Assessment of Solvent Polarity upon Drugs of Abuse in Oral Fluids using Supported Liquid Extraction prior to LC/MS Analysis

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Introduction

Over the past decade, the need for non-invasive drug screening that that precludes sample adulteration has become attractive. As a result, detection using oral fluid devices for Drugs of Abuse (DOA) has come to the vanguard of the scientific community. The use of Supported Liquid Extraction (ISOLUTE® SLE+) prior to LC/MS or GC/MS can improve sample cleanliness without forfeiting sample detection within a diverse panel of DOA's. Here, we demonstrate the effects of altering elution solvent polarity and pH for sample pretreatment upon the simultaneous recovery of 34 compounds comprised of opioids, benzodiazepines, and stimulants to directly measure the effects of the oral fluid buffer, OraSure™, upon extraction and signal intensity at presumed LOQ's.

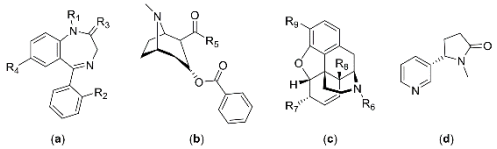


Figure 1. General scheme illustrating various generalized drug classes: benzodiazepines (a), stimulants (b), opioids (c), and plant alkaloids (d). R-groups represent moieties that vary within each drug class

Experimental

Reagents & Materials

All standards were purchased from Cerilliant (Round Rock, TX). HPLC grade water, methanol (MeOH), and acetonitrile (ACN), ethyl acetate (EtOAc), and methyl tert-butyl ether (MTBE) were purchased from Sigma Aldrich (St. Louis, MO) in addition to reagent grade isopropyl alcohol (IPA), dichloromethane (DCM), phosphoric acid, formic acid, and ammonium hydroxide (NH_4OH). ISOLUTE® SLE+ 400 µL sample capacity cartridges (820-0055-BG), Biotage® PRESSURE+ 48 positive pressure manifold (PPM-48), and Biotage® TurboVap LV (C103198) were supplied by Biotage.

Sample Preparation

Supported Liquid Extraction Procedure

Standard Prep: Orasure buffer was spiked with 34 compounds at various concentrations (see results) representing their potential limits of detection (LOQ) and at 100x their LOQ for recovery analyses. All analytes were extracted using ISOLUTE® SLE+.

Sample Pre-treatment: Samples were pretreated and evaluated using 2% and 0.1% formic acid and NH_4OH, in addition to neat buffer.

Load: Samples (400 µL) were loaded at ≥ 0.5 psi onto an ISOLUTE® SLE+ 400 µL cartridge, followed by a five-minute wait using a Biotage® PRESSURE+ 48 positive pressure manifold.

Elution: Analytes eluted into 100 µL of 50 mM methanolic HCl using two sequential 1.0 mL aliquots of one or a mixture of the following solvents with a 5 minute pause between aliquots: EtOAc, EtOAc/IPA [90:10], MTBE, DCM, DCM/IPA [95:5] and [90:10].

Dry Down and Sample Reconstitution: Solvents were evaporated using heated (40°C) nitrogen at 2.0 L/min and subsequently reconstituted in 100 µL of 10% methanol. Recovery was assessed using 100x LOQ for each analyte via LC/MS-MS.

Chromatography Parameters

Table 1. *Agilent 1260 Infinity II HPLC Parameters.*

Column	Resetek Raptor Biphenyl 2.7 µm, 100 x 2.1 mm		
MPA	0.1% Formic Acid (aq)	**Column Temp**	40 °C
MPB	MeOH	**Sample Temp**	rt
Flow Rate	0.6 mL min^{-1}	**Inj Volume**	10 µL

Mass Spectrometry Parameters

Instrument: SCIEX 4000QTRAP triple quadrupole Mass Spectrometer with Turbo Ionspray® Ion interface (Foster City, CA). Optimized source and sMRM parameters detailed in ***Tables 2 and 3,*** *respectively.* Retention window for sMRM set at 60 seconds with target scan time at 1 second.

