Extraction of Synthetic Cannabinoids (SPICE), Opiates, and Benzodiazepine Drugs from Oral Fluid Using Supported Liquid Extraction (ISOLUTE® SLE+)

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Introduction

Drug of abuse testing is typically conducted using urine specimens from patients being screened for illicit drug use. While collection of urine is considered a relatively non-invasive drug testing action, it is not convenient for law enforcement to do in the field. The ability to collect an oral fluid sample in the field can be considered non-evasive and simple to implement. Currently there are oral fluid collection kits (e.g. Immunalysis Quantisal™, Orasure Technologies Intercept®) that facilitate the collection in easy to follow protocols. Oral fluid samples collected using standard collection kits can be qualitatively and quantitatively analyzed by LC-MS/MS, post extraction of the target analytes from the matrix. Drug analytes can be successfully extracted from oral fluid using Supported Liquid Extraction (ISOLUTE SLE+), which offers an efficient alternative to traditional liquid-liquid extraction (LLE). Here, we demonstrate a new rapid and reliable sample preparation method to extract a broad suite of drugs (Figure 1) from neat oral fluid and buffered oral fluid matrices.

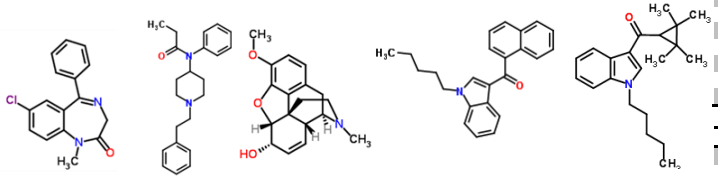

Figure 1. Structure of Diazepam, Fentanyl, Codeine, JWH-018, and XLR-144 representing types of target analytes extracted from oral fluid.

Sample Preparation/Elution Method:

ISOLUTE® SLE+ is a modified diatomaceous sorbent that retains the aqueous matrix, containing the target analytes. A water-immiscible organic solvent is then used to extract out the target compounds and metabolites. The water-soluble endogenous interferences (i.e. proteins, lysophospholipids, phospholipids and salts) are retained on the sorbent, which can subsequently be discarded. The process takes only 3 steps which are outlined in Figure 2. The extraction method and drug groups extracted from oral fluid are shown in Table 1 and Table 2.

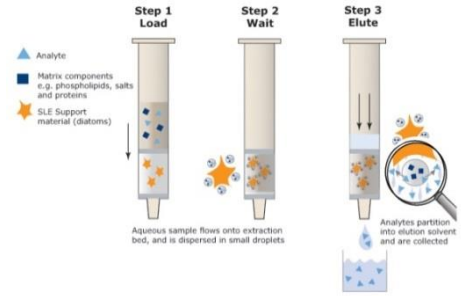

Figure 2: Illustration of the ISOLUTE® SLE+, Supported Liquid Extraction steps.

ISOLUTE SLE+ (400uL cartridge and 96-well plate formats)	Drug Group 1 (Basic Drugs Oral Fluid)	Drug Group 2 (Spice in Oral Fluid)
Sample pretreatment	200uL spiked Oral Fluid 2% NH4OH (1:1 v:v)	200uL Spike Oral Fluid 100mM Amm. Acetate (1:1 v:v)
Sample Load	Total sample (400uL)	Total Sample (400uL)
Process Time	Wait 5 minutes	Wait 5 minutes
Elution	1.2 mL Ethyl Acetate	1.4 mL Ethyl Acetate
Post Extraction	Dry Down and Reconstitute	Dry Down and Reconstitute

Table 1: Extraction protocol for drug groups in oral fluid solution.

	Drug Group 1	Molecular Weight	MRM Transition
1	Alprazolam	307	308.8 > 280.5
2	Clonazepam	314.8	315.8 > 269.8
3	Diazepam	283.9	284.9 > 154
4	Flunitrazepam	312.9	313.9 > 267.9
5	Oxazepam	287	288 > 242
6	Temazepam	299.9	300.9 > 255
7	Nitrazepam	281.1	282.1 > 180
8	Normeperdine	233	234 > 160
9	Naltrexone	341.2	342.2 > 323.8
10	Morphine	285	286 > 165
11	Codeine	299	300 > 199
12	Oxymorphone	301	302 > 227
13	Oxycodone	315	316 > 241
14	Hydrocodone	299	300 > 199
15	6-Acetyl Codeine	341.4	342.4 > 255
16	6-Acetyl Morphine	327	328 > 165.5
17	Fentanyl	336	337 > 188
18	Buprenorphine	467	468.2 > 396.2
19	EDDP	277	278 > 234

	Drug Group 2	Molecular Weight	MRM Transition
1	JWH-073	327	328 > 155
2	JWH-018	341	342.2 . 155
3	JWH-200	384	385 > 155
4	JWH-250	335.5	336.5 > 114
5	XLR-11	329	330 > 125
6	UR-144	311.5	312.5 > 125
7	JWH-018 -4-Hydroxypentyl	357	358 > 155
8	JWH-018-5-Pentanoic Acid	371	372 > 155
9	JWH-073-N-3-Hydroxybutyl	343	344 > 155
10	JWH-250-N-5-Hydroxypentyl	351	352 >120.9
11	JWH-018-N-4-Hydroxypentyl (d5)	362	363.5 > 155
12	UR-144-Pentanoic Acid	341	342.5 > 125
13	UR-144-5-Hydroxypentyl	327.5	328.5 > 125

Table 2. List of extracted drug groups and their mass spectrometry MRM transitions.

