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A LC-MS/MS-Based Method for the Multiplex Detection of 24 Fentanyl Analogs and Metabolites in Whole Blood at Sub ng mL-1 Concentrations

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A LC-MS/MS-BASED METHOD FOR THE MULTIPLEX DETECTION OF 24 FENTANYL ANALOGS AND METABOLITES IN WHOLE BLOOD AT SUB NG ML^{-1} CONCENTRATIONS

A thesis submitted in partial fulfillment of the

requirements for the degree of

Master of Science

By

KRAIG EDWARD STRAYER

B.S., Wright State University, 2015

2018

Wright State University

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WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

February 23, 2018

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Kraig Edward Strayer ENTITLED A LC-MS/MS-Based Method for the Multiplex Detection of 24 Fentanyl Analogs and Metabolites in Whole Blood at Sub ng mL^{-1} Concentrations BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Strayer, Kraig Edward. M.S. Department of Chemistry, Wright State University, 2018. A LC-MS/MS-Based Method for the Multiplex Detection of 24 Fentanyl Analogs and Metabolites in Whole Blood at Sub ng mL^{-1} Concentrations

The United States and numerous other countries worldwide are currently experiencing a public health crisis due to the abuse of illicitly manufactured fentanyl (IMF) and its analogues. This manuscript describes the development of a liquid chromatography-tandem mass spectrometry-based method for the multiplex detection of N = 24 IMF analogues and metabolites in whole blood at concentrations as low as 0.1−0.5 ng mL^{-1}. These available IMFs were fentanyl, norfentanyl, furanyl norfentanyl, remifentanil acid, butyryl norfentanyl, remifentanil, acetyl fentanyl, alfentanil, AH-7921, U-47700, acetyl fentanyl 4-methylphenethyl, acrylfentanyl, para-methoxyfentanyl, despropionyl fentanyl (4-ANPP), furanyl fentanyl, despropionyl para-fluorofentanyl, carfentanil, (±)-cis-3-methyl fentanyl, butyryl fentanyl, isobutyryl fentanyl, sufentanil, valeryl fentanyl, para-fluorobutyryl fentanyl, and para-fluoroisobutyryl fentanyl. Most IMF analogues (N = 22) could be easily distinguished from one another; the isomeric forms butyryl/isobutyryl fentanyl and para-fluorobutyryl/para-fluoroisobutyryl fentanyl could not be differentiated. N = 13 of these IMF analogues were quantified for illustrative purposes, and their forensic quality control standards were also validated for limit of detection (0.017−0.056 ng mL−1), limit of quantitation (0.100−0.500 ng mL−1), selectivity/sensitivity, ionization suppression/enhancement (87−118%), process

efficiency (60−95%), recovery (64−97%), bias (<20%), and precision (>80%). This flexible, time- and cost-efficient method was successfully implemented at the Montgomery County Coroner's Office/Miami Valley Regional Crime Laboratory in Dayton, Ohio, where it aided in the analysis of N = 725 postmortem blood samples collected from February 2015 to November 2016.

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Acknowledgements

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1. Introduction

Fentanyl is a synthetic opioid that was developed for pharmaceutical use in 1960 by Paul Janssen in Belgium.[1−4] Since its introduction into the United States in 1970, fentanyl has rapidly become a leading analgesic and anesthetic agent due to its 50−100 times higher potency than that of morphine, shorter onset, and quicker absorption by the human body.[3,4] Fentanyl causes depression of the respiratory and central nervous system in a dose-dependent manner. Over the past few years, increased availability and abuse of illicitly manufactured fentanyl (IMF) and its analogues emerged as a significant threat to public health in the United States and other countries.[5−10] Ohio is one of several U.S. states that was gravely impacted by the opioid epidemic; the number of IMF-related overdose deaths increased by 526% between 2013 and 2015.[11] Even more alarming is the fact that new IMF analogues are being synthesized in Asian countries and marketed on a regular basis across United States and Europe in an attempt to stay ahead of regulations.[12−14] Many of these analogues have increased potency compared with IMF. For example, carfentanil or the so called "elephant tranquilizer" entered the U.S. market in July 2016 and is known to be 100 times more potent than fentanyl.15,16 From July to November 2016, over 80% of all carfentanil positive cases in the United States (i.e., N = 451 cases) were reported in Ohio.[17]

