Development and application of a High-Resolution mass spectrometry method for the detection of fentanyl analogs in urine and serum

Yu Zhang [a], John C. Halifax [a], Christina Tangsombatvisit [b], Cassandra Yun [a], Shaokun Pang [c], Shirin Hooshfar [a], Alan H.B. Wu [a], Kara L. Lynch [a,*]

[a] *Department of Laboratory Medicine, University of California, San Francisco, and Zuckerberg San Francisco General Hospital, 1001 Potrero Ave, ZSFG Bldg. 5, 2M16 San Francisco, CA 94110, USA*
[b] *University of California, Berkeley, San Francisco, CA 94720, USA*
[c] *SCIEX, 1201 Radio Rd, Redwood City, CA 94065, USA*

ARTICLE INFO

ABSTRACT

Introduction: The use of illicitly manufactured synthetic opioids, specifically fentanyl and its analogs, has escalated exponentially in the United States over the last decade. Due to the targeted nature of drug detection methods in clinical laboratories and the ever-evolving list of synthetic opioids of concern, alternative analytical approaches are needed.

Methods: Using the fentanyl analog screening (FAS) kit produced by the Centers for Disease Control and Prevention (CDC), we developed a liquid chromatography-high resolution mass spectrometry (LC-HRMS) synthetic opioid spectral library and data acquisition method using information dependent acquisition of product ion spectra. Chromatographic retention times, limits of detection and matrix effects, in urine and serum, for the synthetic opioids in the FAS kit (n = 150) were established. All urine and serum specimens sent to a clinical toxicology laboratory for comprehensive drug testing in 2019 (n = 856) and 2021 (n = 878) were analyzed with the FAS LC-HRMS library to determine the prevalence of fentanyl analogs and other synthetic opioids, retrospectively (2019) and prospectively (2021).

Results: The limit of detection (LOD) of each opioid ranged from 1 to 10 ng/mL (median, 2.5 ng/mL) in urine and 0.25–2.5 ng/mL (median, 0.5 ng/mL) in serum. Matrix effects ranged from −79 % to 86 % (median, −37 %) for urine, following dilution and direct analysis, and −80 % to 400 % (median, 0 %) for serum, following protein precipitation. The prevalence of fentanyl/fentanyl analogs in serum samples increased slightly from 2019 to 2021 while it remained the same in urine. There were only 2 samples identified that contained a fentanyl analog without the co-occurrence of fentanyl or fentanyl metabolites. Analysis of the established MS/MS spectral library revealed characteristic fragmentation patterns in most fentanyl analogs, which can be used for structure elucidation and drug identification of future analogs.

Conclusions: The LC-HRMS method was capable of detecting fentanyl analogs in routine samples sent for comprehensive drug testing. The method can be adapted to accommodate testing needs for the evolving opioid epidemic.

Introduction

Reported overdose deaths in the United States related to fentanyl, fentanyl analogs, and synthetic opioids other than methadone increased from 3,105 in 2013 to 56,516 in 2020. Since 2016, synthetic opioids, predominantly fentanyl and analogs, have caused more deaths annually than any other illicit drug [1]. Illicit drug manufacturers modify the structural backbone of fentanyl to generate fentanyl analogs to circumvent drug detection and regulation. Multiple fentanyl analogs have been detected in drug products and biological specimens over the

past decade and their diversity continues to evolve [2–8]. The current prevalence of fentanyl analogs and synthetic opioids in patients seeking medical care is unknown as specific identification methods have not been routinely used in clinical settings.

Automated fentanyl immunoassays have been FDA-approved and implemented in clinical laboratories for qualitative screening of fentanyl, however, these assays have specificity and sensitivity limitations. For example, risperidone and its active metabolite, 9-hydroxyrisperidone, exhibit cross-reactivity with one of these immunoassays, while norfentanyl, the primary metabolite of fentanyl, demonstrates limited cross-reactivity causing false-negatives at standard concentrations in urine [9]. Many fentanyl analogs show good cross-reactivity with the available fentanyl immunoassays [10–12], however, immunoassays capable of detecting other structurally unrelated synthetic opioids are uncommon.

To address the limitations of immunoassays, mass spectrometry (MS)-based methods, including gas chromatography-MS (GC–MS), liquid chromatography-tandem MS (LC-MS/MS), and liquid chromatography-high resolution MS (LC-HRMS), have been developed for screening and confirmation of fentanyl analogs or other synthetic opioids [13–16]. HRMS measures exact masses, which can be used to calculate the empirical formula of the analyzed drugs allowing for the differentiation of compounds with the same nominal mass. With HRMS methods, the data is typically acquired in an untargeted manner, which is favorable for the detection of emerging fentanyl analogs and synthetic opioids.