Table 2. *SCIEX 4000QTRAP ESI (+) Turbo Ionspray® Source Parameters.*

Ionization Spray Voltage	+1500(V)	**CAD**	Medium
Source Temp	600 °C	**GS1**	60
Curtain	20 (V)	**GS2**	50

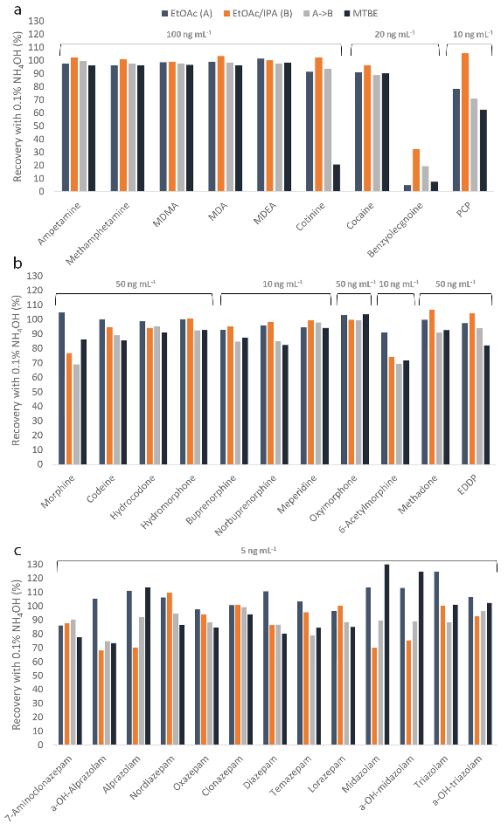
Table 3. *sMRM parameters for all 34 DOA analytes.*

Analyte	RT	Q1	Q3	DP	CE	CXP
Amphetamine	2.79	136.10	119.2/91	41	12/23	20/14
Methamphetamine	3.53	150.15	119/90.9	66	15/27	20/16
MDMA	4.05	194.24	163.1/105.2	71	17/35	16/12
Cotinine	1.60	177.19	80.1/98.1	71	33/29	14/6
MDA	3.57	180.19	163.2/105.1	26	11/31	4/12
MDEA	4.45	208.22	163.2/105.1	76	19/35	12/18
Clonazepam	4.48	316.15	101.9/123.3	26	32/32	6/6
7-aminoclonazepam	5.16	286.00	121.2/222.2	30	50/30	14/12
Alprazolam	5.79	326.11	291.1/209.1	101	37/49	16/16
α-OH-Alprazloam	6.51	309.21	281.1/205.1	101	35/59	18/34
Diazepam	6.67	285.25	193.3/257.1	101	45/31	14/14
Nordiazepam	6.35	271.14	140.1/165.1	106	41/41	24/28
Oxazepam	6.19	287.17	111.1/241.1	81	17/29	18/20
Temazepam	6.47	301.18	255.2/283.0	71	29/19	20/16
Lorazepam	6.47	323.13	277.0/102.0	91	29/73	22/6
Midazolam	5.78	326.15	291.0/223.3	111	37/51	20/38
α-OH-Midazolam	5.95	341.90	203.1/168.0	86	39/53	8/18
Triazolam	6.43	345.04	317.1/308.1	86	41/37	26/22
α-OH-Triazolam	6.23	361.06	333.0/343.0	121	39/29	34/22
Morphine	2.20	286.24	152.2/165.2	106	81/57	24/12
Codeine	3.93	300.29	115.2/165.1	101	99/51	18/26
Hydrocodone	4.25	300.24	199.1/128.1	96	41/79	16/8
Hydromorphone	2.94	286.22	185.0/157.1	121	41/53	10/12
Methadone	5.95	310.30	105.1/77.1	56	79/39	12/12
EDDP	5.79	279.22	235.1/115.2	86	39/97	12/18
Meperidine	5.00	248.17	220.2/70.0	106	29/45	12/10
6-Acetylmorphine	3.97	328.24	165.2/43.0	116	57/91	28/6
Buprenorphine	5.56	469.40	55.2/83.1	121	111/81	8/14
Norbuprenorphine	5.28	414.30	83.0/57.4	131	73/79	14/8
PCP	5.64	244.23	86.0/91.0	61	17/39	14/6
Cocaine	5.12	304.20	182.1/77.0	86	27/83	10/4
Benzyolecgnoine	5.00	290.23	168.1/77.1	61	27/79	10/12

