Prevalence of Novel Psychoactive Substances in Oral Fluid

Final Research Report

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SUMMARY OF PROJECT

Major Goals and Objectives

1. Task 1: To develop and validate a comprehensive analytical method for the detection and quantification of novel synthetic opioids in oral fluid by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Development of such a comprehensive analytical technique will provide a useful methodology to be implemented in forensic toxicology or clinical laboratories.
2. Task 2: To collect anonymous oral fluid specimens from prisoners, arrestees, drivers under the influence of drugs (DUID), and other forensic settings in order to detect and quantify novel synthetic opioids using validated LC-MS/MS method. Analysis of oral fluid specimens from sensitive populations that may be seeking alternative or substitute opioid use allows us to examine the novel synthetic drugs that are available to these populations.
3. Task 3: To examine prevalence of novel synthetic opioids in susceptible populations in order to understand the extent of synthetic opioid abuse. Examination of drug use trends can help direct future drug testing policies in a criminal justice or forensic setting.

Research Questions

1. Can LC-MS/MS be used to analyze novel synthetic opioids?
2. Is solid phase extraction (SPE) suitable for isolation of novel synthetic opioids from oral fluid?
3. Is oral fluid a useful alternative matrix for identifying use of novel synthetic opioids?
4. What is the prevalence of novel synthetic opioid use in sensitive populations (arrestees, prisoners, drug treatment, etc.)?
5. Can oral fluid drug testing be useful in investigating drug impairment?

Research Design, Methods, and Techniques

The project was split into several different aspects in order to 1) develop analytical methods necessary to complete testing and then 2) analyze oral fluid samples. Originally, the project sought to

analyze the novel synthetic opioids AH-7921, MT-45, U-47700, and W-18, in addition to traditional opioids morphine, 6-monoacetylmorphine, and buprenorphine. Targeted analytes were based on prevalence at the time of grant writing. Over time, drug trends shifted, and the project also sought to incorporate several other novel synthetic opioids (both fentanyl- and non-fentanyl related).

Method development: For each assay, parameters for extraction, chromatography, and mass spectral acquisition were thoroughly developed and optimized. Extractions were evaluated in terms of matrix effects and analytical recovery and various parameters were assessed including sample size, extraction cartridge, wash solvents, elution solvents, and elution volumes. Chromatography was optimized for baseline separation of positional isomers to the best of our ability and parameters assessed included mobile phase composition, mobile phase modifiers, and LC columns. For mass spectral data acquisition, parameters were chosen to maximize instrument sensitivity. Internal standards were evaluated as available to compensate for matrix effects and recovery.

Method validation: For analytical methods to be published and applied to analysis of forensic specimens, the techniques need to be fully validated according to strict guidelines. Calibrators and controls need to be prepared and analytically scrutinized to determine the validity of the technique. Method validation experiments were performed in accordance with current standards in the field and investigated sensitivity, linearity, bias, imprecision, matrix effects, extraction recovery, interferences, dilution integrity, carryover, and analyte stability.

Sample preparation: For calibrators and controls, oral fluid specimens (1 mL) were diluted with extraction buffer (3 mL; to mimic oral fluid collected from a subject with a collection device) and then subjected to SPE in order to recover the drugs from the oral fluid and clean up matrix or buffer interferences to allow for sensitive analysis by LC-MS/MS. For oral fluid collected from forensic settings (via Quantisal device, Immunalysis Corp., Pomona, CA), the specimens equilibrated for 12-24 h in extraction buffer, then stored under refrigeration until analysis by the developed, validated methods.

Instrumentation: Non-fentanyl related novel synthetic opioids were analyzed on an Agilent 6470 triple quadrupole (QQQ) mass spectrometer equipped with Jet Streaming technology and electrospray ionization (ESI) coupled to an Agilent 1290 Infinity II liquid chromatography system. Fentanyl-related novel synthetic opioids were analyzed on an Agilent 6530 Accurate Mass Quadrupole Time-of-Flight (QTOF) mass spectrometer coupled to an Agilent 1290 Infinity liquid chromatography. For non-fentanyl related opioids, compounds were quantified using multiple reaction monitoring (MRM). For the fentanyl analog screening method, analytes were detected using Time of Flight (TOF) and All Ions Fragmentation (AIF) modes. For the fentanyl analog quantification method, targeted acquisition was utilized. Chromatographic separation was consistent for both fentanyl analog methods.

