Simultaneous, Automated Extraction of 96 Urine Samples for Drugs of Abuse Analysis by LC-MS/MS Using the Microlab® NIMBUS® Workstation

Application Note

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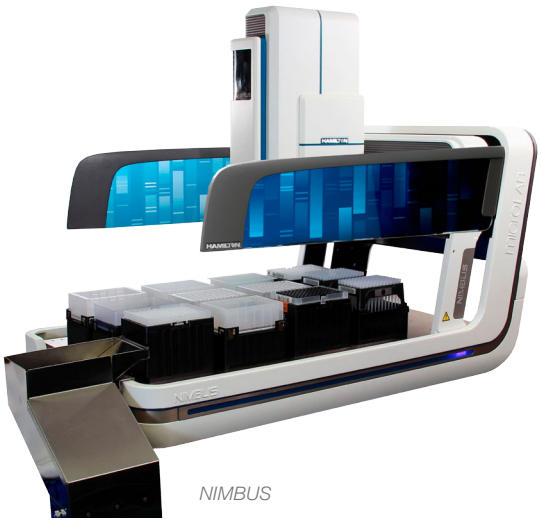
Summary

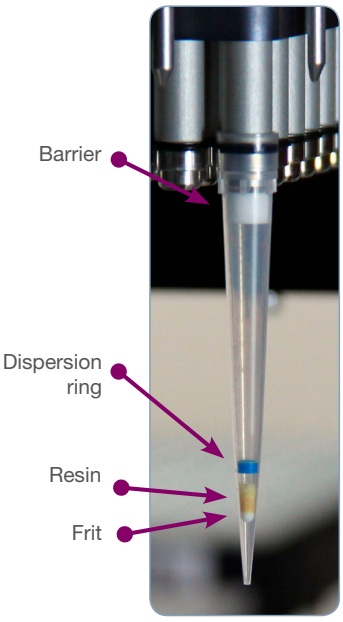
Utilizing Hamilton's CO-RE disposable tips with DPX technology provides a fast, accurate, and simple extraction method for analyzing drugs of abuse in urine. The Microlab NIMBUS equipped with a CO-RE 96-channel Multi-Probe Head (MPH) allows for high-throughput, automated sample processing. Using the NIMBUS96 platform, two sample plates can be ready for LC-MS/MS injection in approximately 15 minutes.



NIMBUS

Introduction

Matrix effects are a major concern in LC-MS/MS analysis. Proper sample preparation can be very time consuming and is generally the bottleneck for sample throughput and analysis of many laboratories. In-tip Dispersive Pipette eXtraction (DPX) is a new solid phase extraction (SPE) technology that minimizes sample processing steps and decreases reagent use compared to typical SPE plate-based techniques. Hamilton's CO-RE tips containing DPX chemistry, now offer a fully automated, high-throughput extraction method that can be used on any Hamilton liquid handling platform. The method reported herein demonstrates the extraction method with high reproducibility and provides the necessary sensitivity for target analyte quantification and analysis for forensic and clinical laboratories.



Workflow

Microplates containing hydrolyzed urine were loaded on to the NIMBUS96. The automated NIMBUS method starts by filling a 96-well microplate with 200 µL of water (for the wash step) 150 µL of 1% FA (formic acid) in methanol (for the elution step). The DPX tips (mixed mode with RP/WAX) were then conditioned by aspirating 30% methanol from a solvent reservoir. After conditioning, the sample solutions, containing 150 µL urine + 100 µL mixture of buffer, enzyme and internal standards, were aspirated and dispensed three times in order to bind the drugs of abuse targets to the sorbent. Water was then aspirated and dispensed to remove sample matrix components such as salts, urea, and creatinine. The analytes of interest were eluted by aspirating and dispensing 1% FA in methanol three times. The eluent is simply diluted until an appropriate percentage of methanol is reached for injection. In this case, 1050 µL of water was added (to make the final solution 12.5% methanol). Analysis was performed on a Thermo TSQ Vantage triple quadrupole instrument with an Agilent 1260 HPLC using an Agilent Poroshell EC-C18 column (3.0 x 50 mm, 2.7 µm) with a 10 µL injection.

