Automated Comprehensive Extraction of Drugs/Metabolites in Oral Fluid Using Hamilton Nimbus96 and LC-MS/MS

HIGHLIGHTS: Less solvent volumes, <10 minutes for extraction

Mixed Mode WAX/RP - XTR

INTRODUCTION

Dispersive Pipette XTRaction technology delivers a rapid, accurate and precise single extraction method for comprehensive analysis of drugs and metabolites in oral fluid. The Hamilton Microlab NIMBUS96 automated liquid handling system is equipped with a 96-channel multi-pipetting head and when coupled with XTR tips high sample throughput is achieved. In approximately ten minutes, one 96 sample microplate is extracted and ready for LC-MS/MS analysis.

Sample preparation is required in order to remove matrix interferences from oral fluid samples prior to LC-MS/MS analysis. Multi-step SPE column preparation and multiple extractions add to turn-around-time and creating a "bottle neck" in sample throughput for laboratories.

To address SPE limitations, we have developed a rapid method for comprehensive analysis of oral fluid using dispersive pipette extraction technology. XTR tips containing a loose mixed mode sorbent WAX/RP bind matrix interferences, which results in clean sample extracts. Dispersive extraction eliminates sample preparation steps associated with traditional SPE methods and requires much less solvent volumes for injection.

MATERIALS AND METHODS

Microplates containing oral fluid (250 µL) are loaded on to the NIMBUS96. Additional microplates are filled with 200 µL water, 500 µL of 0.1% formic acid (FA) in acetonitrile (ACN) (Elution Solvent 1) and 250 µL of 0.1% FA in ACN (Elution Solvent 2). WAX/RP-XTR tips are then conditioned by aspirating 30% methanol from a solvent reservoir. Sample solutions are aspirated and dispensed five times in order to bind the analytes of interest to the sorbent. Water is then aspirated and dispensed to remove common matrix interferences. Target compounds are eluted by thrice aspirating and dispensing 275 µL of 0.1% FA in ACN, then aspirating 250 µL of 0.1% FA in ACN and dispensing into the microplate containing the first eluent. The total volume (750 µL) is evaporated to dryness* and reconstituted in 125 µL of 10% methanol (aqueous).

Hamilton Nimbus96 with XTR Tips

Table 1. Sample Preparation

1	CONDITION	Aspirate Methanol
2	BIND ANALYTES	Aspirate/Dispense Oral Fluid using XTR tips with Mixed Mode WAX/RP Sorbent
3	WASH	Aspirate/Dispense Water
4	ELUTE ANALYTES	Aspirate/Dispense ACN
5	SOLVENT EVAPORATION	750 µL to dryness
6	RECONSTITUTE	120 µL of 10% Methanol

LC-MS/MS analysis was performed using a Thermo TSQ Vantage triple stage quadrupole mass spectrometer attached to an Agilent 1260 HPLC system. Chromatographic separation was performed on an Agilent Poroshell EC-C18 2.7 µm 50 x 3.0 mm column with a 10 µL injection.

RESULTS AND DISCUSSION

Analytical results are linear, accurate and precise. Correlation coefficients (R^2) were greater than 0.99 over the concentration range of 2.5-500 ng/mL, with the majority of analytes exhibiting linearity over the range of 0.625-500 ng/mL. Relative standard deviations (%RSDs) were calculated using 6 replicate extractions (100 ng/mL), and ranged from 1.6-8.0%. Limits of detection (LODs) were calculated as 3.3(σ/m), where σ is the standard of deviation of the lowest non-zero calibrator and m is the slope of the calibration curve. Limits of quantitation (LOQs) was calculated as 10(σ/m) and ranged from 0.069-13 ng/mL. (Table 2)

Note: LODs and LOQs are highly dependent on the laboratory's analytical method and LC-MS/MS sensitivity. To obtain higher sensitivity, evaporated samples may be reconstituted in 62.5 µL of 10% methanol in order to obtain a 1:1 ratio of the original oral fluid concentration.

