Comprehensive Dispersive Pipette XTRaction of Drugs/Metabolites in Urine Using Integra

HIGHLIGHTS: Cleaner extracts <10 minutes

Mixed Mode WAX/RP - XTR

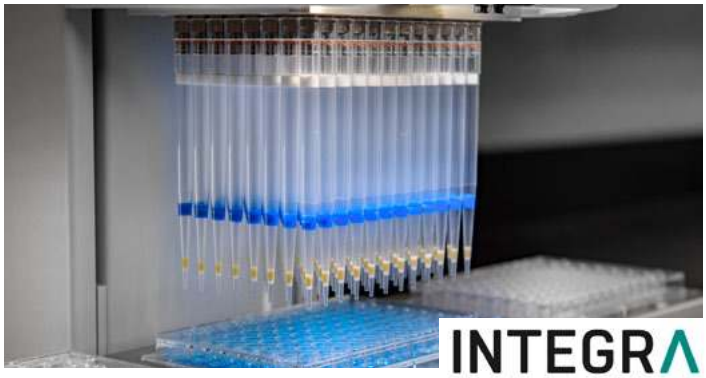
INTRODUCTION

Clinical and forensic laboratories have historically used liquid or solid phase extraction (SPE) methods for analysis of drugs and metabolites in urine. SPE methods produce clean extracts with consistent, high quality data and ensure long term robustness of mass spectrometers. Yet the time-consuming nature and relatively high cost of SPE have pushed laboratories to implement alternative strategies. Approaches have focused on expensive LC-MS/MS instrumentation using various "dilute and shoot" (D/S) sample preparation methods to address "dirty" or complex samples. Although D/S methods are perceived as "inexpensive", high end ultra-sensitive instrumentation, reduced LC column life, frequent LC-MS/MS maintenance, repeat sample injections, and increased data analysis time are often required.

Dispersive Pipette XTRaction 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 solution by simply aspirating and dispensing. Incorporating XTR tips with the Integra Viaflo 96 semi-automated liquid handling system eliminates the tedious and labor intensive elements of sample preparation. In less than 10 minutes, 96 samples are simultaneously extracted, which results in higher throughput when compared to traditional SPE methods.

MATERIALS AND METHODS

Well plates of hydrolyzed urine, water, 30% methanol, and 1% formic acid in methanol are prepared. XTR tips with mixed mode WAX/RP sorbent are conditioned by aspirating 30% methanol. Following this step, the sample solutions (150 µL urine, buffer, enzyme, internal standard; 250 µL total volume) are aspirated and dispensed three times in order to bind the analytes. Water is then aspirated and dispensed to remove any sample matrix components such as free salts, urea and creatinine. Analytes of interest are eluted by aspirating and dispensing 1% FA in methanol.



Integra VIAFLO96 with XTR Tips

Table 1. Sample Preparation

1	CONDITION	Aspirate/Dispense 30% Methanol
2	BIND ANALYTES	Aspirate/Dispense Hydrolyzed Urine
3	WASH	Aspirate/Dispense Water
4	ELUTE ANALYTES	Aspirate/Dispense Acidified Methanol
5	DILUTE INJECT	Add Water Clean, analyte-rich extract ready for analysis

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.

RESULTS AND DISCUSSION

Analytical results are linear, accurate and precise. Correlation coefficients (R^2) were greater than 0.99 over the concentration range of 12.5–400 ng/mL, with the majority of analytes exhibiting linearity over the range of 6.25–1600 ng/mL. Relative standard deviations (%RSDs) were calculated using 4 replicate extractions

(400 ng/mL) and ranged from 1.6–8%. Limits of detection (LODs) were calculated as 3.3(σ/m) where σ is the standard deviation of the lowest nonzero calibrator and m is the slope of the calibration curve. Limits of detection ranged from 0.50–18 ng/mL.

