AUTOMATED COMPREHENSIVE DISPERSIVE PIPETTE EXTRACTION OF DRUGS/METABOLITES IN ORAL FLUID

APPLICATION NOTE

COMPREHENSIVE ORAL FLUID

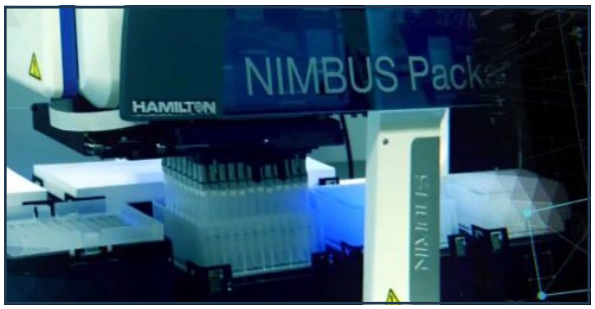
Oral fluid is gaining popularity as an alternative matrix for drug analysis. Oral fluid collection is simple, non-invasive, and can be easily monitored. Like other complex biological matrices, sample preparation is required to remove matrix interferents from oral fluid samples prior to LC-MS/MS analysis. This procedure can be time-consuming and is often the "bottle neck" for laboratory analysis and throughput.

DPX extraction is a dispersive SPE method that requires much less solvent and time-consuming steps compared to other SPE techniques. Automation of the extraction using the NIMBUS96 system with DPX-CO-RE technology makes the technique both rapid and high throughput. The method we describe here is highly reproducible and provides the sensitivity necessary for forensic and clinical purposes.

WORKFLOW

Solvent reservoirs, empty well plates and a well plate containing oral fluid samples (250 µL) were loaded onto the NIMBUS system. The NIMBUS system fills a well plate with 200 µL of water, a well plate with 500 µL of 0.1% FA in acetonitrile, and a well plate with 250 µL of 0.1% FA in acetonitrile. DPX (RP/WAX, 300 µL) CO-RE tips are picked up and conditioned in a solvent reservoir with 30% methanol. After conditioning, DPX tips aspirate and dispense the samples five times to bind the analytes. Water is aspirated and dispensed to rid the tip of any free salts. The analytes of interest are eluted in the following multi-step process. First, 500 µL of 0.1% FA in acetonitrile is aspirated and dispensed three times. Then, 250 µL of 0.1% FA acetonitrile from a fresh well plate is aspirated and dispensed into the well that contains the 500 µL of 0.1% FA in acetonitrile. This additional elution step is needed to increase recoveries for THC.

The total eluent (750 µL) is solvent evaporated to dryness and reconstituted in 125 µL of 10% methanol in water. 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 50mm, 2.7 µm) with a 10 µL injection.



Hamilton NIMBUS96 with DPX-RP/WAX Tips

1 CONDITION TIPS	*Aspirate and Dispense 30% Methanol*
2 BIND ANALYTES	*Aspirate and Dispense Oral Fluid*
3 WASH	*Aspirate and Dispense Water*
4 ELUTE ANALYTES	*Aspirate and Dispense Acetonitrile*
5 EVAPORATE SOLVENT	*750 µL to Dryness*
6 RECONSTITUTE	*10% Methanol*

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 deviation of the lowest non-zero calibrator and m is the slope of the calibration curve. Limits of detection ranged from 0.023-4.3 ng/mL. Limits of quantitation (LOQs) were calculated as 10 (σ/m) and ranged from 0.069-13 ng/mL (Table 1) .

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 undiluted oral fluid concentration. It is also possible that a larger volume of oral fluid can be extracted to improve sensitivity.

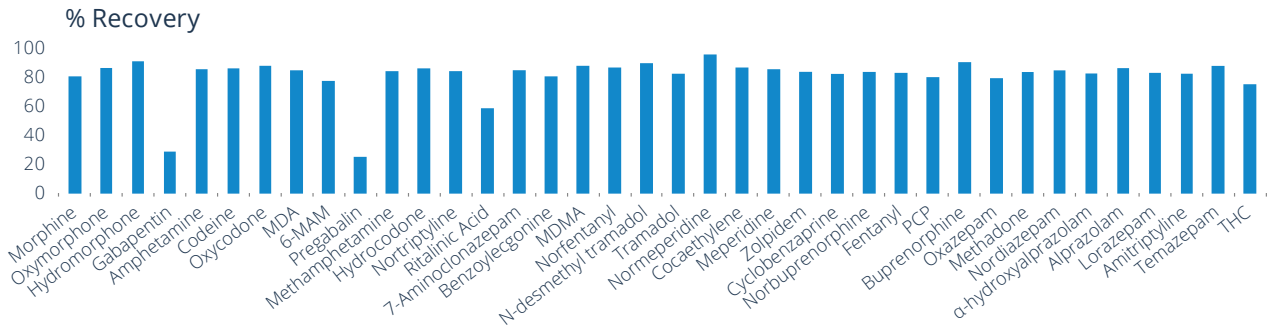
It should be noted that the use of two separate elution steps is necessary in order to optimize THC recovery.

CONCLUSION

When combined with the Hamilton NIMBUS96, DPX technology provides comprehensive, rapid and easy-to-use sample preparation. This method provides analytical sensitivity, accuracy and precision that are ideal for high throughput clinical and forensic laboratories.

Table 1. 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
Oxycodone	0.9993	3.0	0.80	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	0.43	1.3
Cyclobenzaprine	0.9987	3.7	0.010	0.030
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 DPX-RP/WAX tip. Compounds of interest include opiates, opioids, benzodiazepines, common drugs of abuse, non-opioid analgesics, anticonvulsants, sedative-hypnotics, stimulants, antidepressants and metabolites as indicated.