Quantitation of an Oral Fluid Drug Panel Including THC Using High Resolution Accurate-Mass (HRAM) Orbitrap Mass Spectrometry

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Abstract

Purpose: The objective of this work was to accurately confirm and quantitate 31 drugs in oral fluid required by SAMHSA and the National Safety Council (NSC) by liquid chromatography and high-resolution, accurate mass (HRAM) Orbitrap™ mass spectrometry.

Methods: Human oral fluid samples were spiked with the 31 drugs of abuse at nine different concentration levels, extracted using DPX INTip™ SPE, separated chromatographically, and detected on an Orbitrap Exploris™ 120 mass spectrometer.

Results: All drugs achieved lower LOQ's than the cutoffs suggested by the new SAMHSA guidelines. Each of the drugs were also confirmed below those guideline levels using library search, isotopic pattern, and fragment matching.

Introduction

As the clinical and forensic communities move towards oral fluid matrix for ease of collection and roadside testing, it is important to be able to test for a wide range of analytes and achieve the required sensitivity. With the new SAMHSA guidelines providing LOQ levels for a list of drugs, the extraction protocol and instrumentation need to be sensitive enough to accomplish these cut-offs. Including tetrahydrocannabinol (THC) into the assay provides challenges in the extraction procedure as most drugs of abuse are basic and THC is neutral. This extraction workflow, which can extract THC alongside other drugs of abuse, coupled with the Orbitrap™ mass spectrometer generates high-resolution accurate mass data that offers improved sensitivity, selectivity, and accuracy for the detection and quantitation of drugs of abuse in oral fluid.

Materials and methods

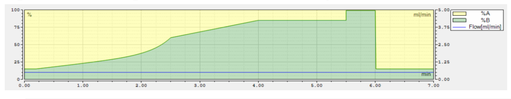
Sample Preparation

Nine calibration levels ranging from 0.5 to 1,000 ng/mL (1.25 to 5,000 ng/mL for Meprobamate and Carisoprodol) were prepared by spiking stock solution of the 31 target analytes into human oral fluid. Samples were diluted with Quantisal™ Buffer 1:3 (oral fluid: buffer) and 500 µL were aliquoted for extraction. Each sample was spiked with 125 µL of internal standard stock. The samples were then extracted using DPX INTip SCX/WAX SPE. The tips were conditioned with 800 µL x2 of 50% MeOH and then the samples were aspirated with the tips 4-5x. The tips were then washed with 500 µL x3 of 30% MeOH. Finally, the analytes were eluted with 500 µL x2 of 48% ACN, 48% MeOH, 4% Ammonium Hydroxide (v/v/v). Samples were dried down at 50°C for 25 minutes. The samples were reconstituted in 20 µL of MeOH + 0.1% Formic Acid and 80 µL of H2O + 0.1% Formic Acid.

Liquid Chromatography

Analytes were separated with the Thermo Scientific™ Vanquish™ Horizon ultra-high performance liquid chromatography (UHPLC) system by a 7-min gradient (Figure 1) using a Thermo Scientific™ Accucore™ Vanquish™ Biphenyl column (2.6 µm, 50 x 2.1 mm). Mobile phases consisted of 0.1% formic acid in both water (mobile phase A) and methanol (mobile phase B). 5-µL of each standard were injected in triplicate.

Figure 1. UHPLC gradient used for data acquisition.



Mass Spectrometry

Targeted analysis and quantitation were performed a Thermo Scientific™ Orbitrap Exploris™ 120 HRAM mass spectrometer. Full-scan, targeted, data-dependent MS2 (ddMS2) mode was used with an inclusion list for the targeted compounds. Resolutions of 60,000 (FWHM at m/z 200) for full scan and 15,000 for MS2 were employed. An isolation window of m/z 1.5 and compound specific collision energies were applied to generate rich HRAM MS2 spectra.

