

## A summary of “Screening and Quantification of Pain and Antidepressant Drugs in Human Urine by Liquid Chromatography-High Resolution Mass Spectrometry”

### Overview:

Widespread drug prescription and abuse have necessitated the monitoring of patients for compliance. Often, patient samples are first screened by immunoassay to identify the presence of drugs, and then identification and quantity is confirmed by mass spectrometry (MS). A way to decrease costs while increasing sample accuracy and number of analytes during screening has been achieved by utilizing high resolution mass spectrometry (HRMS), in particular the Q-Exactive™.

In this study, a method was developed in which 47 drugs in urine were simultaneously screened and quantified by liquid chromatography (LC)-HRMS utilizing data independent acquisition (DIA). IMCSzyme®, a genetically modified β-glucuronidase, was selected for this application because of its non-selective hydrolysis capabilities over a wide range of drugs as well as its efficient and high rate of hydrolysis, reducing the likelihood of obtaining false negatives. In addition, IMCSzyme® is in the form of a pure solution, which reduces the contaminants loaded onto the system.

### Materials and Methods:

Samples were prepared by combining 75 μL of urine with 300 μL of a master mix containing 5000 U/mL IMCSzyme®, Rapid Hydrolysis Buffer and 47 internal standards corresponding to the drug analytes being tested. Hydrolysis was then performed at 65°C for 60 minutes. Samples were centrifuged for 7 minutes at 4000 rpm before injection onto the LC-MS/MS system. Standards and QCs were prepared with all 47 drug analytes and calibration curves with appropriate concentrations were generated. Poroshell 120 SB-C18 column, 2.1 mm x 50 mm with 2.7 micron particle size was used on Waters Acquity UPLC I-Class. The LC was coupled to the Thermo Q-Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer.

### Results:

Both data dependent acquisition (DDA-MS2) and data independent acquisition (DIA-MS2) were evaluated, and DIA was determined to be the better method for collecting at least one scan for all analytes at LLOQ at a resolving power of 70,000. This method showed no interferences for parent mass or isotope. Correlation coefficient was > 0.99. Intra- batch accuracy for quality control samples was less than 20% and inter-batch accuracy was less than 10% at LLOQ (Table 1). Evaluation of internal standard-normalized matrix factor showed ≤14% RSD for all analytes.

The hydrolysis time, temperature, and concentration for IMCSzyme® was optimized, and yielded ≥80% hydrolysis for all glucuronidated analytes tested. Notably, two of the analytes tested, codeine-6-glucuronide and morphine-6-glucuronide are two of the hardest to cleave, but IMCSzyme® consistently hydrolyzed over 80% at the optimized conditions.

### Conclusions:

A successful method for screening and quantifying 47 drugs in patient urine samples was developed and validated. This new method utilizes the efficiency of IMCSzyme® for quick sample hydrolysis as well as its allowance for optional sample clean-up to increase sample throughput. By coupling IMCSzyme® with the DIA methodology using the Q-Exactive Orbitrap™, screening, identifying, and quantifying drugs in patient samples have been simplified and the time for processing samples has been significantly reduced.

**Table 1.** Validation Results Summary

Precision and Accuracy				
QC	Accuracy (%Dev)		Precision (%RSD)	
	Intra (n=6)	Inter(n=18)	Intra(n=6)	Inter(n=18)
LLOQ	≤ 18.8	≤ 9.7	≤ 10.5	≤ 13.4
Low	≤ 17.8	≤ 6.7	≤ 5.7	≤ 13.9
Mid	≤ 6.6	≤ 3.2	≤ 7.4	≤ 7.1
High	≤ 12.9	≤ 8.3	≤ 5.4	≤ 7.6
Correlation Coefficient (r <sup>2</sup> )		≥ 0.997		
Stability				
Stability	Condition		Accuracy (%Dev)	
Freeze/Thaw	3 cycles, - 20°C		≤ 9.8	
Room Temperature	24 hrs.		≤ 15.7	
Autosampler Stability	1 week, 5 °C		≤ 12.0	
Long - Term Storage Stability	4 weeks, 5 °C		≤ 17.1	