Liquid Chromatography Mass Spectrometry

Detection of the target analytes was optimized using an Applied Biosystems /MDS Sciex 4000 Q-Trap triple quadrapole MS (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface. The mass transitions for the drug targets are shown in Table 2. Chromatographic separation was accomplished using the Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK).

Results and discussion

The recoveries for the extracted groups of drugs are shown in Figures 3-5. Figure 3 outlines averaged recoveries (n=7) for Group 1 drugs, fortified into neat oral fluid (e.g. no collection kit buffers) at a concentration of 10ng/mL. The recoveries outlined in Figure 4 (n=7) represent Group 1 drugs fortified in oral fluid, collected with the Intercept and Quantisal commercial collection kits at 10ng/mL.

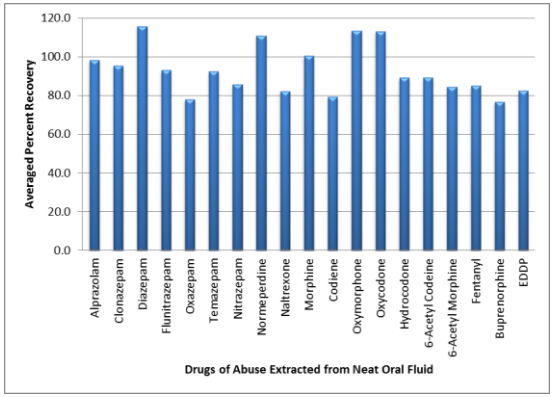

Figure 3: Plot of Group 1 drugs in neat oral fluid.

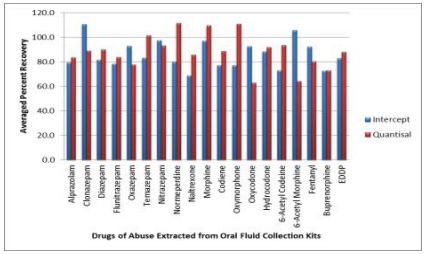

Figure 4. Plot of Group 1 drugs in oral fluid collected with the commercial collection kits.

Similar results were observed for the Group 2 drugs when extracted from neat oral fluid and oral fluid collected with commercial kits and tested with buffering solutions. Figure 5 show recoveries observed for Group 2 analytes extracted from neat oral solution and with commercial kit at 1.0ng/mL to demonstrate efficient extraction of Group 2 target analytes at lower limits of detection.

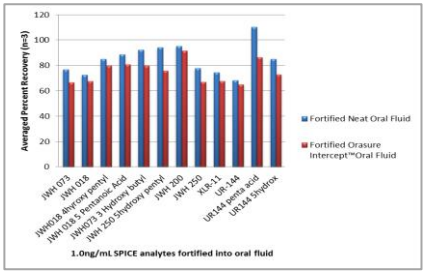

Figure 5. Plot of Group 2 drugs in oral fluid collected with the commercial collection kits at extracted at LOD of 1.0ng/mL

The matrix effects for neat and buffered oral fluid were evaluated for both Group 1 and Group 2 analytes. Similar results were observed for both groups with matrix effects of less than 20% for both neat and buffered fortified oral matrices. Figure 6 shows measured matrix effects for Group 2 drugs in neat and buffered oral fluid. The high level of recovery and low level of ion suppression observed supports cleanliness of extraction method.

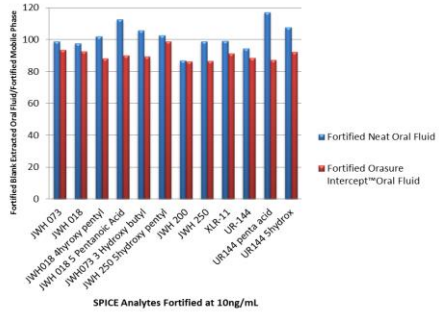

Figure 6. Matrix effect evaluation for neat and buffered oral solutions.

Conclusions/Future Work

The extraction methodologies employed here demonstrate the utility of supported liquid extraction as a viable extraction sorbent a broad range of drugs in complex matrices. Future work will include investigating the utility of SLE+ in the screening of other complex matrices like whole blood for broad ranges of drug across various classifications. The authors of this poster would like to thank Dean Fritch for his assistance with the Orasure Intercept kits.

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