In 2019, the Centers for Disease Control and Prevention (CDC) developed Traceable Opioid Material Kits to support laboratory detection of current and emerging opioids. One published report to-date has developed a HRMS library using these kits, but the method was only validated for the detection of eight fentanyl analogs in spiked samples [17]. We collected product ion mass spectra for 150 fentanyl analog screening (FAS) standards for inclusion in our HRMS library and established retention times for each drug on our routine LC-HRMS comprehensive drug testing method. We validated the limit of detection (LOD) and matrix effects in serum and urine for these 150 FAS standards. For a subset of fentanyl analogs, we determined the LOD for two commercially available fentanyl immunoassays and compared these to the LC-HRMS method. The FAS library we generated was used to retrospectively (2019) and prospectively (2021) analyze data from biological samples sent for comprehensive drug testing to determine the prevalence of these drugs in our patient population. Analysis of the established MS/MS spectral library revealed characteristic fragmentation patterns in most fentanyl analogs, which can be used for structure elucidation and drug identification of future analogs.

Materials and methods

Reagents and standards

Acetonitrile, methanol, and water were of HPLC grade and purchased from Honeywell (Charlotte, NC); ammonium formate and formic acid were purchased from Sigma-Aldrich (St. Louis, MO) and Lichropur (Billerica, MA), respectively. Drug-free urine and serum were purchased from UTAK Laboratories (Valencia, CA) and Bio-Rad Laboratories (Hercules, CA), respectively. Fentanyl-d5 from Cerilliant (Round Rock, TX) was used as an internal standard.

Fentanyl analog screening kits

Two fentanyl analog screening kits, containing 120 synthetic fentanyl analogs (# 9003237) and 30 synthetic opioids (# 9003286), were obtained from Cayman Chemical (Ann Arbor, MI) with support from the CDC. Each drug was reconstituted with 500 μL of methanol to create a stock of 400 μg/ml and kept at −20 °C. For the validation, the 150 drugs were separated into 15 groups with 10 drugs/group. Considering the

high incidence of isomers (~61 %), drugs with the same or similar masses were assigned into different groups to ensure drugs within one group were distinguishable from each other. The minimum mass difference of drugs within the same group was ~2 Da. The grouped samples were prepared with each drug at 100 ng/mL and kept at −20 °C.

Sample preparation

Urine samples were prepared by 1:5 dilution in sample preparation buffer (12.5 % of methanol: acetonitrile (1:1) in H_2O). Serum samples were processed by protein precipitation with acetonitrile (1:4), dried down with nitrogen gas, and reconstituted with 40 % original volume of sample preparation buffer. Fentanyl-d5 was used as an internal standard to compensate for matrix effect and variations during sample preparation and instrumental analysis.

Liquid chromatography-high resolution mass spectrometry (LC-HRMS)

LC-HRMS conditions and data analysis were as described previously [18]. Briefly, liquid chromatographic separation was performed on a Phenomenex Kinetex C18- column (50 × 3.00 mm ID, 2.6 μm) with a Shimadzu Prominence LC-20ADXR system. A gradient program started with 100 % mobile phase A (0.05 % formic acid and 5 mmol/l ammonium formate in H_2O) and gradually switched to 100 % mobile phase B (0.05 % formic acid in methanol: acetonitrile (1:1)) over 10 min. HRMS data were acquired either with a SCIEX TripleTOF®5600 operating in electrospray ionization (ESI) positive-ion mode using a time-of-flight MS (TOF-MS) survey scan with dedicated product ion scan to collect a library spectrum for the library generation, or using information dependent acquisition-triggered collection of product ion spectra for method development, validation and routine testing. The LC-HRMS method identifies drugs based on accurate mass, isotope pattern, retention time, and matching acquired mass spectra to library spectra, which were acquired from each drug standard. Data analysis was performed using PeakView®, MasterView™ and SciexOS™ software (Sciex).

Method validation

The method was validated as a qualitative assay through evaluation of LOD and matrix effects in both urine and serum. The LOD was evaluated by duplicate injections of drug standards at the following concentrations, 1.0, 2.5, 5.0, and 10.0 ng/mL in 6 different drug-free urine matrices, and 0.25, 0.5, 1, 2.5, and 5.0 ng/mL in 6 different drug-free serum matrices. LOD was defined as the lowest concentration for a drug to be identified positive with a combined score (defined below) of >70 % and signal-to-noise ratio >20:1, in all replicates. The combined score was calculated as follows [19]:

$$Combined\ score = 10\%\ mass\ score + 10\%\ retention\ score \\ + 10\%\ isotope\ pattern\ score + 70\%\ library\ score$$

