

## High Throughput Automated Sample Preparation and Analysis of Drugs of Abuse in Human Urine Using LC-MS/MS

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### Background

Due to the widespread abuse of illicit drugs, toxicology/clinical laboratories are facing a significant increase in patient samples. There is a need to develop a high throughput analytical method that can process large numbers of samples in a limited time frame while being sensitive enough to analyze drugs/metabolites at appropriate cut-off levels. Hence, we developed a high throughput LC-MS/MS method for the analysis of drugs of abuse in human urine with automated sample preparation using MicroLab NIMBUS (Hamilton Company).

### Method

Using an 8 channel Microlab NIMBUS, 100  $\mu$ L of samples and 100  $\mu$ L of master solution containing ISTDs and IMCS enzyme/buffer, were transferred into each well of a 96 well plate and hydrolyzed at 60 °C for 30 min. 600  $\mu$ L of methanol was then added into each well. The mixture was diluted 5 times with 0.1% acetic acid in an Isolute<sup>®</sup> filter plate (Biotage) and filtered by a positive pressure device. The filtered samples were analyzed on an Agilent 1290 HPLC coupled with a 6460 QQQ operated in positive dynamic MRM mode. Separation was achieved on an Agilent Poroshell (120 EC-C18, 100X2.1mm, 2.7 $\mu$ m) column using gradient elution of 0.1% acetic acid and acetonitrile at 400  $\mu$ L/min. 5  $\mu$ L was injected.

### Result

The drugs/metabolites analyzed in the method include 16 benzodiazepines, 15 opiates, cocaine and zopiclone. Pipetting techniques and parameters for different liquid classes were optimized on NIMBUS for accurate and reproducible pipetting and thorough mixing. Adding a heating adaptor and hydrolyzing at 60°C improved overall hydrolysis efficiency in the 96 well plate. The filtration was conducted at 8psi/50sec followed by three high pressure pushes to avoid splashing and cross contamination. The method has been fully validated. Compared to the manual method previously used in our lab, the new method produces comparable or better results in terms of precisions (CV%: 1.2—12%), accuracy(97%--114%), recovery (88-112%) and sensitivity(LOD: 0.4-10 ng/mL), while reducing reagent and labor usage, and human variation/error. It is now used routinely for the analysis of patient samples in the opioid dependency program.

### Conclusion

This method is sensitive, reliable, cost-effective, and suitable for large-scale routine analysis.

Key words: automated, NIMBUS, drugs of abuse