Automated extraction and analysis of drugs of abuse in oral fluid using disposable pipette extraction (DPX) and LC/MS/MS

[1]**William E. Brewer,** [2]Oscar G. Cabrices, [2]Fred D. Foster, [2]Edward A. Pfannkoch
[1]DPX Labs, LLC, 151 Powell Rd, Suite 116, Columbia, SC 29203
[2]GERSTEL Inc, 701 Digital Dr, Suite J, Linthicum, MD 21090

Abstract

A completely automated method of analysis of oral fluid for drugs of abuse is presented. The automated extraction utilizes disposable pipette extraction (DPX) tips to isolate and concentrate the drugs of interest from the oral fluid solution. The analytes were eluted into a low volume of organic solution, which was then injected into the LC/MS/MS instrument without solvent evaporation. This provides "just in time sample preparation", with the extractions taking app. 5 min to perform during a 7 min chromatographic analysis.

The study focused on the 5-panel drugs of abuse defined by the Substance Abuse and Mental Health Services Administration (SAMHSA) which includes opiates, THC, amphetamines, cocaine, and PCP. With no solvent evaporation, limits of detection (LOD) and limits of quantitation (LOQ) were found to be app. 5 ng/mL and 10 ng/mL, respectively, for most of the drugs of interest. THC was analyzed separately and had an LOD of 2 ng/mL and LOQ of 5 ng/mL.

Introduction

Oral fluid has become increasingly used as a specimen in many areas of forensic and clinical interest. The biggest challenges associated with oral fluid analysis are the requirement of low detection limits and complex sample matrix interferences. Due to these challenges, sample preparation is required prior to analysis. The solid-phase extraction method developed in this study used disposable pipette extraction (DPX) for comprehensive analysis of drugs of abuse using LC/MS/MS.

The primary objective of this study was to develop an automated, rapid and comprehensive method of extracting and analyzing drugs of abuse in oral fluid using LC/MS/MS. To make the method rapid, it is advantageous to implement a "just in time" sample preparation strategy in which the samples are extracted and ready for analysis in less time than the LC/MS chromatographic run. If the chromatographic run is less than 8 min, there is not ample time for solvent evaporation. Another goal would be to have all analytes separated and analyzed in a single chromatographic run.

Using this "just-in-time" approach, we successfully analyzed opiates, amphetamines, cocaine, and PCP at concentrations as low as 5-10 ng/mL, and THC at 2-5 ng/mL without solvent evaporation. With the exception of THC, all analytes were analyzed in a single chromatographic run using 15% acetonitrile for the elution solvent. To elute THC, acetonitrile was used. Injection of 20 µL of acetonitrile achieved detection limits at levels less than 5 ng/mL.

Incorporation of THC into the same chromatographic run is discussed, as well as achieving even lower detection limits without solvent evaporation.

Key Words

disposable pipette extraction, drugs of abuse, LC/MS/MS, DPX, automation

Figure 1. A picture of a GERSTEL MPS with the DPX option being used with an Agilent LC/MS/MS system. In this study, the LC/MS system was a model 6460 and the samples were extracted and eluted using 96 deep well plates.

Experimental

Materials

All drug standards were purchased from Cerilliant Corporation (Round Rock, TX, USA). The drugs (5 panel) and their corresponding deuterated internal standards included 1) opiates: morphine, oxymorphone, hydromorphone, codeine, oxycodone, hydrocodone); 2) amphetamines: amphetamine, methamphetamine, MDMA, MDEA and MDA ; 3) cocaine and benzoylecgonine,; 4) PCP and 5) THC.

Oral fluid was collected from volunteers using Quantisal collection kits (Immunalysis, Pomona, CA). The DPX tips (WAX-TA-di (20 mg)) were provided by DPX Labs (Columbia, SC). The DPX tips were made using a new patent pending technology to ensure "dispersive" extractions occur reproducibly during the automated extractions.

Methods

Automated DPX extractions were performed from 96 deep well plates using an automated prep sequence which included the steps of:

1. conditioning DPX-WAX tip with 30% methanol
2. aspirating 750 µL oral fluid solution into DPX tip
3. washing with 500 µL DI water
4. eluting with 250 µL 15% acetonitrile
5. eluting with 250 µL acetonitrile (for THC)
6. injecting 2 (or 20 µL) into LC/MS/MS system

Instrumentation

All analyses were performed using a GERSTEL MPS 2XL autosampler (Figure 1) configured with an active washstation coupled to an Agilent 6460 LC/MS/MS instrument with a Poroshell EC-C18 column (3.0 x 50mm, 2.7 µm). Sample injections were made using a 6 port (0.25mm) Cheminert C2V injection valve. Column temperature was set at 55 °C.

