Automated Comprehensive Dispersive Pipette XTRaction of Drugs/ Metabolites in Urine Using Tecan

HIGHLIGHTS: <10 minutes for clean extractions

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 a variety of "dilute and shoot" (D/S) sample preparation methods to address "dirty" samples. While D/S methods are perceived as "inexpensive", high-end ultrasensitive 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. With seamless integration onto robotic liquid handlers, XTR tips eliminate the tedious and labor intensive aspects of sample preparation. In less than 10 minutes, 96 samples are simultaneously extracted, resulting in higher throughput when compared to D/S methods.

MATERIALS AND METHODS

Well plates containing hydrolyzed urine and reservoirs containing water, 30% methanol and 1% formic acid (FA) in methanol are loaded onto the TECAN EVO system. The TECAN system fills a well plate with 200 µL of water and another well plate with 150 µL of 1% FA in methanol. TheXTR tips with mixed mode WAX/RP sorbent are picked up and conditioned in a solvent reservoir with 30% methanol. After conditioning, WAX/RP-XTR tips aspirate and dispense the hydrolyzed samples three times in order to bind the analytes. Water is then aspirated and dispensed to remove any free salts, urea and creatinine. The analytes of interest are concentrated using low volume elution to avoid solvent evaporation.

Based on LC conditions, the eluent is diluted until an appropriate percentage of methanol is achieved. In this case, 1050 µL of water was added by the TECAN system for a total volume of 1200 µL (12.5% methanol). 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.

Table 1. Sample Preparation

1	CONDITION	Aspirate/Dispense 30% Methanol
2	BIND ANALYTES	Aspirate/Dispense Hydrolyzed Urine using WAX/RP - XTR tips
3	WASH	Aspirate/Dispense Water
4	ELUTE ANALYTES	Aspirate/Dispense Acidified Methoanol
5	DILUTE	Add Water
6	INJECT	Clean, Analyte-rich Extract

RESULTS AND DISCUSSION

Analytical results were 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 6.25-800 ng/mL. Relative standard deviations (%RSDs) were calculated using 4 replicate extractions (400 ng/mL) and ranged from 1.7-9.7%. 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. LODs ranged from 0.25-12 ng/mL. Limits of quantitation (LOQs) were calculated as 10 (σ/m) and ranged from 1.0-60 ng/mL (Table 2).

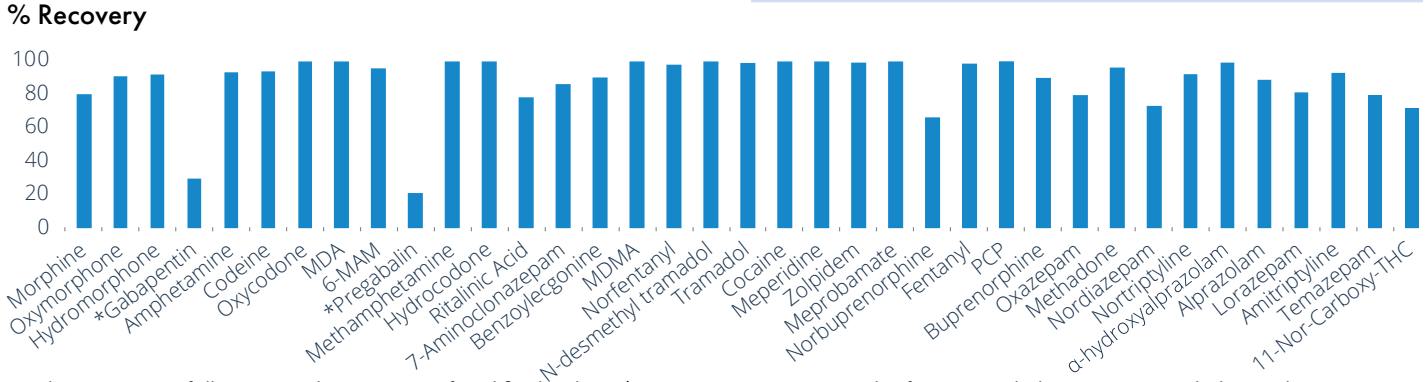
LODs and LOQs are highly dependent on the laboratory's analytical method and LC-MS/MS sensitivity. In order to maximize sensitivity, larger urine volumes may be extracted and/or sample elution volumes may be increased (500 µL) with subsequent solvent evaporation.

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 the automated platform 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 minimized by preventing contamination of the LC-MS/MS system. Dispersive Pipette XTRaction technology provides comprehensive, rapid and easy-to-use sample preparation when combined with the Tecan Freedom EVO automated liquid handling system—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	4.5	13.5
Oxymorphone	0.9956	9.2	4.9	14.7
Hydromorphone	0.9974	6.6	7.4	22.3
Gabapentin	0.9948	4.8	20	60
Amphetamine	0.9965	7.2	12	36
Codeine	0 .9916	9.7	3.75	11.2
Oxycodone	0.9967	4.4	2.7	8.2
MDA	0.9968	6	2.5	7.4
6-MAM	0.9937	7.6	0.73	2.19
Pregabalin	0.9907	6.2	13	39
Methamphetamine	0.9973	2.5	1.11	3.33
Hydrocodone	0.9935	8.2	3	9
Ritilinic Acid	0.996	3.1	5	15
7-Aminoclonazepam	0.9982	7.7	5	15
Benzoylecgonine	0.9945	6.5	5	15
MDMA	0.9969	3.1	2	6.3
Norfentanyl	0.9931	6.5	1	4
N-desmethyl tramadol	0.9925	6.6	8	25
Tramadol	0.9959	8.9	4	12
Cocaine	0.999	5.6	6.7	20
Meperidine	0.9917	5.2	5.5	16
Zolpidem	0.9932	6.5	8.8	27
Cyclobenzaprine	0.9915	8.7	1.15	3.5
Norbuprenorphine	0.9918	8.7	2.8	8.5
Fentanyl	0.9952	8	0.25	1
PCP	0.998	2.9	4.8	14
Buprenorphine	0.9917	8.5	1.5	4.5
Oxazepam	0.9957	4.8	2.3	7
Methadone	0.9955	8.5	3.2	9.7
Nordiazepam	0.9957	9	5.6	16.8
Nortriptyline	0.9943	5.4	1.69	5.07
α-hydroxyalprazolam	0.9925	4.4	1.8	5.5
Alprazolam	0.9952	5.7	12	38
Lorazepam	0.995	3.5	5	15
Amitriptyline	0.9958	5.1	3.39	10.2
Temazepam	0.9974	7	3	9
11-Nor-Carboxy-THC	0.9846	1.7	2.8	8.5



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.