Analysis: Analyte detection or quantification were performed using Agilent MassHunter software. Drug identification criteria included: retention time, Gaussian peak shape, product ions, and ion ratios. Microsoft excel was used to track validation performance and perform statistics necessary to ensure acceptable method validation parameters. Authentic specimens were analyzed alongside fresh calibrators and quality controls.

Authentic specimens: Anonymous oral fluid specimens were collected using the Quantisal device. The device has a volume adequacy indicator for 1.0 ± 0.1mL oral fluid. The pad was swabbed around the mouth until the indicator turns blue or within 10 min, whichever occurs first. Oral intake (smoking, drinking, eating, chewing gum, oral hygiene) was prohibited 10 min prior to collection. Specimens were collected from inmates, arrestees, drivers suspected of driving under the influence of drugs (DUID), and also received from collaborating forensic toxicology laboratories in accordance with SHSU IRB # FY2018-37550. All samples were subjected to the fentanyl screening method and the non-fentanyl related targeted quantification method. When fentanyls were present, samples were refluxed to the quantitative fentanyl analog assay. For some collection sites, drug impairment was assessed by Drug

Recognition Experts (DRE) and results of performance on standardized field sobriety tests were included for comparison to oral fluid toxicology results.

Expected Applicability

The techniques developed in this proposal allow for identification of novel synthetic drugs in oral fluid specimens without the need for specialized, invasive collection like blood and can be easily observed unlike urine. As many drug users prefer to use novel synthetic drugs to evade positive drug testing results, users (particularly in sensitive populations like prisoners) are exposing themselves to potent, harmful, unknown substances. Drug users may not be fully aware or correctly informed of the substances they are purchasing or ingesting. Understanding the prevalence of these compounds in various settings, particularly in the criminal justice realm, may help direct future drug testing policy. Additionally, it will bring awareness to prison administrations the frequency of use for these compounds so that they can educate the correctional officers on the adverse events to be aware of and also to inform their populations of the dangers of ingesting these compounds.

The methodologies developed under this grant proposal contribute significantly to forensic toxicology by offering validated analytical techniques for the screening and quantification of novel synthetic opioids, fentanyl-related and non-fentanyl related, in oral fluid. The methods are comprehensive and offer sensitive analytical approaches to identifying potent and impairing opioids in oral fluid.

The techniques save time and money for many forensic toxicology laboratories that do not have personnel or resources for method development or research efforts. The validated and published methods allow for laboratories to screen and quantify multiple drugs in a single analysis without the need for multiple analyses or expensive private toxicological testing. The methods allow for analysis of novel synthetic opioids in oral fluid, an alternative matrix becoming more popular in clinical and forensic settings, with direct applicability to other traditional matrices such as blood.

PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Participants

Name	Project Role	Institution	Contribution	Funding Support
Madeleine Swortwood	Principal Investigator	SHSU	Analytical laboratory work, sample collection, data analysis, manuscript revision, supervision, project oversight	SHSU, in addition to this award
Kaitlyn Palmquist	Graduate Assistant	SHSU	Laboratory work, data analysis, manuscript preparation	SHSU, in addition to this award
Christina Smith	Graduate Assistant	SHSU	Sample collection, laboratory work, data analysis	SHSU
Michael Truver	Graduate Assistant	SHSU	Laboratory work, sample collection, data analysis, manuscript preparation	SHSU, in addition to this award

Collaborating Partners

Name	Agency	Contribution	Location	Detail
Sumandeep Rana	Redwood Toxicology	Authentic samples	Santa Rosa, CA	Domestic
David Schwope	Aegis Sciences Corp.	Authentic samples	Nashville, TN	Domestic
Christine Moore	Alere Inc.	Discounted supplies Authentic samples	Pomona, CA	Domestic
Cecilia Marquart	Texas DRE Coordinator	Coordinate collection sites	Huntsville, TX	Domestic
San Antonio Detention Center		Sample collection site	San Antonio, TX	Domestic
Dallas County Jail		Sample collection site	Dallas, TX	Domestic
Brazoria County Jail		Sample collection site	Brazoria, TX	Domestic
Montgomery County Jail		Sample collection site	Conroe, TX	Domestic

