APPLICATION NOTE

RAPID, AUTOMATED LC-MS/MS ANALYSIS OF DRUGS OF ABUSE IN ORAL FLUID USING DISPERSIVE PIPETTE XTRACTION

WAX + SCX

INTRODUCTION

Analysis of oral fluid generally requires some type of sample preparation to concentrate the analyte and reduce matrix effects for sensitive and reproducible analyses. The use of Dispersive Pipette XTRaction™ (XTR) tips has been shown to offer advantages over traditional solid-phase extraction (SPE) products in terms of speed and seamless integration with automated liquid handlers.

Most SPE methods for analyzing drugs of abuse in oral fluid incorporate a strong acid in order to achieve high recoveries of basic drugs using strong cation exchange sorbent. However, there have been reports[1] that this acid may lead to conversion of cannabidiol (CBD) to tetrahydrocannabinol (THC). In this research study, we used mixed mode XTR tips without the addition of a strong acid in order to avoid possible conversion of CBD to THC. This study focuses on analyzing 45 drugs of abuse commonly found in driving-under-the-influence (DUI) cases. The analysis uses a single extraction method and high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS). A separate LC-MS/MS method is also presented for differentiation of Δ^8 and Δ^9-THC (for positive THC cases). We evaluated whether XTR tips for INTip™ SPE could be used for simultaneously extracting a 45 drug/metabolite panel from Quantisal™-collected oral fluid suitable for Driving Under the Influence of Drugs (DUID) testing.

WORKFLOW

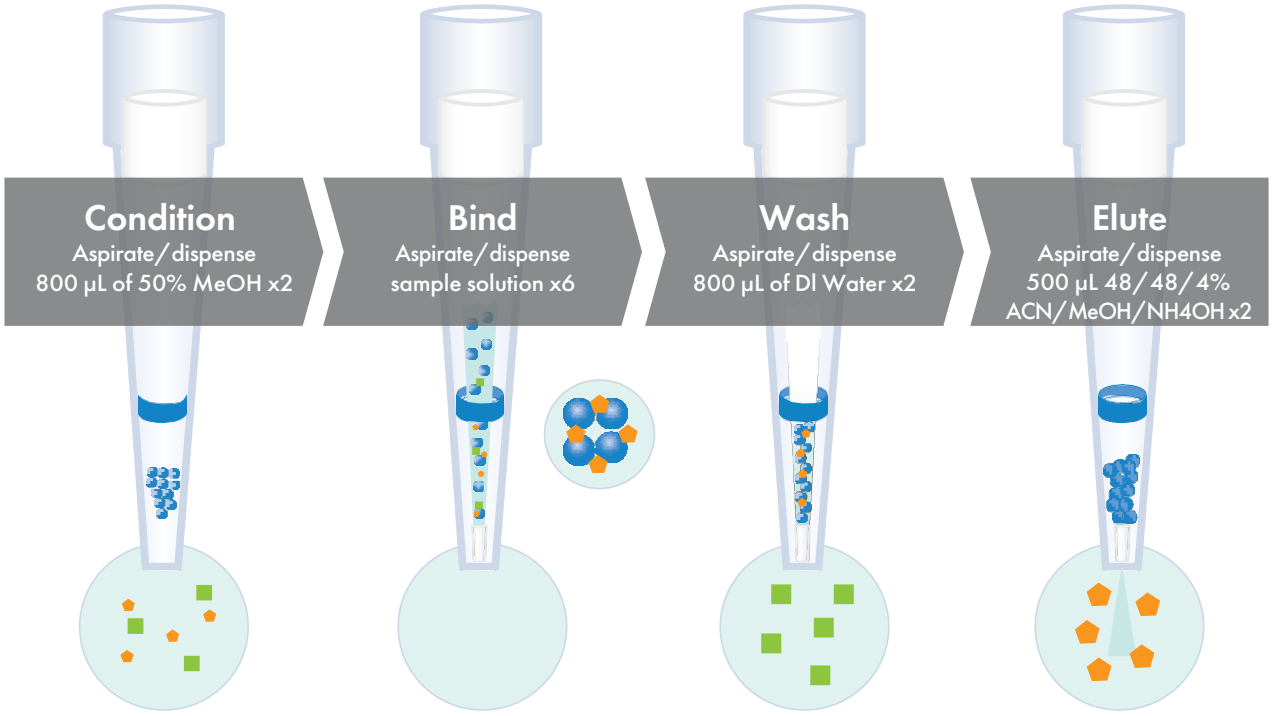

Figure 1. Sample preparation using Dispersive Pipette XTRaction. Aspirate and dispense steps mix loose sorbent chemistry with the oral fluid sample solution. The disperser in XTR tips perturbs the sample solution and loose sorbent creating a highly efficient interaction of the chemistry with the sample solution resulting in ideal analyte recoveries.