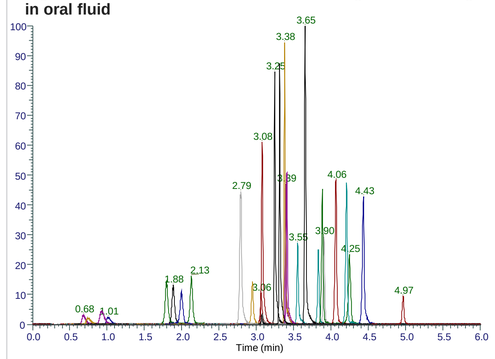
Data Analysis

Data was acquired and processed with Thermo Scientific™ TraceFinder™ software, version 5.2 which utilizes its Compound Database that stores information including molecular formula, exact mass, retention time and fragment ions for all compounds of interest. A mass window of 5ppm was used as the precursor ion criteria of HRAM data.

Results

The combined extracted ion chromatogram of each drug is depicted in Figure 2. This 7-minute method is able to separate each of the isomers and elute each compound in under 6-minutes.

Figure 2. Combined extracted ion chromatogram of 31 drugs in oral fluid



Recovery Study

A brief recovery study was performed to test the amount of analyte recovered from a pre-extraction spike compared to post-extraction spike. Figure 3 highlights the percent recovered per each compound.

Figure 3. Recovery study of the 31 drugs of abuse in oral fluid showing percent recovered

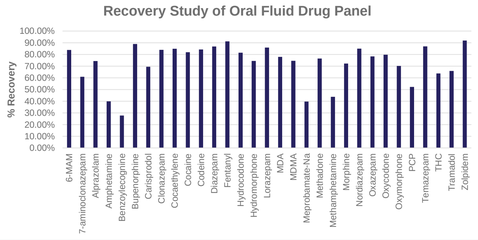


Table 1. Criteria assigned in TraceFinder software for limits.

Parameter	Criteria
Limit of Quantitation (LOQ)	Back-calculated concentration on calibration curve within 20%.
Upper Limit of Linearity (ULOL)	Highest calibrator that achieves linearity
Limit of Identification (LOI)	**Orbitrap**: IP = passing isotopic pattern score (70) FI = presence of diagnostic fragment ions within 5 ppm LS = passing library score (70)

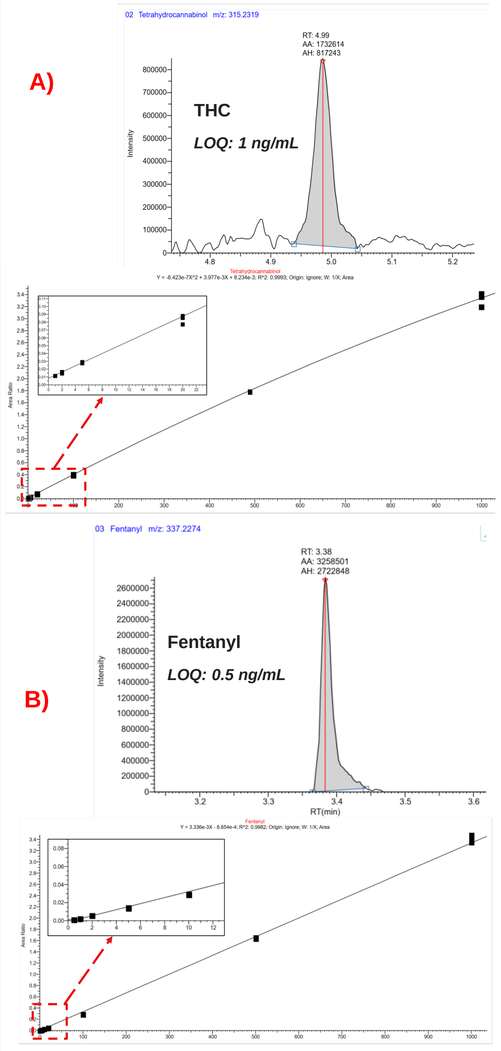
Table 2. Criteria assigned in TraceFinder software for Orbitrap confirmation.

Parameter	Criteria
Isotopic Pattern	< 10 ppm mass deviation, < 20% intensity deviation, fit > 70%
Fragment Ion	At least 2 fragments with < 10 ppm mass deviation in MS^2 spectra
***mz*Vault HRAM Library**	Reverse search with > 70% match of ddMS2 spectra

Table 3. Calibration and confirmation results of the 31 analytes in oral fluid. LOQ, ULOL, and LOI are in ng/mL.