Matrix effects were determined by spiking drug standards into six drug-free urine matrices at 10 ng/mL or into six drug-free serum matrices at 2.5 ng/mL and comparing to drug standard spiked into water before sample processing. This comprehensive matrix effect reflected variations in both sample preparation and matrix effects, including ion suppression or enhancement, caused by co-eluting components during ionization [20]. Samples were injected in triplicate, and signal intensity was represented using peak area. Matrix effects were calculated as following:

$$Matrix\ effect \frac{Mean\ signal\ intensity\ in\ urine\ or\ serum - Mean\ signal\ intensity\ in\ water}{Mean\ signal\ intensity\ in\ water} \\ \times 100\%$$
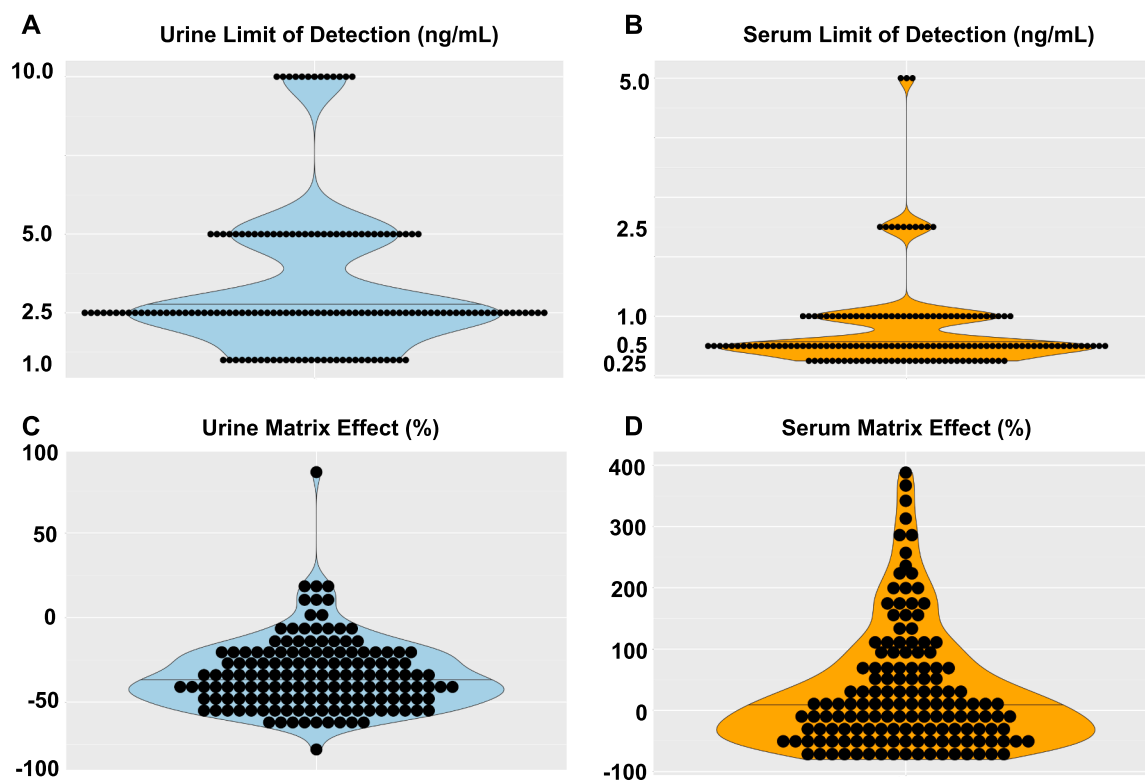
Fig. 1. Limit of detection (LOD) and matrix effect (ME) of fentanyl analogs and synthetic opioids in drug-free urine or serum. Each dot indicates one drug, and y-axis indicates LOD or ME of that drug.

Fentanyl immunoassays

Two fentanyl immunoassays, DRI Fentanyl Assay (Thermo Scientific) and ARK™ Fentanyl Assay (ARK Diagnostics), were performed on the ADVIA Chemistry XPT System (Siemens). The LODs for fentanyl, nor-fentanyl and 18 fentanyl analogs were determined for the immunoassays in the same manner as described above for the LC-HRMS method.

Clinical sample analysis

This study is categorized as "not human research" by the UCSF IRB since the data files that were analyzed were de-identified prior to analysis with no link back to the identifiers (https://irb.ucsf.edu/not-human-subjects-research). All LC-HRMS data collected from urine and serum specimens sent to the toxicology laboratory for comprehensive drug testing in 2019 (n = 856 total, 394 urine, 462 serum) and 2021 (n = 878 total, 395 urine, 483 serum) were analyzed against the FAS library to determine the prevalence of fentanyl analogs and other synthetic opioids. Data analysis was performed using PeakView®, MasterView™ and SciexOS™ software (Sciex). Data was processed in Excel and R, version 4.1.3, and co-occurrences of fentanyl analogues were visualized using the UpSetR package in R, version 4.1.3 [21,22].