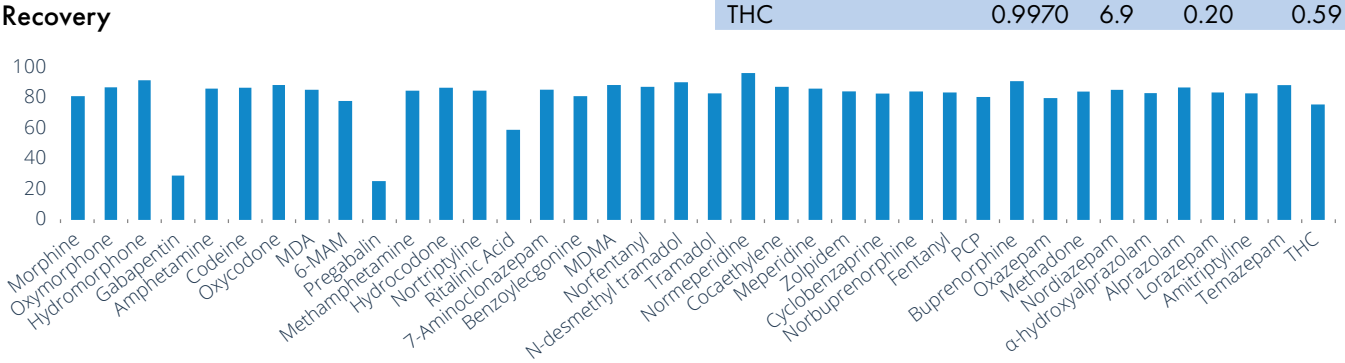
Use of two separate elution steps is necessary in order to optimize THC recovery.

CONCLUSIONS

When combined with the NIMBUS96, Dispersive Pipette XTRaction technology provides comprehensive, rapid and easy-to-use sample preparation. Analytical sensitivity, accuracy and precision make this method ideal in high throughput clinical and forensic laboratory environments.

Table 2. Comprehensive Extraction of Drug and Metabolites-Validation Data

Compound	R^2	% RSD (n=6)	LOD (ng/mL)	LOQ (ng/mL)
Morphine	0.9996	3.3	0.18	0.54
Oxymorphone	0.9962	2.1	0.37	1.1
Hydromorphone	0.9994	1.9	0.24	0.72
Gabapentin	0.9991	2.4	0.34	1.0
Amphetamine	0.9967	2.9	3.0	9.0
Codeine	0.9995	2.3	4.3	13.0
Oxycodone	0.9993	3.0	0.8	2.4
MDA	0.9965	5.1	1.7	5.1
6-MAM	0.9943	7.7	1.1	3.3
Pregabalin	0.9988	3.3	0.76	2.3
Methamphetamine	0.9994	2.6	0.28	0.84
Hydrocodone	0.9992	1.5	0.29	0.87
Nortriptyline	0.9984	2.3	0.37	1.1
Ritalinic Acid	0.9982	3.7	0.12	0.36
7-Aminoclonazepam	0.9960	2.6	0.42	1.3
Benzoylecgonine	0.9978	1.8	0.16	0.48
MDMA	0.9995	1.9	0.14	0.42
Norfentanyl	0.9972	3.6	3.0	8.9
N-desmethyl tramadol	0.9936	2.8	1.8	5.5
Tramadol	0.9939	2.5	0.46	1.4
Normeperidine	0.9979	3.1	0.17	0.51
Cocaethylene	0.9978	3.1	0.023	0.069
Meperidine	0.9979	1.9	0.061	0.18
Zolpidem	0.9903	11.0	0.43	1.3
Cyclobenzaprine	0.9987	3.7	0.01	0.03
Norbuprenorphine	0.9933	7.4	0.59	1.8
Fentanyl	0.9988	1.7	0.17	0.51
PCP	0.9975	6.9	0.65	2.0
Buprenorphine	0.9956	8.0	1.1	3.3
Oxazepam	0.9990	2.2	0.53	1.6
Methadone	0.9988	6.0	0.18	0.53
Nordiazepam	0.9993	1.4	0.42	1.3
α-hydroxyalprazolam	0.9984	2.7	3.0	8.9
Alprazolam	0.9989	4.2	0.11	0.33
Lorazepam	0.9989	5.4	0.46	1.4
Amitriptyline	0.9991	1.6	0.35	1.1
Temazepam	0.9991	3.1	0.18	0.54
THC	0.9970	6.9	0.20	0.59



Analyte recoveries following single extraction of oral fluid with RP/WAX- XTR tips. Compounds of interest include opiates, opioids, benzodiazepines, common drugs of abuse, non-opioid analgesics, anticonvulsants, sedative-hypnotics, stimulants, antidepressants and metabolites as indicated.