Practical Considerations Using Oral Fluid Collection Devices and SPE Method Development for Drugs of Abuse and with THC and Metabolites

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Introduction

Oral fluid represents a complex, heterogeneous biological fluid primarily produced by the parotid, submandibular, and sublingual salivary glands. Together, these glands make the majority of saliva, which excretes into the oral cavity through a collective network of striated ducts. Although only the major glands possess a collective secretive orifice, all salivary glands produce a secrete that varies in complexity. With the resurgence of oral fluids (OF) as testing matrix for drugs of abuse (DOA), the need to provide larger and more comprehensive panels for drugs is required. However, to reach the lower limits of quantitation necessary for basal analyte detection in OF, both the biological matrix and the storage buffers present obstacles for DOA detection. Specifically, the use of excipients or emulsifying agents in OF storage buffer, e.g. polyethylene glycol (PEG), are generally disruptive to the purification process of oral fluids because they act as a chemical bridge between the biphasic layers under liquid-liquid and solid phase extractions (LLE and SPE, respectively). Herein, we describe the relationship between ***85 DOA*** and their subsequent response to the recovery and matrix effects of Immunalysis' Quantisal buffer as used with water as a surrogate oral fluid, synthetic oral fluid from UTAK, and the Quantisal device. Moreover, we examine the impact upon recovery and matrix effects upon modulating solvent polarity of the organic wash to improve analyte detection and SPE method ruggedness upon a large and diverse panel of analytes.

Experimental

Reagents and Materials

Standards

All standards were purchased from Cerilliant (Round Rock, TX). HPLC grade water, methanol (MeOH), and acetonitrile (MeCN) were purchased from Sigma Aldrich (St. Louis, MO) in addition to reagent grade isopropyl alcohol (IPA), dichloromethane (DCM), formic acid, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), methyl tert-butyl ether (MTBE), tetrahydrofuran (THF), acetone, and ammonium hydroxide (NH_4OH). Synthetic oral fluid (P/N: 43409) and Quantisal extraction devices (P/N: QS-0025) were generously supplied from UTAK and Immunalysis, respectively. EVOLUTE® EXPRESS CX (60 mg bed) cartridges (611-0006-BXG), Biotage® PRESSURE+ 48 position positive pressure manifold (PPM-48), and Biotage® TurboVap® LV (415000) were supplied by Biotage.

Sample Preparation

Water as Surrogate Oral Fluid

For water as a surrogate oral fluid, each sample analyzed comprised of at 1:3 mixture of water to Quantisal to simulate manufactures OF:Buffer ratio. The buffer was spiked with all 85 standards for a final concentration of 100 ng/mL and then adjusted to 4% formic acid. All samples were loaded (1.0 mL) post column conditioning and equilibration.

Synthetic Oral Fluid from UTAK & Immunalysis Quantisal Device

For synthetic oral fluid, each sample analyzed comprised of 200 µL of Quantisal buffer with 100 µL of synthetic oral fluid. Quantisal extraction devices were used per manufactures instructions with a total of 300 µL (~100 µL oral fluid) used for analysis. Each was subsequently spiked with 100 µL of standards at 20 ng/mL followed by the addition of 100 µL of 4% formic acid. All samples were loaded post column conditioning and equilibration.

THC and Metabolites LC-MS/MS Stability Studies

For THC and metabolites, several different stabilizers and mobile phases were tested to determine if a 1 ng/mL LOQ was achievable. Mobile phases included acetonitrile, methanol, and methanol with 0.1% formic acid. Glass inserts in a 96-well plate and no glass inserts were used. Silanized inserts were also tested. Lastly, reconstitution of methanol, acetonitrile, and 50:50 water/methanol was used. Biphenyl and C18 columns were also tested.