CHANGES IN APPROACH

The analytical approach for this project changed slightly in terms of analytical scope, instrumentation, and sample collection. Originally, the project sought to analyze the novel synthetic opioids AH-7921, MT-45, U-47700, and W-18, in addition to traditional opioids morphine, 6-monoacetylmorphine, and buprenorphine. Targeted analytes were based on prevalence at the time of grant writing. Over time, drug trends shifted, and the project shifted to incorporate several other novel synthetic opioids (both fentanyl- and non-fentanyl related). As the fentanyl analogs are quite potent and new analogs are constantly emerging, analytical methods incorporated use of high-resolution mass spectrometry for increased sensitivity, selectivity, and for a more screening-based approach as it allows for library matching (as opposed to targeted quantification on a triple quadrupole mass spectrometer).

Project implementation was significantly delayed and impacted sample collection and progress. First, IRB approval took several months and impacted release of funds, which also delayed method development. Second, our laboratory instrument suffered a catastrophic failure and parts were backordered for several months, further delaying method validation. Sample collection was unable to begin until analytical methods were in place. Original grant proposal sought to collect samples within Texas prisons and the request for research collection was under review for 16 months and ultimately denied. As this was intended to be a major source of samples, the project shifted to acquiring samples from other sites. Later, COVID-19 university policies halted in-person sample collection for close to one year and required significant modifications to IRB protocols. For these reasons, number of authentic samples was lower than projected (n=245).

OUTCOMES

Activities/Accomplishments

Task 1: Three analytical methods were developed, optimized, and validated for detection of novel synthetic opioids in oral fluid using LC-MS/MS. 1) Quantitative analysis of novel synthetic opioids, morphine, and buprenorphine in oral fluid by LC-MS/MS; 2) Data-independent screening method for 14 fentanyl analogs in whole blood and oral fluid using LC-QTOF-MS; and 3) Quantification of fentanyl analogs in oral fluid using LC-QTOF-MS. This resulted in 3 peer-reviewed publications and 4 conference posters/presentations (See "Artifacts" below).

Task 2: Oral fluid samples (n=245) were collected or obtained and analyzed by the above analytical methods. Samples were collected from San Antonio Detention Center (n=43), Dallas County Jail (n=2), Brazoria County Jail (n=71), and Montgomery County Jail (n=1) between May 2018 and September 2021. Of the samples collected, DRE examinations accompanied n=37 cases. Samples were obtained via collaborating agencies from Aegis (n=100), Redwood Toxicology (n=17), and Immunalysis Corp (n=11) between October 2018 and January 2021 from pain management patients and probationers/parolees. This resulted in 1 peer-reviewed publication and one conference poster.

Task 3: Prevalence of novels synthetic opioids was assessed in authentic specimens. This resulted in 1 webinar (FTCOE).

Results and Findings

Method 1: The purpose of this research was to develop and validate a comprehensive analytical method for the detection and quantification of morphine, 6-acetylmorphine, buprenorphine, U-47700, U-49900, U-50488, AH-7921, MT-45, W-18 and W-15 in oral fluid collected via Quantisal. This was achieved by solid-phase extraction followed by liquid chromatography–tandem mass spectrometry. The limits of detection and quantitation were 5 ng/mL and 10 ng/mL, respectively. Linearity was observed between 10 and 500 ng/mL ($R^2 \geq 0.9959$). Bias and imprecision were <±11.1%. Matrix effects ranged

from −21.1 to 13.7%. No carryover was detected following injection of the highest calibrator. All analytes were stable (within ±15% change from baseline) under all tested conditions (24 h at room temperature, 72 h at 4°C, and in the autosampler for 60 h at 4°C).

