condition (Optional)	• 100 µL MeOH • 100 µL 100 mM pH 10.0 Sodium carb/bicarbonate buffer	• 100 µL MeOH • 100 µL 100 mM pH 6.0 Phosphate buffer
3 Load	• 400 µL	• 400 µL
4 Wash	• 100 µL 5% MeOH in DI H_2O	• 100 µL 100 mM Glacial acetic acid in DI H_2O • 100 µL 40% MeOH
5 Elute	• 50 µL 2% Formic acid in MeOH	• 50 µL 2% NH_4OH in MeOH
6 Post Elution (Optional)	• Evaporate & Reconstitute in mobile phase or • Add 50 µL DI H_2O	• Evaporate & Reconstitute in mobile phase or • Add 50 µL 2% Formic acid in DI H_2O

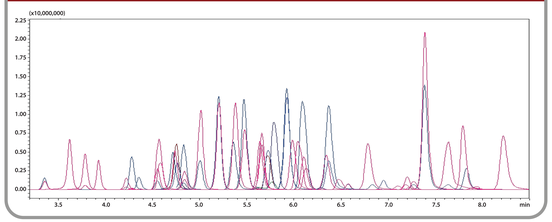
Micro-Prep™ Part Numbers

Micro-Prep™ **HLB** - 96 Well Microelution Plate	Part Number: W96-XTMC-HLB
Micro-Prep™ **MMCX** - 96 Well Microelution Plate	Part Number: W96-XTMC-MMCX
Micro-Prep™ **SAX** - 96 Well Microelution Plate	Part Number: W96-XTMC-SAX
Micro-Prep™ **SCX** - 96 Well Microelution Plate	Part Number: W96-XTMC-SCX

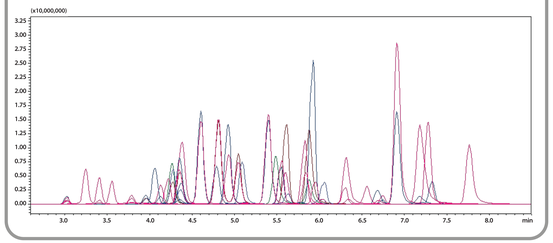
LC-MS/MS PARAMETERS

HPLC system	Shimadzu Nexara LC-30AD w/MS-8050
HPLC column	UCT Selectra® PFPP (50 × 2.1 mm, 1.8 µm) (p/n: SLPFPP50ID21-18UM)
Guard column	UCT Selectra® PFPP (5 × 2.1 mm, 1.8 µm) (p/n: SLPFPPGDC20-18UMOPT)
Guard column holder	UHPLC Direct Connect Guard (p/n: SLGRDHLDR-HPOPT)
Column temperature	40°C
Flow rate	0.4 mL/min
Injection volume	5 µL
Auto-Sampler temperature	10°C
Mobile Phase	Bottle A: 5 mM Amm. Formate + 0.1% Formic Acid in Water Bottle B: 5 mM Amm. Formate + 0.1% Formic Acid in Methanol
Gradient	0 min (0% B), 0-8 min (100% B), 8-9 min (100% B), 9-9.01 min (0% B), 9.01-13.0 min (0% B)

HLB / Chromatogram of 50 ng/mL Extracted QC sample



MMCX / Chromatogram of 50 ng/mL Extracted QC sample



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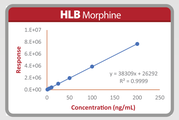
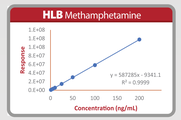
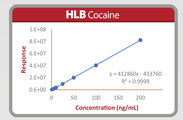
RESULTS

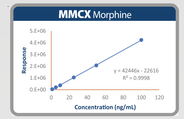
HLB microelution plate utilized to extract urine quality control samples yielded excellent recoveries for a majority of the analytes in the panel. From a total of 47 drugs, >80% recoveries were achieved for 37 drugs fortified at 5 ng/mL and for 43 drugs spiked at 50 ng/mL. Corresponding RSD values were <10% at both concentration levels. From a total of 50 drugs extracted on the MMCX microelution plate, 45 and 48 drugs showed >80% recoveries at 5 ng/mL and 50 ng/mL respectively. The RSD values for both concentrations were <20%.

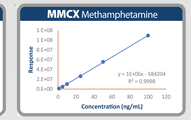


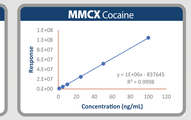




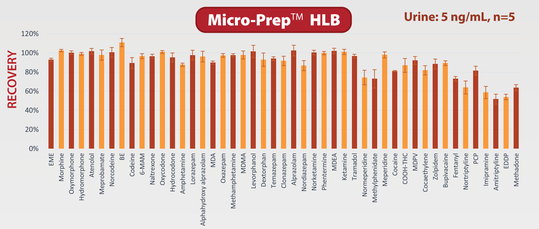
Calibration Curve Examples (5, 10, 25, 50, 100, 150 & 200 ng/mL)

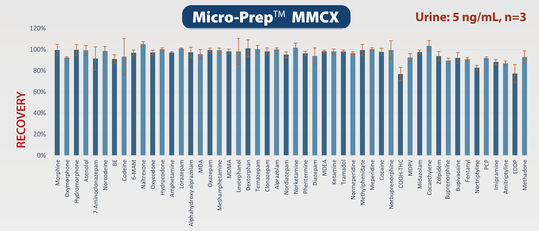


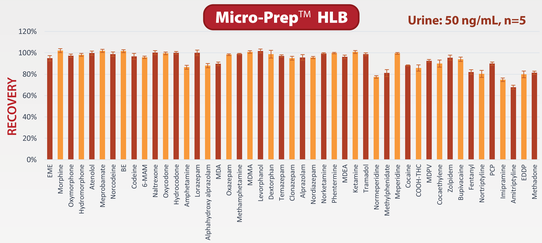


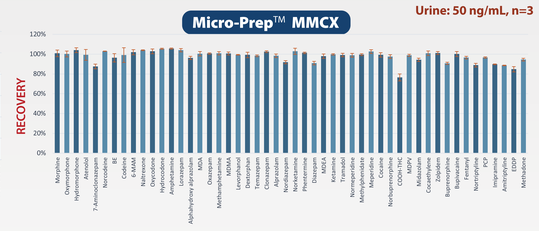


Calibration Curve Examples (1, 5, 10, 25, 50 & 100 ng/mL)









CONCLUSION

The use of UCT Selectra® PFPP UHPLC column resulted in excellent peak shape and good linear calibration curves for all the analytes. Excellent recoveries and relative standard deviation (RSD) values confirm both the microelution extraction methods to be efficient. 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.

Questions / Comments: **methods@unitedchem.com**

Disclosure: The speaker, author, moderator, planning member and/or presenter/s do have financial relationships with UCT, Inc. , as defined in the AACC policy on potential bias or conflict of interest. The specific product/s Micro-Prep™ HLB and MMCX microelution plates will be mentioned and/or discussed.