The U.S. Drug Enforcement Agency (DEA) has responded to this epidemic by declaring IMF a public health safety factor on March 18th, 2015.[8] Unfortunately, IMF and its analogues are not always part of routine toxicology testing in the United States. Thus, there is an urgent need for developing sensitive, multiplex detection methods that could be easily modified to include newly emerging IMF analogues. A successful method was reported in 2017 by the Miami-Dade County Medical Examiner Department, where an ultrahigh performance liquid chromatography ion trap mass spectrometry system with MSn capabilities (UHPLC-Ion-Trap-MSn) was employed for the qualitative identification of N = 13 IMF analogues (i.e., acetyl fentanyl, alfentanil, β-hydroxythiofentanyl, butyryl fentanyl, carfentanil, despropionyl fentanyl, fentanyl, furanyl fentanyl, norfentanyl, 4-fluoroisobutyryl fentanyl, 4-fluorobutyryl fentanyl, sufentanil, and U-47700) in postmortem samples.[18]

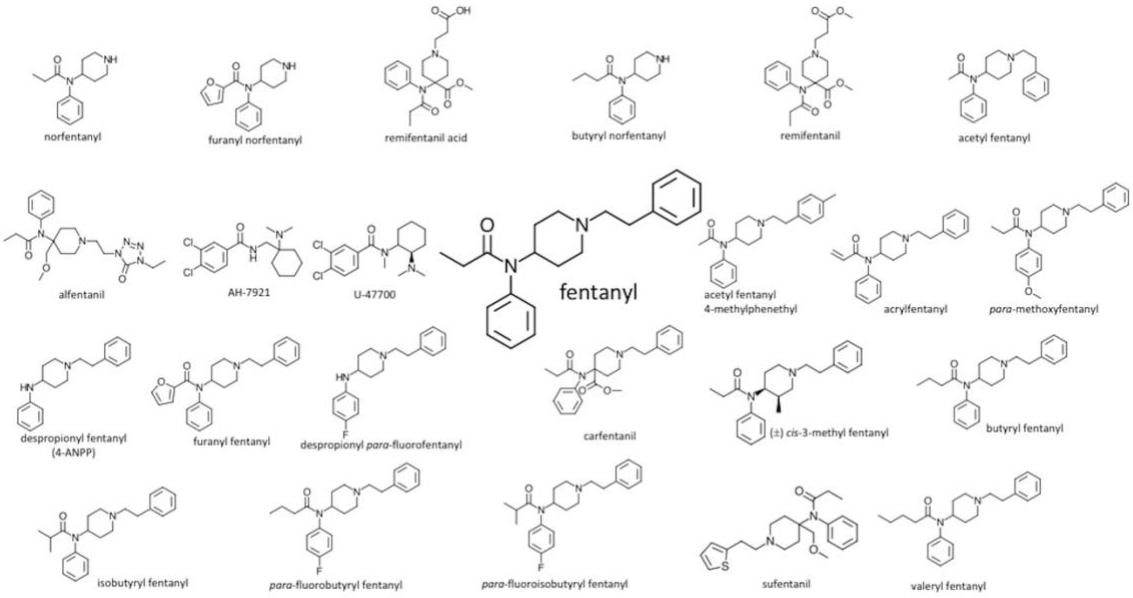

Figure 1. Molecular structure of N = 24 IMF analogues, metabolites, and synthetic opioids used for the development of the LC-MS/MS-based method.

The **Key Aim** of this study is to describe the development and validation of a new liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based method for the multiplex detection of N = 24 IMF analogues, metabolites, and synthetic opioids. The IMF analogues were selected on the basis of previous forensic reports and their presence on the Dark Web:[19] (1) norfentanyl, (2) furanyl norfentanyl, (3) remifentanil acid, (4) butyryl norfentanyl, (5) remifentanil, (6) acetyl fentanyl, (7) alfentanil, (8) AH-7921, (9) U-47700, (10) acetyl fentanyl 4-methylphenethyl, (11) acrylfentanyl, (12) fentanyl, (13) para-methoxyfentanyl, (14) despropionyl fentanyl (4-ANPP), (15) furanyl fentanyl, (16) despropionyl para-fluorofentanyl, (17) carfentanil, (18) (±)-cis-3-methyl fentanyl, (19) butyryl fentanyl, (20) isobutyryl fentanyl, (21) para-fluorobutyryl fentanyl, and (22) para-