EVOLUTE® EXPRESS CX SPE Procedure

Solvent ID	Solvent	% Aqueous (a)	%Aqueous (b)
S1	MeOH	50	0
S2	MeCN	50	0
S3	Acetone	50	0
S4	IPA	50	0
S5	MTBE	5	0
S6	DMSO	50	N/A
S7	DMF	50	N/A
S8	THF	50	N/A

Table 1. *Wash #2 solvent parameters.*

Step	Volume (µL)	Solvent	Time (min)	Pressure (psi)
Condition	1000	MeOH	≤ 0.2	≤ 0.5
Equilibration	1000	4% Formic Acid	≤ 0.2	≤ 0.5
Sample Load	500	Sample	1-2.0	≤ 0.5
Wash #1	2000	4% Formic Acid	≤ 0.5	0.5
Wash #2	2000	Solvents S1-8 (a & b)	0.5-1.5	0.5
Plate Dry	N/A	N/A	5.0	40
Elution	2000	DCM/MeOH/NH_4OH [78:20:2]	~2-3.0	Grav.
Plate Dry	N/A	Quick Pulse	x2	40

Table 2. *Biotage 48 Position Positive Pressure Processing Parameters.*

Dry Down and Sample Reconstitution: Elution solvent was collected into 100 µL of 50 mM methanolic HCl and evaporated in 10 minutes at 40 °C with 2.0 L/min of nitrogen using a Biotage® TurboVap LV. Extracts were subsequently reconstituted with 100 µL of 20% methanol (aq) in 0.1% formic acid and immediately analyzed via LC/MS-MS.

Post-Column Infusion (PIC) Parameters

All PIC analyses were performed using the chromatographic parameters noted below without the use of the column. A Harvard apparatus pump delivered all 85 analytes (20 ng/mL) directly into the LC flow path at 20 µL/min.

Chromatography Parameters

HPLC Metric(s)	Parameter
Column	Restek Raptor Biphenyl 2.7 µm, 50 x 3.0 mm
MPA	0.1% Formic Acid (aq)
MPB	0.1% Formic Acid in MeOH
Flow Rate	0.5 mL min^{-1}
Column Temp.	40 °C
Sample Temp.	20 °C
Injection Volume	10 µL

Table 3. *Agilent 1100 Series HPLC Parameters.*

Mass Spectrometry Parameters

Instrument: SCIEX 4000QTRAP® triple quadrupole Mass Spectrometer with Turbo Ionspray® Ion interface (Foster City, CA). Optimized source parameters shown in table 3 (sMRM transition parameters not shown, but available upon request). Retention window for sMRM set at 45 seconds with target scan time at 2.85 seconds.

Ionization Spray Voltage	+1500(V)	CAD	Medium
Source Temp	600 °C	GS1	50
Curtain	30 (V)	GS2	70

Table 4. *SCIEX 4000QTRAP® ESI (+) Turbo Ionspray® Source Parameters.*

Results

Using water as a surrogate oral fluid, a frequency distribution analysis revealed 44% of the 85 analytes yieled > 20% disparity in peak area among all solvents used in wash step #2. Further analysis showed S1a/b-S4a/b were superior wash systems for all analytes (data not shown) and were examined under PIC for matrix effects.

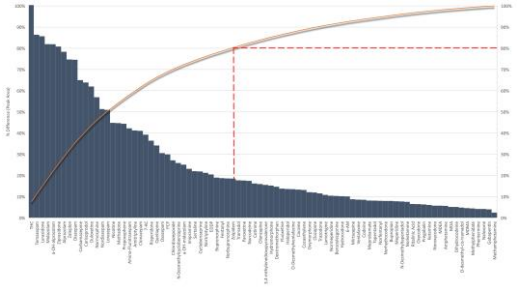

Figure 1. *Frequency distribution analysis of all 85-analytes extracted with water as a surrogate oral fluid (n=3).*