	LOQ	ULOL	LOI
6-MAM	0.5	1000	0.5
7-aminoclonazepam	0.5	1000	0.5
Alprazolam	0.5	1000	0.5
Amphetamine	1	1000	1
Benzoylecgonine	2	1000	2
Buprenorphine	0.5	1000	0.5
Carisoprodol	10	5000	10
Clonazepam	1	1000	1
Cocaethylene	1	1000	1
Cocaine	2	1000	2
Codeine	1	1000	1
Diazepam	1	1000	2
Fentanyl	0.5	1000	0.5
Hydrocodone	0.5	1000	0.5
Hydromorphone	1	1000	1
Lorazepam	1	1000	1
MDA	5	1000	5
MDMA	5	1000	5
Meprobamate-Na	25	5000	25
Methadone	2	1000	2
Methamphetamine	0.5	1000	0.5
Morphine	1	1000	1
Nordiazepam	0.5	1000	0.5
Oxazepam	0.5	1000	0.5
Oxycodone	0.5	1000	0.5
Oxymorphone	0.5	1000	0.5
PCP	0.5	1000	0.5
Temazepam	0.5	1000	0.5
THC	1	1000	1
Tramadol	2	1000	2
Zolpidem	1	1000	1

Figure 4. Extracted ion chromatograms of A) THC and B) fentanyl at LOQ along with their respective calibration curves



Quantitation

The limit of quantitation (LOQ), upper limit of linearity (ULOL), and limit of identification (LOI) were evaluated for each of the 31 analytes. Table 1 shows the criteria for each of these limits. For confirming each drug, isotopic pattern, fragment ion matching, and library search were employed. Table 2 defines these specific identification parameters.

Quantitation continued

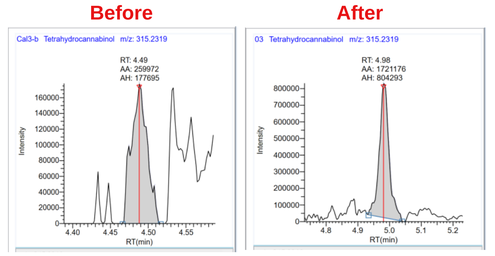
Each of the 31 drugs of abuse achieved lower LOQ's and LOI's than the recommended confirmation cutoffs in the new 2023 SAMHSA guidelines. Additionally, each of the compounds had lower LOQ's and LOI's than the National Safety Council's Tier 1 drug cutoffs except for Diazepam which achieved an LOI of 2 ng/mL. More work will need to go into fine tuning to achieve the 1 ng/mL cutoff.

Figure 4 depicts extracted ion chromatograms of two of the analytes, THC and fentanyl, at their respective LOQ concentrations with corresponding calibration curves.

THC Troubleshooting

Throughout this study, some initial challenges presented themselves in regards to detecting THC. One of the most important factors to consider in extracting THC is that it is a neutral compound and sticky to glass vials and other consumables. To combat this, silanized glass tubes and vial inserts were used to help prevent THC from sticking. It is also imperative to add MeOH to every step of the extraction process. This means adding enough volume of MeOH diluted internal standard to the sample (roughly 30%) and a wash solvent that contains 30% MeOH. This will improve the binding of THC to the SPE resin prior to its elution. When reconstituting, add the MeOH portion of the reconstitution solvent and vortex before adding the remaining aqueous portion. A before and after of these changes can be seen in Figure 5. The significant increase in signal shows the importance of incorporating these steps for THC.

Figure 5. Extracted ion chromatograms of THC before and after adjustments made. THC after achieved a 7x increase in intensity.



Conclusions

- Fast and quantitative method developed around 31 drugs of abuse specified by SAMHSA and the National Safety Council
- Linearity achieved from LOQ's as low as 0.5 ng/mL up to 1,000 ng/mL which exemplifies the sensitivity of these instruments and extraction procedure
- Extraction and LCMS protocol sufficiently passes the SAMHSA guidelines

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