The LC mobile phase used was: A -- 5mM ammonium formate with 0.05% formic acid; B -- 0.05% formic acid in methanol. The gradient started at 98% A for 0.5 min, ramped to 30% B at 1.5 min, ramped to 70% B at 3.5 min, ramped to 95% B at 4.5 min and held to 6.4 min, then ramped back to 98% A. The flow rate was 0.5 mL/min.

Table 1. MS parameters of all drugs used in this analysis. Parameters were not optimized for this particular instrument.

Compound	Prec Ion	Prod Ion	Frag	CE	Cell Acc	Ret Time
6-monoacetyl	328.2	165.1	158	41	7	1.9
6-monoacetyl	328.2	58.1	158	29	7	1.9
Benzoylecgonine	290.1	168.1	118	17	7	2.38
Benzoylecgonine	290.1	105	118	29	7	2.38
Cocaine	304.2	182.1	138	17	7	2.56
Cocaine	304.2	77	138	61	7	2.56
Codeine	300.2	165.1	158	45	7	1.86
Codeine	300.2	128	158	60	7	1.86
D3-Cocaine	307.1	185.1	138	17	7	2.56
D5-Amphetamine	141.1	124.1	70	5	7	2.1
D5-Amphetamine	141.1	93.1	70	33	7	2.1
d-Amphetamine	136.1	119.1	66	5	7	2.1
d-Amphetamine	136.1	91	66	17	7	2.1
Hydrocodone	300.2	199	159	29	7	1.98
Hydrocodone	300.2	128	159	65	7	1.98
Hydromorphone	286.2	185	159	29	7	1.58
Hydromorphone	286.2	157	159	45	7	1.58
MDA	180.1	163	61	5	7	2.11
MDA	180.1	105	61	21	7	2.11
MDEA	208.1	163	107	9	7	2.26
MDEA	208.1	135	107	21	7	2.26
MDMA	194.1	163	97	9	7	2.13
MDMA	194.1	105	97	25	7	2.13
methamphetamine	150.1	119	92	5	7	2.14
methamphetamine	150.1	91	92	17	7	2.14
Morphine	286.2	165.1	158	41	7	1.43
Morphine	286.2	152	158	60	7	1.43
Morphine-D3	289	165.1	153	40	7	1.43
Morphine-D3	289	152	153	68	7	1.43
Oxycodone	316.2	298.1	143	17	7	1.92
Oxycodone	316.2	241.1	143	29	7	1.92
Oxymorphone	302.1	227.1	133	28	7	1.5
Oxymorphone	302.1	198.1	133	48	7	1.5
PCP	244.2	91	86	41	7	2.98
PCP	244.2	86.1	86	9	7	2.98
PCP-D5	249.3	96.1	97	36	7	2.98
PCP-D5	249.3	86.1	97	8	7	2.98
THC	315.9	193.6	120	20	7	5.35
THC	315.9	123.4	120	30	7	5.35
THC-d3	318.9	196.6	120	20	7	5.35
THC-d3	318.9	123.4	120	40	7	5.35

The LODs were calculated based on S/N ratios for target and qualifier ions being greater than 3 and ion ratios being within 20% of expected values. The LOQs were determined by calculating the accuracies of the quantitative results from the calibration plots to be within the range of 80% to 120% for acceptance (in addition to meeting the LOD standards).

Results & Discussion

Oral fluid analysis is a challenge for 4 primary reasons. First, the detection limits need to be low (around 10 ng/mL for most of the drugs). Second, the volume of oral fluid collected is low (1 mL at most). In addition, the solution is diluted by 4-fold during collection. Finally, the analysis is further exasperated by interferences found in oral fluid such as enzymes and peptides.

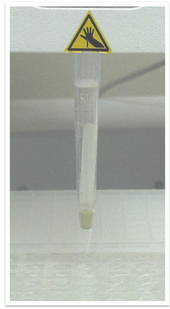

Figure 2. DPX tip positioned over a "sealed well" of a 96 deep well plate following the 2nd elution. The white disperser is visible in the DPX tip, just above the sorbent bed.

For these reasons, oral fluid is generally extracted using some type of solid-phase extraction (SPE) method. In our method, we performed SPE using DPX technology. We found that addition of a "disperser" into the DPX tip (patent pending) ensured consistent "dispersive" SPE in the tip, which resulted in higher recoveries and better reproducibility.