Results and discussion

Method development and validation

The retention time and product ion spectra of each fentanyl analog or other synthetic opioid were collected and added to our HRMS FAS library (Supplemental Table 1). Results of the method validation are shown in Fig. 1 and Supplemental Table 1. The LODs in urine ranged from 1 to 10 ng/mL (median 2.5 ng/mL) (Fig. 1A) with an LOD ≤ 5.0 ng/mL for most drugs (137 out of 150). In serum, the LODs ranged from 0.25 to 5.0 ng/mL (median 0.5 ng/mL) with an LOD ≤ 1.0 ng/mL for

most drugs (147 out of 150) (Fig. 1B). In fentanyl overdose cases, urine concentrations have been reported to range from 5 to 95 ng/mL (23). The serum concentrations of fentanyl required to achieve analgesia range from 0.6 to 3.0 ng/mL, concentrations >2.0 ng/mL are associated with respiratory depression, and fatal concentrations are reported to range from 3 to 50 ng/mL (average 25 ng/mL) [23–26]. The HRMS FAS method provides sufficient sensitivity for cases of fentanyl exposure with a urine LOD of 2.5 ng/mL and serum LOD of 0.5 ng/mL. Pharmacokinetic information on most fentanyl analogs is currently unknown. Three fentanyl analogs that have been studied, acetyl fentanyl, butyryl fentanyl and valeryl fentanyl, are less potent than fentanyl, meaning that a higher concentration is required to elicit the same effect. The potencies for these three analogs range from 0.03 to 0.29 compared to fentanyl, while the urine LODs (2.5 ng/mL) and serum LODs (0.5–1 ng/mL) are similar to fentanyl. For drugs with higher potency, they can perform biological functions at a lower dosage and blood concentration and, therefore, higher sensitivity is needed for detection. Carfentanil, the most potent analog known to date, was found to have an LOD of 1 ng/mL in urine and 0.25 ng/mL in serum. In case studies, carfentanil concentrations were found to be 0.03–12 ng/ml in urine and 0.1–120 ng/mL in blood [27]. Therefore, given the limited information available for fentanyl analog pharmacokinetics, the FAS method is expected to provide adequate sensitivity to detect most, if not all, fentanyl analogs at physiologically relevant concentrations in clinical cases.

The matrix effects in urine for the fentanyl analogs ranged from −79 % to 86 % with a median of −37 % (Fig. 1C), while matrix effects in serum ranged from −80 % to 400 % with a median of 0 % (Fig. 1D). For all drugs, the matrix effects in both urine and serum were >−85 %, falling into an acceptable range, as reported [20]. For a few analogs, extremely low signals were obtained from standards spiked into water and processed using protein precipitation, signifying low stability or solubility in water. In these cases, serum matrix effect estimates are less reliable; however, the serum LOD for these drugs ranged from 0.25 to 0.5 ng/mL, indicating that matrix effects or other interferences are
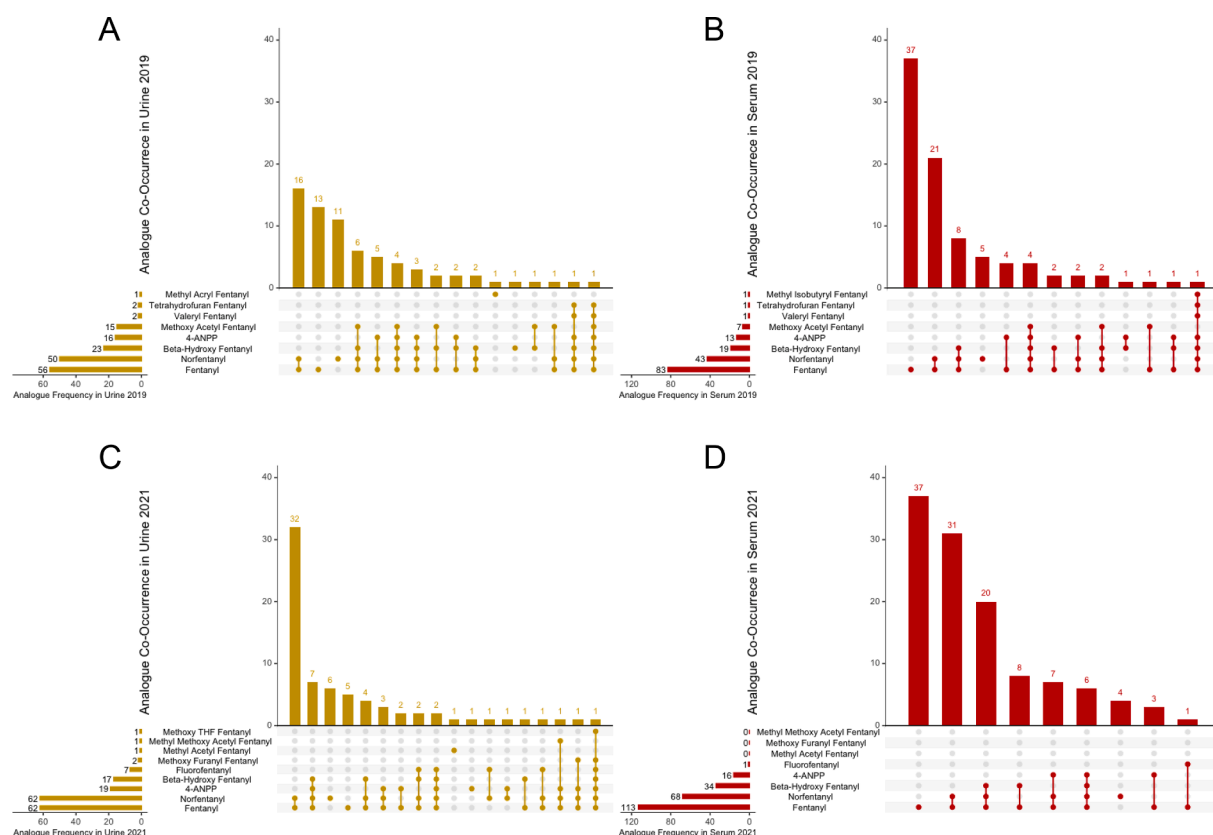
Fig. 2. The frequency and co-occurrence of fentanyl and analogs sent for comprehensive drug testing in 2019 in urine (A, n = 394) and serum (B, n = 462) and in 2021 in urine (C, n = 395) and serum (D, n = 483).