fluoroisobutyryl fentanyl, (23) sufentanil, and (24) valeryl fentanyl (Figure 1). U-47700 is not an analogue of fentanyl and is not approved as a pharmaceutical agent, but it is typically included in fentanyl studies because of its similar, potent analgesic activity and combination with IMF in cases of overdose deaths.[20] AH-7921 is also a synthetic opioid analgesic that was placed into schedule I of the U.S. Controlled Substances Act in 2016.[21] It is usually incorporated in fentanyl-related studies due to its structure being similar to that of IMF and potency comparable to that of morphine.[22]

In this study, LC-MS/MS is the analytical method of choice because of its common use in numerous forensic and toxicology laboratories across the nation.[23] LC has become the leading separation technique in chromatography due to its flexibility, reproducibility, and efficiency. Although LC achieves the physical separation of multiple components in a mixture, MS offers information about their structural identity. The addition of tandem MS technology further improves the specificity and accuracy of the detection method. The triple-quadrupole mass spectrometry (QQQ) capability of the selected system facilitates the simultaneous identification and quantification of fentanyl analogues. QQQ performs a true multiple-reaction monitoring (MRM) mode scan because both mass analyzers can simultaneously monitor quantitative and qualitative ion transitions. Running dynamic MRM[24,25] is desired for rapid and simple quantifications due to its dynamic/noble range and sensitivity.[26] Pairing LC-MS/MS with solid phase extraction (SPE)[27] allows for the identification and quantification of IMF analogues from postmortem blood.

2. Methods

2.1 Materials.

High-performance liquid chromatography (HPLC) grade water and acetonitrile (ACN) were purchased from Honeywell (Morris Plains, NJ). Formic acid (88%), methanol, ammonium formate, potassium phosphate mono-basic-sodium hydroxide buffer solution (phosphate-buffered saline (PBS), pH 6.0), glacial acetic acid, ammonium hydroxide, isopropanol, and methylene chloride were obtained from Fisher Scientific (Pittsburgh, PA). Certified reference standards of acetyl fentanyl, acetyl norfentanyl, alfentanil, sufentanil, fentanyl, and norfentanyl were acquired from both Cerilliant (Round Rock, TX) and Lipomed (Cambridge, MA). Butyryl fentanyl and (±)-cis-3-methyl fentanyl were procured from both Lipomed and Cayman Chemical. Butyryl norfentanyl, para-fluorofentanyl, para-fluorobutyryl fentanyl, furanyl fentanyl, furanyl norfentanyl, valeryl fentanyl, acrylfentanyl, isobutyryl fentanyl, despropionyl para-fluorofentanyl, 4-ANPP, U-47700, 4-fluoroisobutyryl fentanyl, para-methoxyfentanyl, acetyl fentanyl 4-methylphenethyl analogue, and AH-7921 were purchased from Cayman Chemical (Ann Arbor, MI). Remifentanil and remifentanil metabolite were obtained from Cerilliant. Internal standards were acetyl fentanyl-$^{13}C_6$, fentanyl-d_5, and norfentanyl-d_5 from Cerilliant. Carfentanil was donated by DEA. Clean screen drugs of abuse (DAU) SPE columns were acquired from United Chemical Technologies Worldwide Monitoring (Bristol, PA).

2.2 Instrumentation.

Two different LC-MS/MS systems (Agilent Technologies, Santa Clara, CA) were employed for validation purposes: (1) a 1200 series LC system (Binary HPLC Pump, high-performance autosampler, and vacuum degasser) equipped with a 6410 triple quadrupole, and (2) an HPLC 1260 Infinity system (binary pumps, a six-port valve, and high-performance autosampler) coupled to a 6420 triple quadrupole HPLC-MS/MS system.

Figure 2. Left: Image of a 1200 series LC system coupled with a 6410 triple quadrupole. Right: Image of an HPLC 1260 Infinity system coupled to a 6420 triple quadrupole (reproduced with permission of the Montgomery County Coroner's Office laboratories).

The analytical column on both instruments was a Raptor biphenyl LC column (150.0 mm x 3.0 mm, 2.7 µm) that was purchased from Restek (Bellefonte, PA). SPE was done on a UCT Positive Pressure Manifold.

