Comprehensive Dispersive Pipette XTRaction of Drugs/Metabolites in Oral Fluid

HIGHLIGHTS: Less solvent volumes, <8 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 INTEGRA VIAFLO96 semi-automated liquid handling system coupled with XTR tips reduces manual processing steps. In less than 8 minutes 96 samples can be extracted, and this method provides the sensitivity necessary for clinical and forensic analysis.

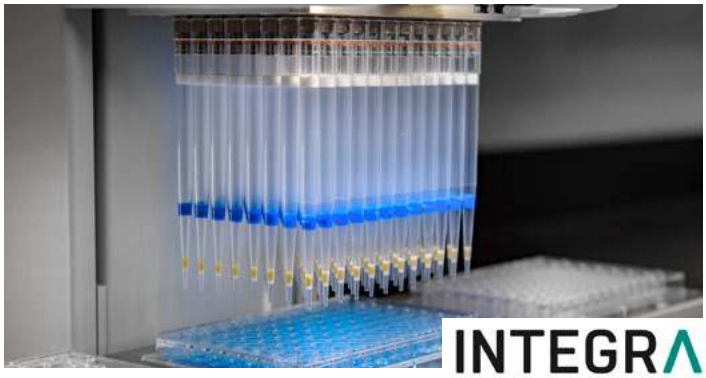
Sample preparation is required in order to remove matrix interferences from oral fluid samples prior to LC-MS/MS analysis. Multi-step SPE column preparation and multiple extractions add to turn-around-time and creating a "bottle neck" in sample throughput for laboratories.

To address SPE limitations, we have developed a rapid method for comprehensive analysis of oral fluid using Dispersive Pipette XTRaction technology. XTR tips containing a loose mixed mode WAX/RP sorbent 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

Well plates containing oral fluid samples, water, 30% methanol, and elution solvent (1% formic acid in acetonitrile) are prepared. 1 mL XTR tips with mixed mode WAX/RP sorbent 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



INTEGRA VIAFLO96 with XTR Tips

Table 1. Sample Preparation

1	CONDITION	Aspirate/Dispense 30% 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 Acetonitrile
5	EVAPORATE/ RECONSTITUTE	400 µL in 10% Methanol

Poroshell EC-C18 column (3.0 x 50 mm, 2.7 µm) 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 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 2).

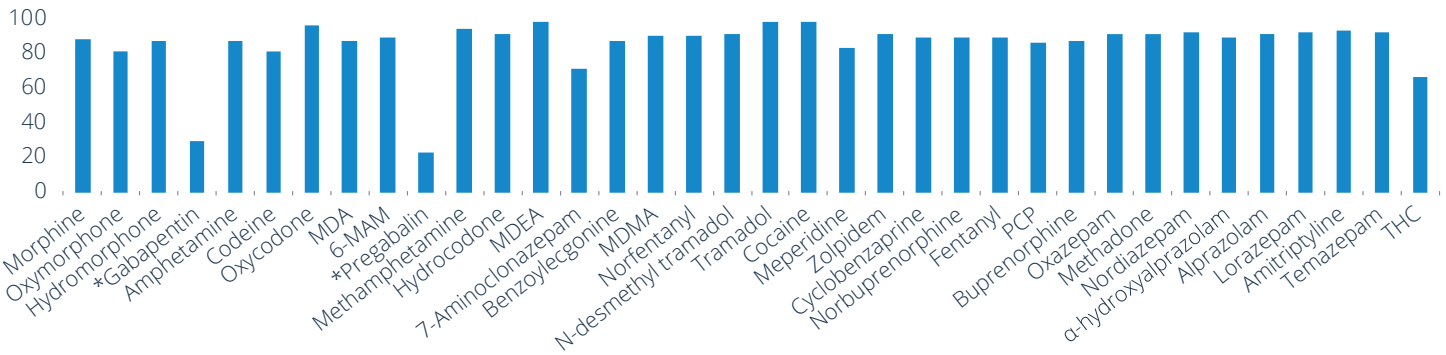
Note that LODs and LOQs are highly dependent on the laboratory's analytical method and LC-MS/MS sensitivity.

CONCLUSIONS

When combined with the INTEGRA VIAFLO96 semi-automated liquid handling system, Dispersive Pipette XTRaction 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 2. Comprehensive Extraction of Drug and Metabolites-Validation Data

Compound	R^2	3.2% RSD (n=6)	LOD (ng/mL)	LOQ (ng/mL)
Morphine	0.9986	4.8	1.2	3..5
Oxymorphone	0.9996	4.8	0.3	1
Hydromorphone	0.9989	4.3	0.4	1.2
Gabapentin	0.9984	5.2	10	30
Amphetamine	0.9927	8.9	2.5	7.5
Codeine	0.9989	3.9	0.4	1.3
Oxycodone	0.9989	4.9	1.5	4.5
MDA	0.9940	9.1	2	7
6-MAM	0.9951	7.7	0.4	1.2
Pregabalin	0.9892	2.1	10	30
Methamphetamine	0.9996	2.8	0.3	0.9
Hydrocodone	0.9987	2.1	0.5	1.6
MDEA	0.9944	6.7	10	30
7-Aminoclonazepam	0.9939	2.5	0.6	1.6
Benzoylecgonine	0.9992	2.8	0.5	1.5
MDMA	0.9986	5.7	0.5	1.6
Norfentanyl	0.9981	4.7	0.3	0.9
N-desmethyl tramadol	0.9924	5.1	1.1	3.3
Tramadol	0.9961	5.9	0.3	1
Cocaine	0.9940	5.3	0.5	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	1.5	4.6
Norbuprenorphine	0.9935	7.9	0.5	1.5
Fentanyl	0.9961	2.4	0.12	0.36
PCP	0.9959	6.5	0.5	1.5
Buprenorphine	0.9917	6.1	0.5	1.5
Oxazepam	0.9987	2.8	0.8	2.5
Methadone	0.9988	4.7	0.5	1.6
Nordiazepam	0.9989	3.5	0.8	2.4
α-hydroxyalprazolam	0.9922	6.3	1	3
Alprazolam	0.9987	3.7	2.5	7.5
Lorazepam	0.9942	3.7	0.5	1.7
Amitriptyline	0.9983	3.1	0.8	2.5
Temazepam	0.9994	3.2	0.8	2.4
THC	0.9912	5.0	1.5	4.5



Analyte recoveries following single extraction of oral fluid with WAX/RP - 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.