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

INTEGRA

COMPREHENSIVE URINE

APPLICATION NOTE

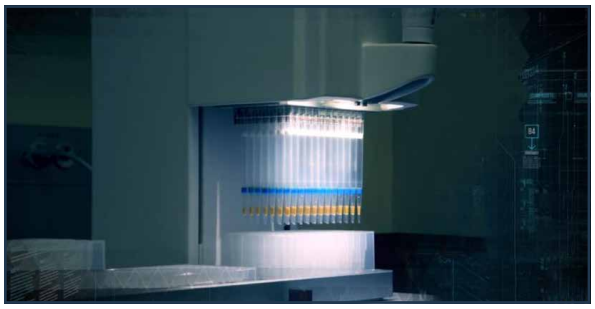
Clinical and forensic laboratories have historically used liquid or solid phase extraction (SPE) methods for analysis of drugs and metabolites in urine. SPE methods produce clean extracts with consistent, high quality data and ensure long term robustness of mass spectrometers. Yet the time-consuming 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 ultrasensitive instrumentation, reduced LC column life, frequent LC-MS/MS maintenance, repeat sample injections, and increased data analysis time are often required.

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 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 10 minutes, 96 samples are simultaneously extracted-resulting in higher throughput when compared to traditional SPE methods.

WORKFLOW

Well plates of hydrolyzed (IMCSzyme® β-glucuronidase) urine, water, 30% methanol, and 1% formic acid in methanol are prepared. DPX tips (WAX/RP) 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.

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.



Integra Viaflo 96 with DPX WAX/RP Tips

1 CONDITION TIPS	*Aspirate and Dispense 30% Methanol*
2 BIND ANALYTES	*Aspirate and Dispense Hydrolyzed Urine*
3 WASH	*Aspirate and Dispense Water*
4 ELUTE ANALYTES	*Aspirate and Dispense Acidified Methanol*
5 DILUTE	*Add Water*
6 INJECT	*Clean, Analyte-Rich Extract*

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 non-zero 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 1).

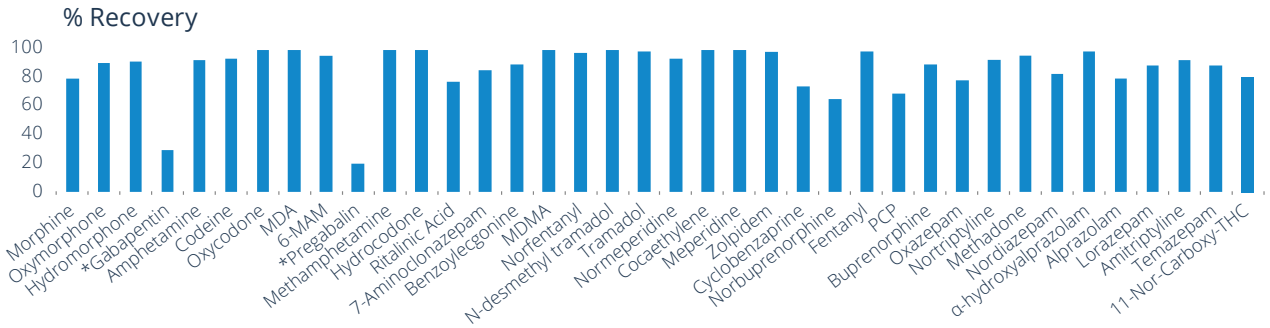
LODs and LOQs are highly dependent on the laboratory's analytical method and LC-MS/MS sensitivity. In order to maximize sensitivity, larger urine volumes may be extracted and/or sample elution volumes may be increased (500 µL) with subsequent solvent evaporation.

CONCLUSION

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 semi-automated platforms and miniaturization of DPX 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, DPX technology provides comprehensive, rapid and easy-to-use sample preparation—a custom solution that is ideal for high throughput clinical and forensic laboratories.

Table 1. Validation Data

Compound	R^2	% RSD (n=4)	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	3.0	5.7	17
Gabapentin*	0.9989	1.7	10	30
Amphetamine	0.9944	3.1	9.3	28
Codeine	0.9972	7.1	7.7	23
Oxycodone	0.9968	8.0	9.8	29
MDA	0.9940	6.4	13	39
6-MAM	0.9932	4.6	1.0	3.0
Pregabalin*	0.9972	1.1	10	30
Methamphetamine	0.9993	5.3	13	38
Hydrocodone	0.9949	2.2	6.5	19
Ritalinic Acid	0.9945	5.2	3.5	10
7-Aminoclonazepam	0.9959	1.3	3.8	11
Benzoylecgonine	0.9943	2.4	5.5	16
MDMA	0.9975	1.7	12	35
Norfentanyl	0.9970	7.9	2.0	6.0
N-desmethyl tramadol	0.9956	9.3	9.0	28
Tramadol	0.9962	4.5	8.7	26
Normeperidine	0.9937	3.3	5.0	16
Cocaethylene	0.9981	8.9	8.2	25
Meperidine	0.9960	3.3	4.4	13
Zolpidem	0.9975	3.4	7.8	23
Cyclobenzaprine	0.9935	6.1	7.5	22
Norbuprenorphine	0.9912	4.9	3.0	10
Fentanyl	0.9979	3.4	0.50	1.5
PCP	0.9967	4.7	1.0	4.0
Buprenorphine	0.9914	7.6	1.0	4.0
Oxazepam	0.9981	7.7	4.4	13
Nortriptyline	0.9943	6.7	10	30
Methadone	0.9932	3.1	7.7	23
Nordiazepam	0.9970	7.4	7.2	22
α-hydroxyalprazolam	0.9922	6.3	6.4	19
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
11-Nor-Carboxy-THC	0.9985	9.3	4.0	12



Analyte recoveries following single extraction of hydrolyzed urine with 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.