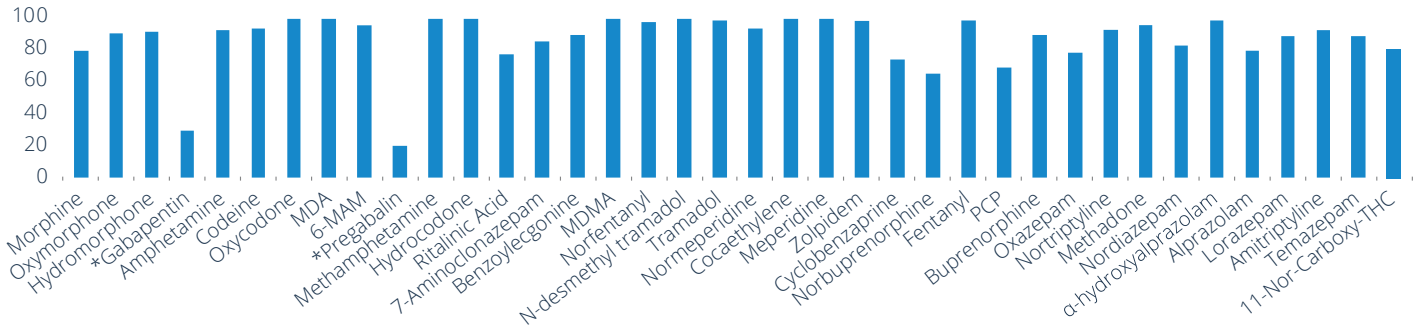
Limits of quantitation (LOQs) were calculated as 10(σ/m) and ranged from 1.5–39 ng/mL (Table 2). LODs and LOQs are highly dependent on the laboratory's analytical method and LC-MS/MS sensitivity. In order to maximize sensitivity, larger urine volumes may be extracted and/or sample elution volumes may be increased (500 µL) with subsequent solvent evaporation.

CONCLUSIONS

Reduced turnaround time and increased throughput are essential to reduce costs since "time is money." When compared to traditional SPE methods, lower direct costs are achieved with semiautomated platforms and miniaturization of XTR tips and from less solvent and waste volumes. Cleaner extracts minimize the likelihood of repeat analyses due to matrix interferences and/or low sensitivity, as well as the need to purchase more expensive LC-MS/MS systems, often seen with "dilute and shoot" methods. Instrument downtime is also reduced by preventing contamination of the LC-MS/MS system. When combined with the Integra Viaflo 96 semi-automated liquid handling system, Dispersive Pipette XTRaction technology provides comprehensive, rapid and easy-to-use sample preparation—a custom solution that is ideal for high throughput clinical and forensic laboratories.

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

Compound	R^2	% RSD (n=6)	LOD (ng/mL)	LOQ (ng/mL)
Morphine	0.9974	5.6	1.5	4.5
Oxymorphone	0.9982	4.9	2.5	7.5
Hydromorphone	0.9982	3	5.7	17.1
Gabapentin	0.9989	1.7	10	30
Amphetamine	0.9944	3.1	9.3	28.2
Codeine	0.9972	7.1	7.7	23.1
Oxycodone	0.9968	8	9.8	28.9
MDA	0.9940	6.4	13	39
6-MAM	0.9932	4.6	1	3
Pregabalin	0.9972	1.1	10	30
Methamphetamine	0.9993	5.3	12.7	38.1
Hydrocodone	0.9949	2.2	6.5	19.5
Ritilinic Acid	0.9945	5.2	3.5	10.3
7-Aminoclonazepam	0.9959	1.3	3.8	11.2
Benzoylecognine	0.9943	2.4	5.5	16.4
MDMA	0.9975	1.7	11.8	35.3
Norfentanyl	0.9970	7.9	2	6
N-desmethyl tramadol	0.9956	9.3	9	28
Tramadol	0.9962	4.5	8.7	26
Normeperidine	0.9937	3.3	5	15.7
Cocaethylene	0.9981	8.9	8.2	24.8
Meperidine	0.9960	3.3	4.4	13.2
Zolpidem	0.9975	3.4	7.8	23.4
Cyclobenzaprine	0.9973	3.4	10	30
Norbuprenorphine	0.9912	4.9	3	10
Fentanyl	0.9979	3.4	0.5	1.5
PCP	0.9967	4.7	1	4
Buprenorphine	0.9914	7.6	1	4
Oxazepam	0.9981	7.7	4.4	13.1
Nortriptyline	0.9943	6.7	10	30
Methadone	0.9932	3.1	7.7	23.2
Nordiazepam	0.9970	7.4	7.2	21.7
α-hydroxyalprazolam	0.9922	6.3	6.4	19.2
Alprazolam	0.9937	3.7	13	40
Lorazepam	0.9984	3.7	2.4	7.3
Amitriptyline	0.9983	3.6	10	30
Temazepam	0.9954	4.3	6.8	20.3
11-Nor-Carboxy-THC	0.9985	9.3	4	12



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.