negligible. In addition, there were a few outliers with high matrix effects; this is to be expected given that the sample preparation approaches are intentionally non-specific (dilution for urine, and protein precipitation for serum) in attempts to detect a broad range of drugs.

LC-HRMS and fentanyl immunoassay comparison

To evaluate the performance of this LC-HRMS method versus readily available fentanyl screening methods in clinical laboratories, we compared it with two FDA-approved fentanyl immunoassays. Fentanyl, norfentanyl, and 18 representative fentanyl analogs were selected to compare the LODs in urine samples using these three methods. For the immunoassays, 13–14 drugs were found to have LODs $\leq$ 10 ng/mL while the other 6–7 drugs, including norfentanyl, were found to have LODs $\geq$ 100 ng/mL. With the LC-HRMS method, the LODs were $\leq$ 10 ng/mL for all fentanyls (Table 1). This highlights the sensitivity limitations of available immunoassays compared to mass spectrometric methods, such as the described LC-HRMS method.

Analysis of data from clinical samples

The LC-HRMS synthetic opioid method and library were used to determine the frequency and co-occurrence of fentanyl and analogs in urine and serum samples sent for comprehensive drug testing in 2019 (Fig. 2A and B) and 2021 (Fig. 2C and D). A total of 856 (462 serum, 394 urine) samples from 2019 and 878 (483 serum, 395 urine) samples from 2021 were submitted for testing and analysis. Fentanyl and/or fentanyl analogs were identified in 19 % of serum specimens and 18 % of urine specimens in 2019. In 2021, 24 % of serum specimens and 18 % of urine specimens were fentanyl and/or fentanyl analog positive. The prevalence of fentanyl/fentanyl analogs in serum samples increased slightly

from 2019 to 2021 while it remained the same in urine. There were only two samples identified that contained a fentanyl analog (2019 urine – methyl acryl fentanyl, 2021 urine – methy acetyl fentanyl) without the co-occurrence of fentanyl or fentanyl metabolites, norfentanyl and β-hydroxy fentanyl, suggesting that fentanyl was the primary constituent in fentanyl containing drug products in our geographical region from 2019 through 2021. In 2019, the highest frequency analog was methoxy acetyl fentanyl (15 urines, 7 serums) followed by valeryl fentanyl (2 urines, 1 serum) and tetrahydrofuran (THF) fentanyl (2 urines, 1 serum). In 2021, these analogs were no longer present. Fluorofentanyl, was the predominate fentanyl analog in 2021 (7 urines, 1 serum), followed by isolated occurrences of methoxy furanyl fentanyl, methyl acetyl fentanyl, methyl methoxy acetyl fentanyl, and methoxy THF fentanyl, all identified in either one or two urine specimens.