Method 2: A data-independent screening method for 14 fentanyl analogs in whole blood and oral fluid was developed and validated using liquid chromatography-quadrupole-time-of- flight mass spectrometry (LC-QTOF-MS). Data were acquired using Time of Flight (TOF) and All Ions Fragmentation (AIF) modes. The limits of detection (LOD) in blood were 0.1–1.0 ng/mL and 0.1–1.0 ng/mL in TOF and AIF modes, respectively. In oral fluid, the LODs were 0.25 ng/mL and 0.25–2.5 ng/mL in TOF and AIF modes, respectively. Matrix effects in blood were acceptable for most analytes (1–14.4%), while the nor-metabolites exhibited ion suppression >25%. Matrix effects in oral fluid were -11.7 to 13.3%. Stability was assessed after 24 h in the autosampler (4 °C) and refrigerator (4 °C). Processed blood and oral fluid samples were considered stable with -14.6 to 4.6% and -10.1 to 2.3% bias, respectively. For refrigerated stability, bias was -23.3 to 8.2% (blood) and -20.1 to 20.0% (oral fluid). Remifentanil exhibited >20% loss in both matrices. For proof of applicability, postmortem blood (n = 30) and oral fluid samples (n = 20) were analyzed. As a result, six fentanyl analogs were detected in the blood samples with furanyl fentanyl and 4-ANPP being the most prevalent. No fentanyl analogs were detected in the oral fluid samples. This study presents a validated screening technique for fentanyl analogs in whole blood and oral fluid using LC-QTOF-MS with low limits of detection.

Method 3: The purpose of the present study was to develop and validate a quantitative method for fentanyl analogs in oral fluid (collected via Quantisal™) using liquid chromatography-quadrupole-time-of-flight-mass spectrometry (LC-QTOF-MS). Validation resulted in limits of detection and quantification ranging from 0.5 to 1 ng/mL. Established linear range was 1-100 ng/mL for all analytes, except acetyl fentanyl at 0.5-100 ng/mL ($R^2 > 0.994$). Within-and between-run precision and bias were considered acceptable with maximum values of ±15.2%CV and ±14.1%, respectively. Matrix effects exhibited

ionization enhancement for all analytes with intensified enhancement at a low concentration (9.3-47.4%). No interferences or carryover was observed. Fentanyl analogs were stable in processed extracts stored in the autosampler (4^{0}C) for 48h. The validated method was used to quantify fentanyl analogs in authentic oral fluid samples (n=17) from probationers/parolees. Fentanyl and 4-ANPP concentrations were 1.0-104.5 ng/mL and 1.2-5.7 ng/mL, respectively.

Oral Fluid & DRE: This study analyzes paired oral fluid and urine with drug recognition expert (DRE) observations. Authentic oral fluid samples (*n* = 20) were collected via Quantisal™ devices from arrestees under an institutional review board-approved protocol. Urine samples (*n*=18) were collected with EZ-SCREEN® cups that presumptively screened for Δ9-tetrahydrocannabinol (cannabinoids), opiates, methamphetamine, cocaine, methadone, phencyclidine, amphetamine, benzodiazepines, and oxycodone. Impairment observations (*n* = 18) were recorded from officers undergoing DRE certification. Oral fluid samples were screened using an Agilent Technologies 1290 Infinity liquid chromatograph coupled to an Agilent Technologies 6530 Accurate Mass Time-of-Flight mass spectrometer. Personal compound and database libraries were produced in-house containing 64 drugs of abuse. An Agilent 1290 Infinity LC system equipped with an Agilent 6470 Triple Quadrupole MS was used for quantification of buprenorphine, heroin markers (6-acetylmorphine, morphine) and synthetic opioids. Subjects were 23–54 years old; 11 (55%) were male and 9 (45%) were female. Evaluator opinion of drug class was confirmed in oral fluid 90% of time and in urine 85% of the time in reference to scope of testing by the LC–MS methods employed (excludes cannabis and central nervous system depressants). Data indicate that oral fluid may be a viable source for confirming driving under the influence of drugs.