Sample Preparation: 500 µL of synthetic negative saliva from Immunalysis Corp. and buffer solutions were fortified with 50 µL of a comprehensive drug standard (45 drugs) including: opiates, opioids, benzodiazepines, barbiturates, THC (Δ^8, Δ^9, and CBD), antidepressants, amphetamines, and cocaine. Concentrations ranged, depending on the analytes, as low as 0.1 ng/mL to as high as 500 ng/mL. A Hamilton Microlab Nimbus-96 was used to automate extractions of oral fluid sample using XTR tips with mixed mode sorbent, 3 mg of WAX + 3 mg of SCX, from DPX Technologies.

The extraction steps are shown in Figure 1. The solutions were subsequently evaporated (using a Biotage SPE dry 96 system at 50°C) and reconstituted in 125 µL of 10% methanol in water.

LC-MS/MS Method: All analyses were performed using a SCIEX 6500+ MS system coupled to an Agilent 1260 LC system using mobile phases of pH 3.6 ammonium formate and LC/MS grade methanol.

Comprehensive Analysis:
Phenomenex Kinetex 2.6 µm biphenyl 50x3.0mm

Time	Flow	%A	%B
0.00	500	85.00	15.0
0.10	500	85.00	15.0
6.00	500	5.0	95.0
8.00	500	5.0	95.0
8.20	500	85.0	15.0
9.50	500	85.0	15.0

Δ^9-THC and Δ^8-THC Analysis:
Restek Raptor FluoroPhenyl 2.7 µm 150x3.0mm

Time	Flow	%A	%B
0.00	800	28.0	72.0
7.5	800	28.0	72.0
7.6	800	0.0	100.0
8.50	800	0.0	100.0
8.60	800	28.0	72.0
11.0	800	28.0	72.0

RESULTS

The sample preparation using XTR tips on the Hamilton Microlab Nimbus-96 was performed in less than 10 minutes, extracting 96 samples
at a time. Figure 2 shows the recoveries for the comprehensive analysis of drugs of abuse with most drugs having greater than 80% recovery. Drugs fortified at suggested cutoffs[2] were readily detected at less than 8% C.V., as shown in Table 1. The comprehensive

LC-MS/MS method was used to detect THC and CBD, but a separate LC-MS/MS method was used (on the same extract from the comprehensive analysis) to detect Δ^8 and Δ^9-THC at levels as low as 0.25 ng/mL as shown in Figure 3.

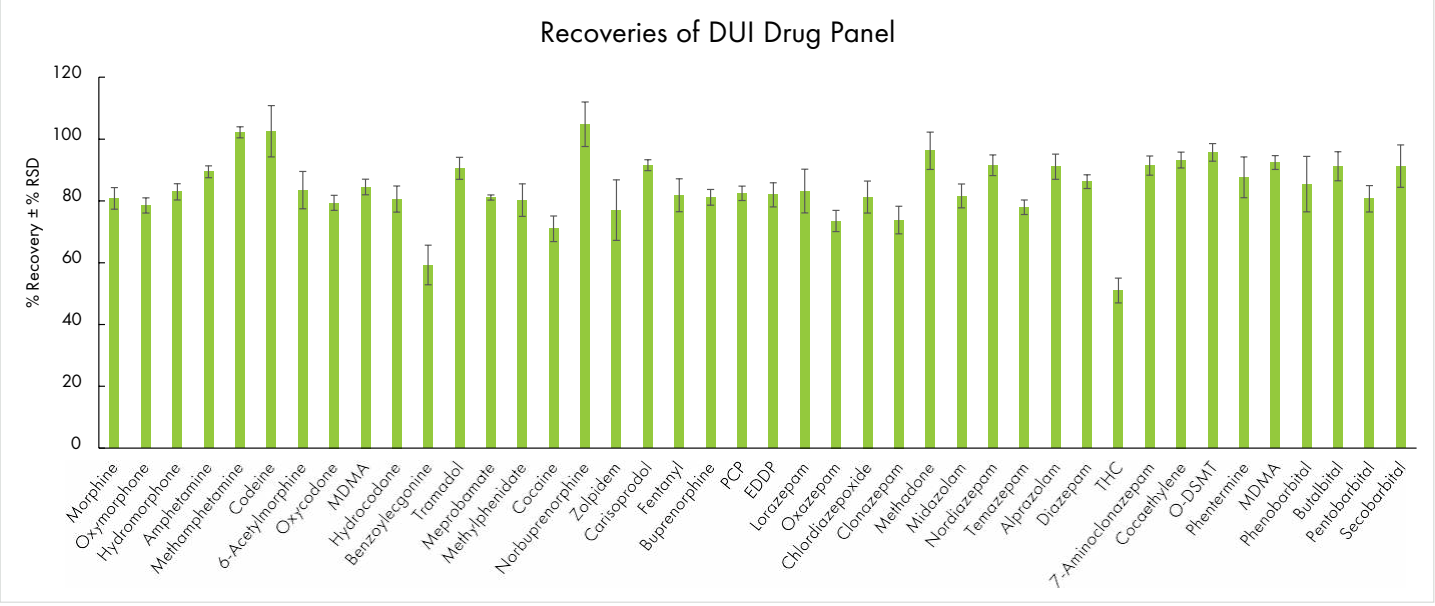

Figure 2. % recovery of drugs of abuse ± the % RSD.