While fentanyl continues to be the major player in the opioid epidemic, the ability to identify emerging fentanyl analogs has value for individual clinical cases and from a public health perspective. In opioid overdose cases, rule-in or rule-out of the presence of a fentanyl analog may inform and facilitate life-saving harm reduction interventions relating to future drug use for that patient. When a patient presents clinically with an opioid overdose, but has additional unexpected clinical manifestations, identification of a fentanyl analog may lead to a better understanding of its clinical effects, whereas, specific identification of fentanyl may indicate the need for further medical work-up to understand the underlying cause of the other symptoms. Disruptions in the drug supply chain can result in significant changes in the potency and purity of drug products resulting in an increase in overdose risk. Early identification of an emerging potent fentanyl analog in a clinical case can inform early public health interventions in attempts to avoid a potential overdose outbreak.
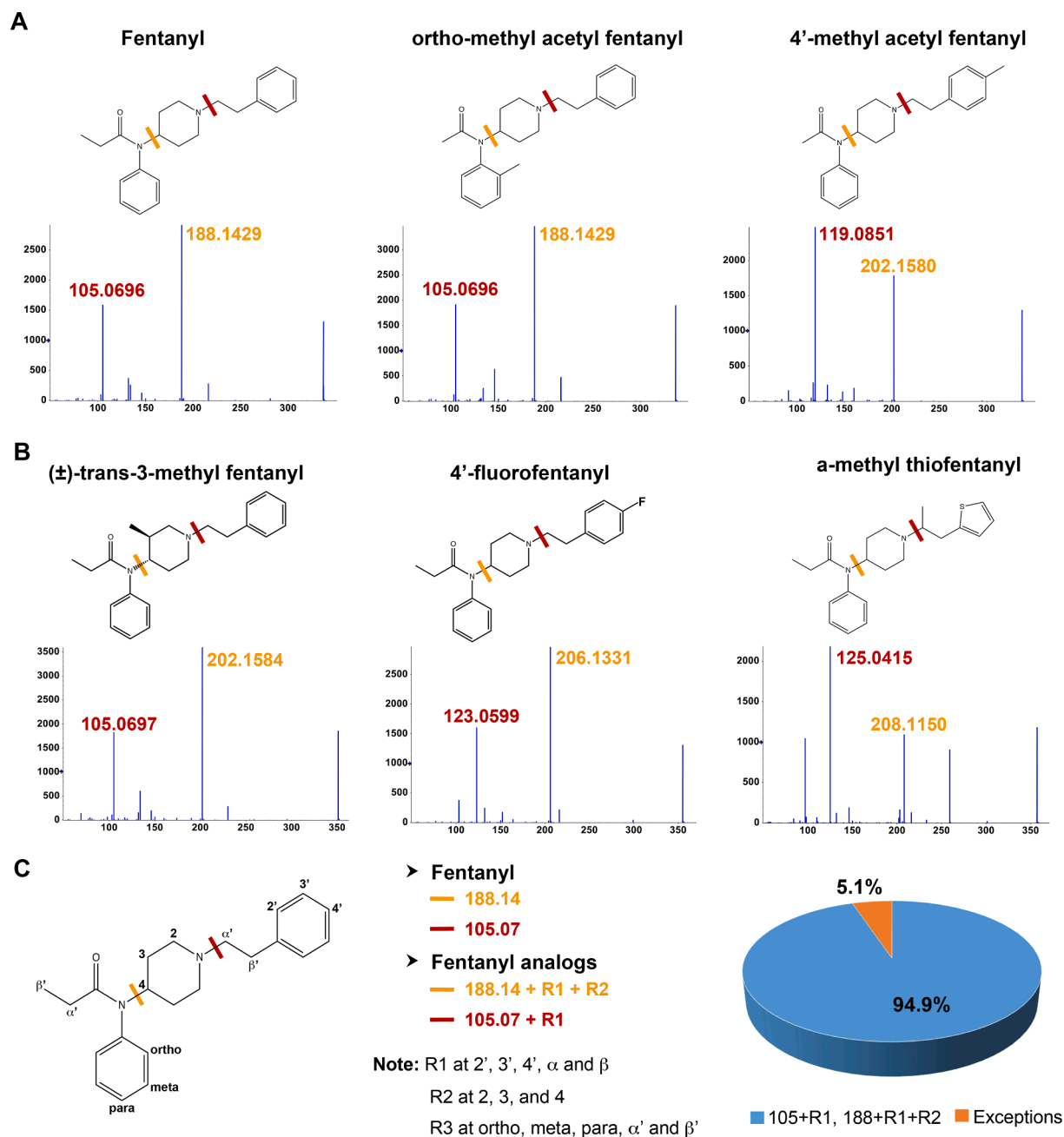
Fig. 3. The molecular structure and MS/MS of fentanyl, fentanyl isomers (A), and other fentanyl analogs (B). The two most common fragmented bonds and product ion prediction based on structure (C). The percentage of fentanyl analogs that follow the fragmentation prediction (D).

Product ion prediction for fentanyl analogs and differentiation of isomers

There are two dominant product ions of fentanyl with a mass-to-charge ratio of 188.1429 and 105.0696. Predicted MS/MS fragmentation suggests that fragmentation of fentanyl takes places at bonds N-4C and N-αC (Fig. 3A), consistent with previous reports [28]. In the synthetic opioid library, there are five isomers of fentanyl, including para-, ortho-, α-, β-, and 4′-methyl acetyl fentanyl, which have a methyl at different loci. All fentanyl isomers break at the same two bonds and generate similar or different fragments depending on the location of the methyl group. Fig. 3A shows ortho methyl acetyl fentanyl and 4′-methyl acetyl fentanyl, both isomers of fentanyl, as examples. Fentanyl and ortho methyl acetyl fentanyl cannot be distinguished by their product ion spectra, but are chromatographically distinct with retention times (RT) of 5.84 and 5.66 min, respectively. Fentanyl and 4′methyl acetyl

fentanyl (RT = 5.88 min) have similar retention times, but produce different product ion spectra. In general, the described LC-HRMS method can differentiate between structural isomers by RT and/or fragmentation pattern, however, it cannot differentiate stereoisomers. For positional isomers, differentiation is dependent upon where the modification is located in relationship to the two predominate bonds that are fragmented. In most cases, the LC-HRMS method cannot distinguish between meta-, ortho-, and para- isomers, but can differentiate between α- and β- isomers. In clinical practice, when isomers cannot be distinguished by different retention times or fragmentation patterns they are not reported out separately. For example, meta-, ortho-, and para- fluorofentanyl all have the same RT and fragmentation pattern and will all be identified as "fluorofentanyl".

