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

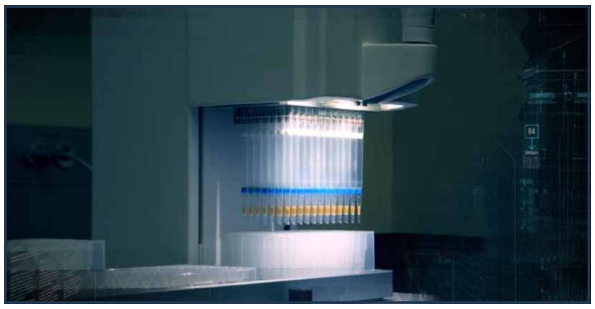
INTEGRA

COMPREHENSIVE ORAL FLUID

APPLICATION NOTE

Oral fluid continues to gain popularity as an alternative matrix for drug analysis. Oral fluid collection is simple, non-invasive, and can be easily monitored. Sample preparation is commonly used to remove matrix interferences from saliva and to address the dilution factor associated with the oral fluid collection device. By solvent evaporating and reconstituting prior to LC-MS/MS analysis, lower sensitivity requirements can be obtained. However, this process is time-consuming and often the "bottle neck" for laboratory analysis and throughput.

Dispersive Pipette Extraction (DPX) tip technology addresses the drawbacks of traditional SPE methods. Loose sorbent is contained between two porous barriers inside a pipette tip. The sorbent is mixed with the sample solution by simply aspirating and dispensing. Incorporating DPX tips with the Integra Viaflo 96 semi-automated liquid handling system eliminates the tedious and labor intensive elements of sample preparation. In less than 8 minutes, 96 samples can be simultaneously extracted—resulting in higher throughput when compared to other sample preparation methods. The method we describe is highly reproducible and provides the sensitivity necessary for clinical and forensic purposes.



Integra Viaflo 96 with DPX WAX/RP 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/ RECONSTITUTE	*400 µL in 10% Methanol*
6 INJECT	*Clean, Analyte-Rich Extract*

WORKFLOW

Well plates containing oral fluid samples, water, 30% methanol, and elution solvent (1% formic acid in acetonitrile) are prepared. DPX tips (WAX/RP, 1 mL) are conditioned by aspirating 30% methanol. Following this step, the sample solutions (400 µL oral fluid, 10 µL ISTD) are aspirated and dispensed four times in order to bind the analytes of interest to the sorbent. Water is then aspirated and dispensed to rid the tip of any free salts. Analytes of interest are eluted by aspirating and dispensing 1% FA in acetonitrile.

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.

APPLICATION NOTE

RESULTS AND DISCUSSION

Analytical results are linear, accurate and precise. Correlation coefficients (R^2) were greater than 0.99 over the concentration range of 0.4–250 ng/mL, with the majority of analytes exhibiting linearity over the range of 1.5–500 ng/mL. Relative standard deviations (%RSDs) were calculated using 3 replicate extractions (50 ng/mL) and ranged from 1.2–9%. 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 0.30–10 ng/mL. Limits of quantitation (LOQs) were calculated as 10(σ/m) and ranged from 0.9–30 ng/mL (Table 1).

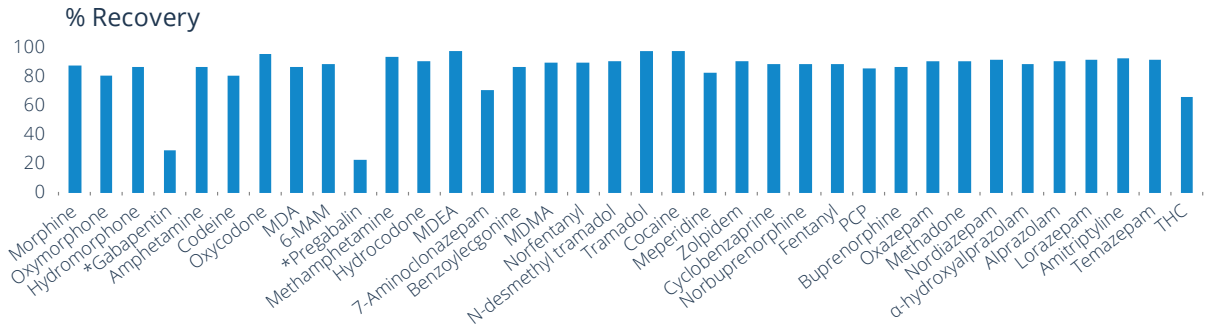
Note that LODs and LOQs are highly dependent on the laboratory's analytical method and LC-MS/MS sensitivity.

CONCLUSION

When combined with the Integra Viaflo 96 semi-automated liquid handling system, 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=3)	LOD (ng/mL)	LOQ (ng/mL)
Morphine	0.9986	4.8	1.2	3.5
Oxymorphone	0.9996	4.8	0.30	1.0
Hydromorphone	0.9989	4.3	0.40	1.2
Gabapentin*	0.9984	5.2	2.4	7.2
Amphetamine	0.9927	8.9	2.5	7.5
Codeine	0.9989	3.9	0.40	1.3
Oxycodone	0.9989	4.9	1.5	4.5
MDA	0.9940	9.1	2.3	7.0
6-MAM	0.9951	7.7	0.40	1.2
Pregabalin*	0.9892	2.1	12	36
Methamphetamine	0.9996	2.8	0.30	0.90
Hydrocodone	0.9987	2.1	0.50	1.6
MDEA	0.9944	6.7	2.7	8.2
7-Aminoclonazepam	0.9939	2.5	0.60	1.6
Benzoylecgonine	0.9992	2.8	0.50	1.5
MDMA	0.9986	5.7	0.50	1.6
Norfentanyl	0.9981	4.7	0.30	0.90
N-desmethyl tramadol	0.9924	5.1	1.1	3.3
Tramadol	0.9961	5.9	0.30	1.0
Cocaine	0.9940	5.3	0.50	1.5
Meperidine	0.9956	3.9	2.8	8.2
Zolpidem	0.9975	1.2	1.2	3.5
Cyclobenzaprine	0.9914	5.3	1.0	3.0
Norbuprenorphine	0.9935	7.9	0.50	1.5
Fentanyl	0.9961	2.4	0.07	0.21
PCP	0.9959	6.5	0.50	1.5
Buprenorphine	0.9917	6.1	0.86	2.6
Oxazepam	0.9987	2.8	0.50	1.6
Methadone	0.9988	4.7	0.70	2.0
Nordiazepam	0.9989	3.5	0.80	2.4
α-hydroxyalprazolam	0.9922	6.3	1.0	3.0
Alprazolam	0.9987	3.7	1.2	3.7
Lorazepam	0.9942	3.7	0.90	2.6
Amitriptyline	0.9983	3.1	0.80	2.5
Temazepam	0.9994	3.2	0.40	1.1
THC	0.9912	5.0	1.5	4.5



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