SEMI-AUTOMATED SIMULTANEOUS EXTRACTION OF 96 URINE SAMPLES UTILIZING THE INTEGRA VIAFLO 96 FOR ANALYSIS OF DRUGS OF ABUSE BY LC-MS/MS

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Clinical Toxicology, Application Note

INTEGRA

SUMMARY

DPX Mixed Mode tips provide a fast, accurate, and simple extraction method for analyzing drugs of abuse in urine. The Integra Viaflo 96 system allows for high throughput semi-automated sample processing. One 96 well plate can be extracted and ready for LC-MS/MS injection in under ten minutes with no need for evaporation.

INTRODUCTION

Sample preparation is required to remove matrix interferents from urine samples prior to LC-MS/MS analysis. This procedure is typically very time-consuming and is generally the "bottle neck" for laboratory analysis and throughput. DPX extraction is a dispersive SPE method that requires much less solvent and time-consuming steps compared to other SPE techniques. The method established herein is highly reproducible and provides the necessary sensitivity for forensic and clinical purposes.

EQUIPMENT

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 50mm, 2.7um) with a 10 uL injection. Extractions were performed on a Integra Viaflo 96 (part# 6001), with a 300ul head (part# 6103) with the 3-position stage (part # 6230) using DPX mixed mode tips (2mg WAX, 1mg RP).

EXPERIMENTAL

Well plate containing hydrolyzed urine are loaded on to the Integra system. Pre-filled well pates for water wash and elution solvent are also loaded onto the Integra. The DPX tips were then conditioned by aspirating 30% methanol from a solvent reservoir. After conditioning, the sample solutions (150 uL urine, 250 uL total volume (buffer, enzyme, internal std mix)) were aspirated and dispensed three times in order to bind the drugs of abuse to the sorbent.

BIND ANALYTES
Aspirate and Dispense 150ul Hydrolyzed Urine with Mixed Mode DPX tip

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WASH RESIN
Aspirate and Dispense Water

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ELUTE ANALYTES
Aspirate and Dispense 150ul Acidified Methanol

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DILUTE
Add 750ul D.I. Water

↓

INJECT
Clean Analyte-Rich Sample

Extraction In Seconds

Water was then aspirated and dispensed. The analytes of interest are eluted by aspirating and dispensing the 1% formic acid in methanol three times.

RESULTS and DISCUSSION

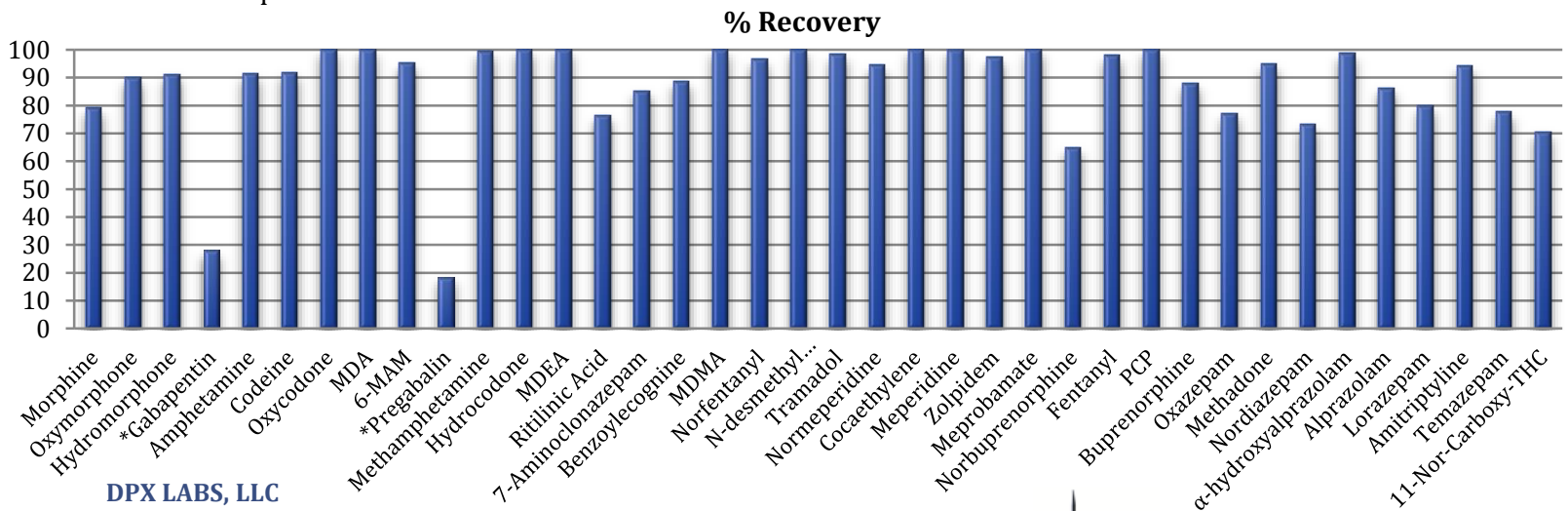
From start to finish, this semi-automated dispersive SPE method takes under ten minutes to prepare one 96 well plate. Results from this method are linear, accurate, and reproducible. All correlation coefficients were greater than 0.99 for the range of at least 12.5-400 ng/mL, with most analytes being linear from 6.25-800 ng/mL. Relative standard deviation was calculated using 4 replicate extractions at 400 ng/mL and ranged from 1.1 to 9.3. Limits of detection 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 line. Limit of quantitation was calculated as 10*σ/m. Limits of detection ranged from 0.50 to 13 ng/mL and limits of quantitation ranged from 1.5 to 38 ng/mL.

It should be emphasized that the LOD and LOQ are highly dependent on the sensitivity of the LC/MS instrumentation and method. If sensitivity needs to be improved to lower LODs and LOQs, solvent evaporation can be employed to concentrate the extracts. The elution volume can also be increased to 500 uL to increase recoveries. Alternatively, a larger volume of sample solution can also be extracted to provide lower LODs/LOQs.

CONCLUSION

The method described herein provides the necessary recoveries, sensitivity, and reproducibility for a reliable sample preparation method in a high throughput setting. Semi-automation of the sample preparation method makes the analysis non-tedious as well as rapid.

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	17.1
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	8.0	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
Nortriptyline	0.9943	6.7	10	30
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	.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
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



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Extraction In Seconds