Novel Synthetic Opioid Prevalence: A total of 245 oral fluid specimens were analyzed by LC-MS/MS (screening and confirmatory approaches) for detection of novel synthetic opioids (fentanyl-related and non-fentanyl related). Samples were collected from arrestees, inmates, and those suspected of driving under the influence of drugs. Additional specimens were obtained from pain management patients and

parolees. No synthetic opioids were detected within the analytical scope of our assays, despite increasing analytical scope and using screening approaches. Heroin markers were detected and quantified and confirmed by DRE findings (when DRE examination was performed). Fentanyl was detected and quantified in 17 parolee samples (that previously tested positive for fentanyl from submitting laboratory) but no analogs were detected within the analytical scope of our methods. While these data are limited, they provide useful interpretive value to forensic laboratories performing oral fluid drug testing. Comparison of oral fluid results to DRE findings supports use of oral fluid as an alternative toxicology matrix for identifying recent drug use and impairing substances.

Limitations

There are several limitations to this work including analytical scope and small sample size limited to small geographic regions. Targeted analytical approaches are limited in scope and sensitivity. Targeted data acquisition techniques do not allow for identification of other compounds. Steps were taken to broaden scope and sensitivity where possible, including screening approaches utilizing high resolution mass spectrometry (with library matching and retrospective data analysis opportunities). Due to unanticipated obstacles in sample collection, specimens were limited in number and location over the three-year period and may not be representative of novel synthetic opioid use in those regions, timepoints, or populations.

ARTIFACTS

List of Products

- K.B. Palmquist, M.J. Swortwood. Quantification of Fentanyl Analogs in Oral Fluid Using LC-QTOF-MS. ORAL PRESENTATION. Society of Forensic Toxicologists Annual Meeting. Nashville, TN. September 2021.
- Palmquist, KB, Swortwood, MJ. Quantification of Fentanyl Analogs in Oral Fluid Using LC-QTOF-MS. J Forensic Sci. 2021; 66(5):1871-1878. https://doi.org/10.1111/1556-4029.14813
- Truver MT, Palmquist KB, Swortwood MJ. Oral Fluid and Drug Impairment: Pairing Toxicology with Drug Recognition Expert Observations. *Journal of Analytical Toxicology* 2019; 43:637-643. https://doi.org/10.1093/jat/bkz075
- Palmquist L, Swortwood MJ. Data-independent Screening Method for 14 Fentanyl Analogs in Whole Blood and Oral Fluid using LC-QTOF-MS. *Forensic Science International* 2019; 297:189-197. https://doi.org/10.1016/j.forsciint.2019.02.006
- Truver MT and Swortwood MJ. Quantitative Analysis of Novel Synthetic Opioids, Morphine, and Buprenorphine in Oral Fluid by LC-MS/MS. *Journal of Analytical Toxicology* 2018;42:554-561. https://doi.org/10.1093/jat/bky053
- K. Palmquist and M.J. Swortwood. LC-QTOF screening for fentanyl analogs in whole blood and oral fluid. POSTER PRESENTATION. American Academy of Forensic Sciences Annual Conference. Baltimore, MD. February 2019.
- M.T. Truver and M.J. Swortwood. Quantitative analysis of novel synthetic opioids, morphine, and buprenorphine in oral fluid by LC-MS/MS. POSTER PRESENTATION. Society of Forensic Toxicologists Annual Meeting. Minneapolis, MS. October 2018.

Data Sets Generated

Data from each method validation were generated. Data from analysis of authentic specimens were also generated by liquid chromatography-tandem mass spectrometry. All samples were anonymized, and no personally identifiable information was collected.

Dissemination Activities

- Swortwood, MJ. Novel Synthetic Opioids in Oral Fluid: Analytical Methods and Prevalence. WEBINAR. Forensic Technology Center of Excellence. February 2021. https://forensiccoe.org/novel-synthetic-opioids-oral-fluid/
- M.T. Truver, K. Palmquist, M.J. Swortwood. Detection and quantification of synthetic opioids in oral fluid. ORAL PRESENTATION. 2019 National Institute of Justice Forensic Science Research & Development Symposium at AAFS. Baltimore, MD. February 2019.
- M. T. Truver and M.J. Swortwood. Drug Impairment: Pairing Toxicology with Drug Recognition Expert Observations. POSTER PRESENTATION. 2019 National Institute of Justice Forensic Science Research & Development Symposium at PittCon. Philadelphia, PA. March 2019.