To further explore the relationship of fentanyl analog structure and fragmentation patterns, we went through the spectra of all fentanyl

analogs and found that the fragmentation predominately occurred at the same two bonds as fentanyl for the fentanyl analogs (Fig. 3B). Therefore, most generate two dominant fragments of 188.14 + R1 + R2 and 105.07 + R1. Of note, R1 and R2 are side moieties at different locations on the fentanyl backbone (Fig. 3C). Among the fentanyl analogs in the synthetic opioid library, 94.9 % (129 out of 136) follow this rule (Fig. 3D).

Conclusion

The LC-HRMS FAS method was successfully validated and implemented in a clinical toxicology laboratory. LODs were comparable to the reported analgesia concentration and in most cases lower. Therefore, this method can detect synthetic opioids and fentanyl analogs at clinically significant concentrations. We performed retrospective and prospective analyses in clinical toxicology cases at our institution. Most cases had fentanyl only or a combination of fentanyl metabolites and/or by-products. The prevalence of fentanyl analogs is relatively low in our geographic area, and the clinical implications are yet to be determined. However, moving forward, this method enables us to identify or distinguish between different fentanyl analogs which may aid in a better understanding of the prevalence and exposure risks in our geographical region. This capacity for routine analysis of synthetic opioids and fentanyl analogs has the potential to reveal insights into the synthetic opioid crisis and inform the public health response.

We were able to characterize the relationship between fentanyl analog structures and their product ions in which fragmentation occurs at two specific bonds generating two dominate ions. This information can be used to identify novel fentanyl analogs, for which standards are not available. It can also be used for elucidation of fentanyl analog metabolism. The LC-HRMS method we have developed is not only able to detect a large number of synthetic opioids but also adaptable to accommodate the rapidly changing landscape of fentanyl analogs being introduced.

IRB and ethics statement

This study was approved by the UCSF IRB who deemed patient consent was not required for analysis of de-identified data acquired for routine clinical purposes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmsacl.2022.07.005.

References

[1] Centers for Disease Control and Prevention/National Center for Health Statistics, National Vital Statistics System, CDC Wonder, Atlanta, GA: US Department of Health and Human Services, CDC; 2020. https://wonder.cdc.gov/, accessed May 12, 2022.

[2] P. Armenian, K.T. Vo, J. Barr-Walker, K.L. Lynch, Fentanyl, fentanyl analogs and novel synthetic opioids: a comprehensive review, Neuropharmacology 134 (2018) 121–132.

[3] C.E. Cowles Jr., J. Mitchell, J.E. Stepp, V.Z. Bewley, Carfentanil: a new and often unrecognized threat, J. Spec. Oper. Med. 17 (2017) 120–122.

[4] K. Waite, A. Deeken, S. Perch, L.J. Kohler, Carfentanil and current opioid trends in Summit County, Ohio, Acad. Forensic Pathol. 7 (4) (2017) 632–639.

[5] M. Bäckberg, O. Beck, K.-H. Jönsson, A. Helander, Opioid intoxications involving butyrfentanyl, 4-fluorobutyrfentanyl, and fentanyl from the Swedish STRIDA project, Clin. Toxicol. (Phila) 53 (7) (2015) 609–617.

[6] A.D. Le, S.K. Alzghari, Systematic review of the clinical consequences of butyrfentanyl and corresponding analogues, Interdiscip. Toxicol. 12 (2019) 83–88.

[7] M.J. Lozier, M. Boyd, C. Stanley, L. Ogilvie, E. King, C. Martin, L. Lewis, Acetyl fentanyl, a novel fentanyl analog, causes 14 overdose deaths in Rhode Island, March-May 2013, J. Med. Toxicol. 11 (2) (2015) 208–217.

[8] J.B. Dwyer, J. Janssen, T.M. Luckasevic, K.E. Williams, Report of increasing overdose deaths that include acetyl fentanyl in multiple counties of the southwestern region of the commonwealth of Pennsylvania in 2015–2016, J. Forensic Sci. 63 (1) (2018) 195–200.

[9] B.T. Wang, J.M. Colby, A.H. Wu, K.L. Lynch, Cross-reactivity of acetylfentanyl and risperidone with a fentanyl immunoassay, J. Anal. Toxicol. 38 (2014) 672–675.

[10] A. Helander, K. Stojanovic, T. Villén, O. Beck, Detectability of fentanyl and designer fentanyls in urine by 3 commercial fentanyl immunoassays, Drug Test Anal. 10 (8) (2018) 1297–1304.

[11] D. Guerrieri, F. Kjellqvist, R. Kronstrand, H. Green, Validation and cross-reactivity data for fentanyl analogs with the immunalysis fentanyl ELISA, J. Anal. Toxicol. 43 (1) (2019) 18–24.