Table 1. The LOQ values compared to the DUI Screening cut-off[2]. Note the LOQ for some of the drugs was the lowest calibrator utilized in this study.

Analyte	LOQ (ng/mL)	R^2	Cut-off for DUI Screen (ng/mL)
6-Acetylmorphine	1.0	0.999	4.0
Alprazolam	0.05	0.999	N/A
7-Aminoclonazepam	0.10	0.995	N/A
Amphetamine	1.0	0.996	20
Benzoylecgonine	0.25	0.998	15
Buprenorphine	0.03	0.997	1
Butalbital	2.0	0.995	10
Carisoprodol	0.50	0.999	500
Chlordiazepoxide	0.03	0.998	1
Clonazepam	0.03	0.999	N/A
Cocaethylene	0.50	0.999	N/A
Cocaine	0.25	0.999	15
Codeine	2.5	0.998	30
Diazepam	0.05	0.999	N/A
EDDP	0.50	0.999	20
Fentanyl	0.10	0.986	1
Hydrocodone	1.0	0.999	30
Hydromorphone	0.50	0.999	30
Lorazepam	0.10	0.997	N/A
MDA	2.0	0.988	20
MDMA	1.0	0.998	20
Meprobamate	0.50	0.999	100
Methadone	0.03	0.997	20
Methamphetamine	1.0	0.997	20
Methylphenidate	0.25	0.999	1
Midazolam	0.03	0.999	1
Morphine	1.0	0.996	30
Norbuprenorphine	0.03	0.999	N/A
Nordiazepam	0.03	0.999	N/A
O-Desmethyltramadol	1.0	0.998	N/A
Oxazepam	0.05	0.998	N/A
Oxycodone	0.50	0.997	30
Oxymorphone	1.0	0.998	30
PCP	1.0	0.999	10
Phentermine	5.0	0.988	20
Pentobarbital	5.0	0.991	N/A
Phenobarbital	10	0.993	10
Secobarbital	0.50	0.999	10
Temazepam	0.10	0.998	N/A
THC	0.25	0.999	4
Tramadol	2.0	0.999	50
Zolpidem	2.5	0.999	10

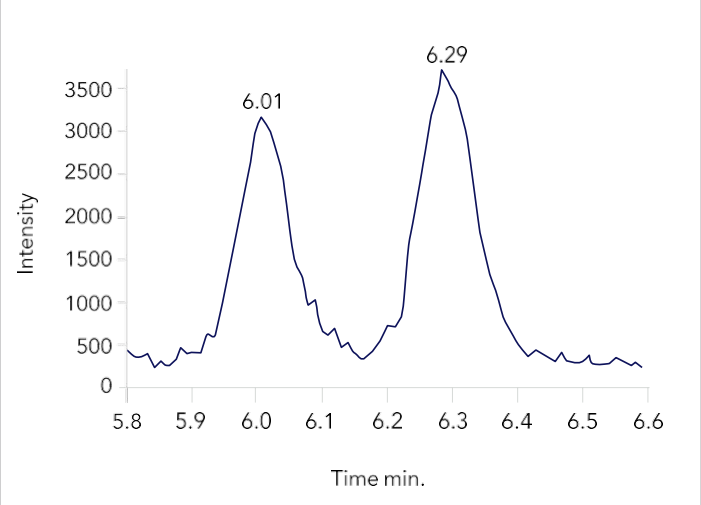

Figure 3. Chromatogram of extract of negative saliva fortified at 1 ng/mL delta-8 (6.01 m) and delta-9 THC (6.29 m). CBD (not shown) has a higher response at this concentration with a retention time at 3.20 m. The 150 mm length column was needed to get baseline resolution between the 2 isomers, which allows for accurate integrations. LOQ and LOD were 0.5 and 0.25 ng/mL for both compounds using this method.

CONCLUSION

This study demonstrates a rapid, efficient and automated method for analyzing comprehensive drugs of abuse in oral fluid using mixed-mode XTR tips. These data demonstrate an effective simultaneous extraction process for 45 drugs and metabolites from Quantisal™-collected oral fluid using XTR™ tips that could be suitable for DUID oral fluid testing.

REFERENCES

1. *C. Coulter, J.R. Wagner, ""Cannabinoids in Oral Fluid: Limiting Potential Sources of CBD Conversion to Δ9- and Δ8-Tetrahydrocannabinol", Journal of Analytical Toxicology, June 17, 2021; 00:1-6.*
2. *A. L. D'Orazio, et al., "Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities—2021 update.", Journal of Analytical Toxicology, June 4, 2021; 00:1-8.*