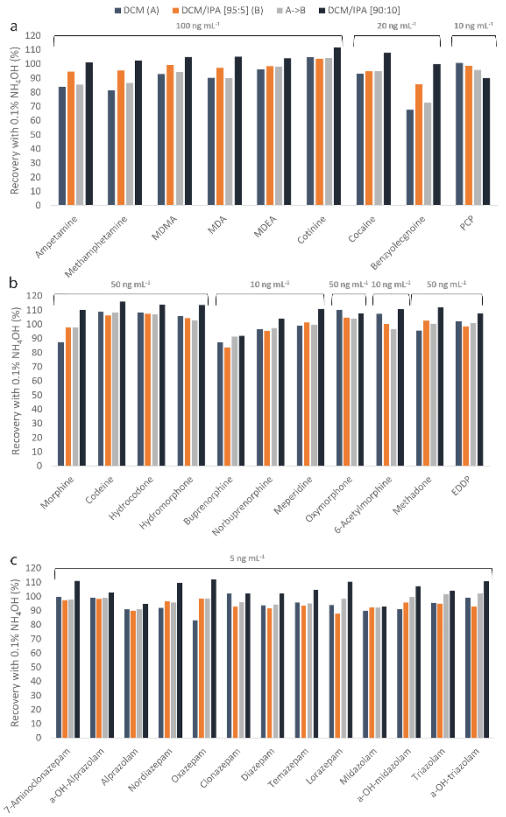
Results

Initial pretreatment with 2.0% and 0.1% formic acid yielded a white, insoluble precipitate upon sample dry down. Reconstitution in 10% methanol did not abrogate the precipitant. Conversely, pretreatment with 2.0% or 0.1% NH_4OH stabilized the samples and demonstrated slightly superior signal intensities under all solvent elution conditions when compared to neat sample pretreatment (data not shown).

Recovery analyses at 100x LOQ (n = 3) were assessed upon the 34-analyte panel using ISOLUTE® SLE+ for sample cleanup with the following elution systems: EtOAc, EtOAc/IPA [90:10], MTBE and EtOAc followed by EtOAc/IPA [90:10]. Results for each class of analytes showed the following recoveries under the various solvent elution systems: opioids (>70-105%), benzodiazepines (70-115%), alkaloid (>90%), and stimulants (70-100%), **(Figure 2a-c)**.



However, the metabolite of cocaine, benzoylecgonine, yielded recoveries ranging from 5-32% among all aforementioned solvent systems **(Figure 2a)**. Similarly, EtOAc/IPA [90:10] universally yielded only marginal recoveries of the benzodiazepines accept when using EtOAc **(Figure 2c)**. Similar trends were noted when examining all analytes at their respective LOQ's (opioids 0.1 and 0.5 ng/mL, benzodiazepine 0.5 ng/mL, stimulants 0.2 and 1 ng/mL, and alkaloids 1.0 ng/mL), (data not shown). Conversely, when examining the effects of slightly increasing the polarity of the elution systems (substituting DCM for EtOAc and DCM/IPA [90:10] for MTBE) it was noted that averaged recoveries (100x LOQ) and LOQ signal (data not shown) increased for benzodiazepines **(Figure 3c)**. Notably, recoveries for benzoylecgonine transitioned from sub-30% under elution conditions with EtOAc to 65-100% with the substitution of DCM. Average recoveries for all opioids and benzodiazepines ranged from 80-115% and 80-110%, respectively, when using DCM. Matrix effects, using only the basified buffer, were within ± 10% for stimulants, except cocaine, benzoylecgonine, and PCP, which indicated enhancement when eluting with DCM/IPA at 90:10. All benzodiazepines yielded substantial suppressive effects (~70%).



Conclusions

- On average, ISOLUTE® SLE+ produced better recoveries for opioids, stimulants, and benzodiazepines when using DCM compared to EtOAc as the main elution solvent
- Recoveries for benzoylecgonine increased from sub-30% to 65-100% when eluting with EtOAc and DCM, respectively.
- Matrix effects were pronounced for all benzodiazepines regardless of solvent system. Stimulants generally remained within 85-115% with opioids producing mixed results.

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