Matrix Effects by Post-Column Infusion

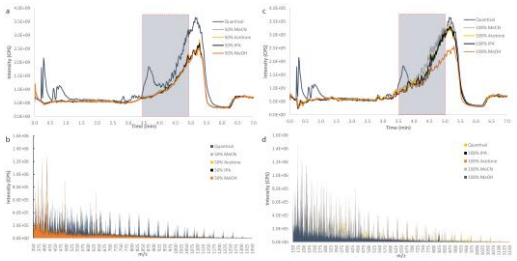

Figure 2. *(2a) PIC TIC from SPE-CX extraction using S1a-S4a. (2b) Full scan MS extracted from 2a (Grey box). (21c) PIC TIC from SPE-CX extraction using S1b-S4b. (2d) Full scan MS extracted from 2c (Grey box).*

THC and Metabolites LC-MS/MS Stability

Using a stock standard that was dried down using a TurboVap and reconstituted, LC-MS/MS stability was studied using various mobile phases, reconstitution solvents, LC columns, and inserts. Sample concentrations were 1 ng/mL and 50 ng/mL. The best signal required mobile phase to be prepared fresh daily. It was also noted that peak intensity decreased over the course of the day. When comparing mobile phases, acetonitrile had a lower intensity than did methanol with 0.1% formic acid. When comparing silanized inserts, glass inserts, and no inserts, the silanized inserts resulted in the greatest intensity, while no inserts had the lowest intensity. The biphenyl column produced higher intensities than the C18 column (the C18 column resulted in poor chromatography). Lastly, for reconstitution solvents, using methanol with 0.1% formic acid yielded the highest signal intensities. While consistent peaks were not seen for the 1 ng/mL sample, progress was made in determining optimum LC-MS/MS conditions for THC and metabolites.

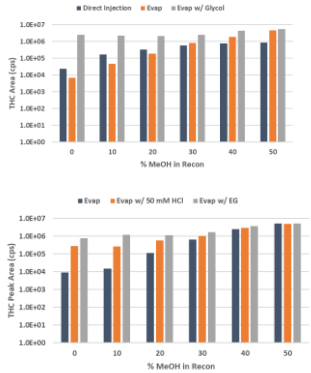

Figure 3. *Varying amounts of methanol were used in the reconstitution solvent. Stabilizers (50 mM HCl and ethylene glycol) were also added to determine if the stability of THC was increased.*

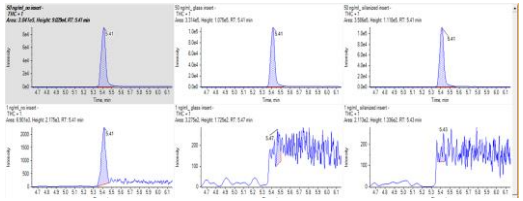

Figure 4. *Use of silanized inserts, glass inserts, and no inserts for THC parent compound at 50 ng/mL and 1 ng/mL.*

Conclusions

- EVOLUTE® EXPRESS CX produced excellent recoveries for analytes with disparate non-covalent and columbic profiles among the 85-analyte DOA panel.
- Frequency distribution analysis of the water surrogate SPE extraction demonstrated that 44% of the 85-member panel responded best when organic wash systems S1 (a&b) through S4 (a&b) were used. The remaining 54% were indifferent to all wash systems.
- Sample matrix effects were generally high; however, 50% MeOH, 50% MeCN, 50% Acetone, 50% IPA, and neat MeOH showed enhanced removal of suspected polyglycol/detergent.
- Sample recovery using EVOLUTE® EXPRESS CX did not discriminate between synthetic (UTAK) oral fluids or user submitted oral fluids when using Quantisal buffer (recovery and matrix effects data not shown).
- Regardless of matrix or buffer, carbamate based analytes responded poorly to mixed-mode system when moderate to high levels of organic washes were employed.
- To increase LC-MS/MS sensitivity for THC and its metabolites, a reconstitution solvent containing methanol, a biphenyl column and silanized glass inserts should be used. Ethylene glycol can also be added to the reconstitution solvent as a stabilizer.

Disclosure

Neither I nor any member of my immediate family has a financial relationship with a company as defined in the AACC policy on disclosure of potential bias or conflict of interest. Biotage pays the salaries of those on the poster.

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