A picture of the DPX extraction during the elution step into a 96 deep well plate is shown in Figure 2. A few things are noteworthy from this picture. The first is that the sorbent, which is typically a light yellow brown, is discolored with the blue dye (from the collection buffer solution). This dye can be viewed as a potential matrix interference (similar to enzymes and peptides in the solution), and hence the extraction was efficient at removing this interference. It is also noteworthy that the deep well plate has a "seal", so the samples and elution solvents are effectively covered during the extraction process.

To perform the analysis of the polar drugs, such as opiates and amphetamines, it is important to note that organic solvent composition at greater than approximately 15% will cause losses of chromatographic resolution on the early eluting compounds. Fortunately, we found that elution with 15% acetonitrile provides high recoveries of all of the 5 panel drug classes with the exception of THC. Hence, we performed the analysis of the polar drugs using 15% acetonitrile for elution solvent. Results from this study are shown in Table 2. The detection limits achieved meet the standard requirements for most laboratory guidelines including SAMHSA.

For THC the detection limits were met by using a 20 µL injection loop (instead of 2µL). Acetonitrile is not ideal for eluting THC, and not surprisingly we found that a large percentage of THC was still retained on the sorbent. Nevertheless, we were able to detect 2 ng/mL of THC (Fig. 3) using this automated method. The calibration plot for THC is shown in Figure 4, which was linear from 5 to 100 ng/mL.

Future Research

Further studies will include more replicates, reproducibility data, and case samples. It is also necessary to optimize the MS parameters for all of the target drugs with this instrument in order to achieve optimal results.

Future research will also focus on improving the recovery of THC. We believe we can recover significantly more drug by incorporating a "top" elution (and perhaps a change of solvent).

Table 2. Approximate LODs and LOQs for drugs of abuse detected by this method without solvent evaporation. THC was analyzed separately with a 20 uL injection.

compound	Ret. Time	LOD (ng/mL)	LOQ (ng/mL)
6-MAM	2.01	10	20
amphetamine	2.13	2	5
benzoylecgonine	2.40	5	5
cocaine	2.58	1	10
codeine	2.11	5	10
hydrocodone	1.99	5	10
hydromorphone	1.60	2	10
MDA	2.15	5	10
MDEA	2.28	5	5
MDMA	2.16	2	5
methamphetamine	2.17	1	5
morphine	1.45	2	5
oxycodone	1.94	5	10
oxymorphone	1.53	2	5
PCP	3.00	1	2
THC (20 uL)	5.32	2	5

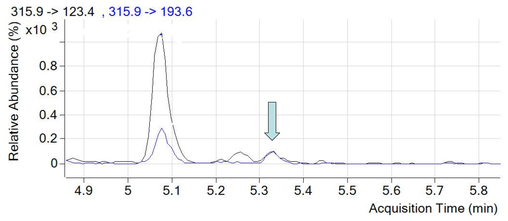

Figure 3. Ion chromatograms of THC at 2 ng/mL, clearly showing the target (black) and qualifier (blue) ions (with an acceptable ion ratio). The accuracy at this level from the calibration plot was 130%, just outside the limit for quantitation.

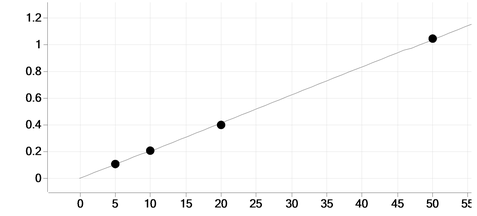

Figure 4.Calibration plot of THC showing linear data from 5 ng/mL to 100 ng/mL (cropped in this figure).

In addition, we plan to incorporate the second injection (of the acetonitrile elution solvent) during the chromatographic analysis of the polar drugs (15% acetonitrile). By doing this, we plan to include THC and other more nonpolar drugs such as benzodiazepines, buprenorphine/ norbuprenorphine and fentanyl within the same chromatographic run. It should be practical to perform a comprehensive analysis using automated DPX with no solvent evaporation to provide high throughput.

Conclusions

We were able to analyze opiates, cocaine (and BE), PCP, amphetamines, and THC at targeted confirmation levels of app. 10 ng/mL (2 ng/mL for THC) with no solvent evaporation. "Just-in-time" sample preparation is clearly a viable method for analysis of the 5-panel drugs of abuse in oral fluid using DPX technology.