Workflow: In-tip Solid Phase Extraction

Results and Discussion

From start to finish, this fully automated dispersive SPE method takes approximately 15 minutes to prepare two 96-well microplates of samples. Results from this method are linear, accurate, and reproducible. All correlation coefficients were greater than 0.99 for the range of at least 12.5-400 ng/mL, with most analytes being linear from 6.25-800 ng/mL. Relative standard deviation was calculated using 7 replicate extractions at 400 ng/mL and ranged from 1.6 to 8.0. Limits of detection (LOD) were calculated as 3.3*σ/m, where σ is the standard deviation of the lowest non-zero calibrator and m is the slope of the calibration line. Limit of quantitation (LOQ) was calculated as 10*σ/m. LOD ranged from 0.50 to 18 ng/mL and LOQ ranged from 1.5 to 54 ng/mL.

While it should be emphasized that confirmatory tests and cutoff levels are highly dependent on LC-MS/MS instrumentation and procedures, for improved sensitivity and lower LODs and LOQs, solvent evaporation can be employed to concentrate the extracts. Elution volume can also be increased to 500 µL to increase recovery as well.

Conclusion

The method described herein demonstrates adequate recoveries, sensitivity, and reproducibility for reliable sample preparation in a high-throughput setting. Complete automation of the sample preparation method provides a less tedious and faster process compared to manual processing for analysis of drugs of abuse; two 96-well microplates are processed in 15 minutes.

Compound	R^2	% RSD (n=7)	LOD (ng/mL)	LOQ (ng/mL)	% Recovery
Morphine	0.9969	4.0	5.0	15	79.18
Oxymorphone	0.9982	3.8	1.8	5.4	89.95
Hydromorphone	0.9982	4.1	2.4	7.2	91.23
Gabapentin	0.9989	2.8	0.5	1.5	28.14*
Amphetamine	0.9944	5.9	3.1	9.4	91.54
Codeine	0.9972	4.1	2.2	6.7	91.89
Oxycodone	0.9968	4.9	1.2	3.7	100.00
MDA	0.9940	7.3	5	15	100.00
6-MAM	0.9932	8	0.73	2.2	95.31
Pregabalin	0.9972	4.7	2.5	7.6	18.41*
Methamphetamine	0.9993	2.5	1.1	3.3	99.60
Hydrocodone	0.9949	5.8	0.93	2.8	100.00
Ritalinic Acid	0.9945	8	0.77	2.3	76.51
7-Aminoclonazepam	0.9959	6.1	1.7	5	85.29
Benzoylecgonine	0.9943	6	0.93	2.8	88.79
MDMA	0.9975	5.0	1	3.1	100.00
Norfentanyl	0.9970	3.1	1.1	3.4	96.74
N-desmethyl tramadol	0.9956	5	18	53	100.00
Tramadol	0.9962	5.4	2.1	6.3	98.34
Normeperidine	0.9937	5.9	1.8	5.3	94.53
Cocaethylene	0.9981	3	1.7	5	100.00
Meperidine	0.9960	6.3	2.5	7.6	99.99
Zolpidem	0.9975	4.8	0.96	2.9	97.31
Cyclobenzaprine	0.9973	3.4	1.4	4.1	93.00
Norbuprenorphine	0.9912	6.6	3.1	9.4	64.79
Fentanyl	0.9979	4.7	0.48	1.4	98.07
PCP	0.9967	4.8	0.98	2.9	100.00
Buprenorphine	0.9914	6.9	1.2	3.5	88.13
Oxazepam	0.9981	2.5	0.61	1.8	77.35
Methadone	0.9932	6.8	0.56	1.7	95.01
Nordiazepam	0.9970	4.1	1.7	5	73.15
α-hydroxyalprazolam	0.9922	5.6	6	18	98.78
Alprazolam	0.9937	3.8	3.9	12	86.39
Lorazepam	0.9984	3.7	0.86	2.6	79.95
Amitriptyline	0.9983	3.6	1.8	5.3	94.23
Temazepam	0.9954	4.3	3	8.9	77.72

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