[12] R.E. Wharon, J. Casbohm, R. Hoffmaster, B.N. Brewer, M.G. Finn, R.C. Johnson, Detection of 30 fentanyl analogs by commercial immunoassay kits, J. Anal. Toxicol. 45 (2) (2021) 111–116.

[13] S. Sofalvi, H.E. Schueler, E.S. Lavins, C.K. Kaspar, I.T. Brooker, C.D. Mazzola, D. Dolinak, T.P. Gilson, S. Perch, An LC-MS-MS method for the analysis of carfentanil, 3-methylfentanyl, 2-furanyl fentanyl, acetyl fentanyl, fentanyl and norfentanyl in postmortem and impaired-driving cases, J. Anal. Toxicol. 41 (6) (2017) 473–483.

[14] A.L. Mohr, M. Friscia, D. Papsun, S.L. Kacinko, D. Buzby, B.K. Logan, Analysis of novel synthetic opioids U-47700, U-50488 and Furanyl Fentanyl by LC-MS/MS in postmortem casework, J. Anal. Toxicol. 40 (2016) 709–717.

[15] A. Wohlfarth, K.B. Scheidweiler, S. Pang, M. Zhu, M. Castaneto, R. Kronstrand, M.A. Huestis, Metabolic characterization of AH-7921, a synthetic opioid designer drug: in vitro metabolic stability assessment and metabolite identification, evaluation of in silico prediction, and in vivo confirmation, Drug Test Anal. 8 (8) (2016) 779–791.

[16] A.L. Patton, K.A. Seely, S. Pulla, N.J. Rusch, C.L. Moran, W.E. Fantegrossi, L.D. Knight, J.M. Marraffa, P.D. Kennedy, L.P. James, G.W. Endres, J.H. Moran, Quantitative measurement of acetyl fentanyl and acetyl norfentanyl in human urine by LC-MS/MS, Anal. Chem. 86 (3) (2014) 1760–1766.

[17] L.C. Krajewski, K.D. Swanson, W.A. Bragg, R.L. Shaner, C. Seymour, M.D. Carter, E.I. Hamelin, R.C. Johnson, Application of the fentanyl analog screening kit toward the identification of emerging synthetic opioids in human plasma and urine by LC-QTOF, Toxicol. Lett. 320 (2020) 87–94.

[18] K.L. Thoren, J.M. Colby, S.B. Shugarts, A.H.B. Wu, K.L. Lynch, Comparison of information-dependent acquisition on a tandem quadrupole TOF vs a triple quadruple linear ion trap mass spectrometer for broad-spectrum drug screening, Clin. Chem. 62 (1) (2016) 170–178.

[19] J.M. Colby, K.L. Thoren, K.L. Lynch, Optimization and validation of high-resolution mass spectrometry data analysis parameters, J. Anal. Toxicol. 41 (1) (2017) 01–05.

[20] W. Zhou, S. Yang, P.G. Wang, Matrix effects and application of matrix effect factor, Bioanalysis 9 (23) (2017) 1839–1844.

[21] J.R. Conway, A. Lex, N. Gehlenborg, J. Hancock, UpSetR: an R package for the visualization of intersecting sets and their properties, Bioinformatics 33 (18) (2017) 2938–2940.

[22] A. Lex, N. Gehlenborg, H. Strobelt, R. Vuillemot, H. Pfister, UpSet: visualization of intersecting sets, IEEE Trans. Vis. Comput. Graph. 20 (12) (2014) 1983–1992.

[23] A. Poklis, Fentanyl: a review for clinical and analytical toxicologists, J. Toxicol. Clin. Toxicol. 33 (5) (1995) 439–447.

[24] K. Kumar, D.J. Morgan, D.P. Crankshaw, Determination of fentanyl and alfentanil in plasma by high-performance liquid chromatography with ultraviolet detection, J. Chromatogr. 419 (1987) 464–468.

[25] P.W. Peng, A.N. Sandler, A review of the use of fentanyl analgesia in the management of acute pain in adults, Anesthesiology 90 (1999) 576–599.

[26] T.L. Martin, K.L. Woodall, B.A. McLellan, Fentanyl-related deaths in Ontario, Canada: toxicological findings and circumstances of death in 112 cases (2002–2004), J. Anal. Toxicol. 30 (8) (2006) 603–610.

[27] N. Allibe, N. Fouilhe Sam-Lai, T. Willeman, J.-F. Jourdil, M. Bartoli, M. Mallaret, B. Nemoz, F. Stanke-Labesque, H. Eysseric-Guerin, Norcarfentanil: carfentanil misuse or remifentanil treatment, Forensic Toxicol. 37 (2) (2019) 488–495.

[28] W. Wichitnithad, T.J. McManus, P.S. Callery, Identification of isobaric product ions in electrospray ionization mass spectra of fentanyl using multistage mass spectrometry and deuterium labeling, Rapid Commun. Mass Spectrom. 24 (17) (2010) 2547–2553.