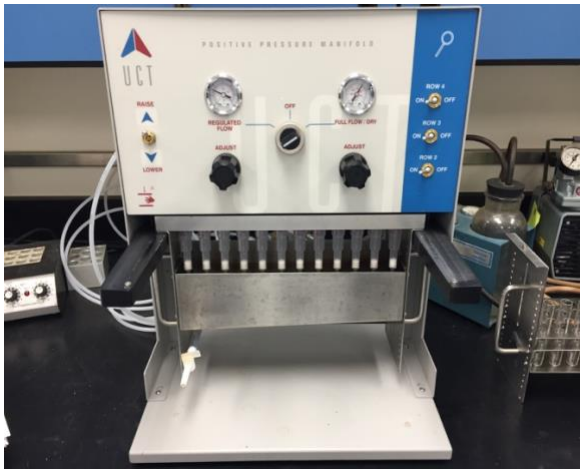

Figure 3. Image of a UCT Positive Pressure Manifold used for extractions (reproduced with permission of the Montgomery County Coroner's Office laboratories).

2.3 Preparation of Calibration and Quality Control Solutions.

Stock standards and stock controls of 1 and 100 ng mL^{-1} were prepared for all IMF analogues by dilution of the purchased certified reference material in methanol and were stored at −4 °C for up to 3 months. All standards (0.1−50.0 ng mL^{-1}) and quality controls (0.35, 2.5, and 25.0 ng mL^{-1}) were made by serial dilution from stocks directly into treated blank whole blood (see Biological Matrices). The quality control concentrations were selected to fit the low (quality control low concentration (QCLO)), medium (medium concentration (QCMED)), and high (high concentration (QCHI)) ends of the calibration range. Additional controls included blank water and blank whole blood. All standards and quality controls excluding blank water were spiked with three internal standards to a final concentration of 10.0 ng mL^{-1}. The norfentanyl-d_5 (stock of 100.0

μg mL^{-1}), fentanyl-d_5 (100.0 μg mL^{-1}), and acetyl fentanyl-$^{13}C_6$ (50.0 μg mL^{-1}) working internal standards were prepared by 5−10-fold volumetric dilution of stock internal standard to 100.0 ng mL^{-1} in methanol. $^{2}H_1$ and $^{13}C_6$ internal standards were selected for use due to their structural similarity and physicochemical properties compared to those of the IMFs. Fentanyl-d_5 was used as the internal standard for all IMF analogues without a stable, labeled internal standard on the market due to the limited availability of most analogues and the structural similarities to fentanyl. Controls (triplicate) and calibration standards were extracted daily. Post extraction controls (spiked after separation) and neat controls (directly evaporated and not extracted) were also made for method validation purposes.

2.4 Biological Matrices.

Whole blood free of pathogens was obtained from the Community Blood Center, Dayton, OH. Blank whole blood was preserved with sodium fluoride (1%) and was refrigerated (~4 °C) or frozen (−10 to −20°C). Before use, the acquired blood was analyzed for over 70 potential contaminants and drugs of abuse (Table S1) by running a blank sample through multiple extractions and quantifications. Verified whole blood was diluted with water at a 1:1 ratio. Because of limited blood supply, the product was diluted to extend the amount of blood needed for each analysis; however, proficiency blind tests were carried out to demonstrate accurate analyte quantitation for accreditation purposes.

2.5 Sample Preparation and Solid Phase Extraction.

Calibrants, controls, and samples were treated the same throughout all experiments for method validation. Briefly, 1.0 mL of whole blood was added to 4.0 mL of PBS and 2.0 mL of water in a 16 x 125 Pyrex Screw Cap Tube. Each sample was then spiked with 100.0 μL of internal standard. Calibrators and controls were administered to additional stock solutions, resulting in seven calibration concentrations (0.1, 0.25, 0.5, 1.0, 5.0, 10.0, and 50.0 ng mL^{-1}) and three quality controls (0.35, 2.5, and 25.0 ng mL^{-1}). Afterward, calibrants, controls, and samples were vortexed and centrifuged at 3000 rpm (1811g) for 10 min to remove particulate matter prior to SPE.

The isolation of IMFs was selectively achieved using CLEAN SCREEN DAU columns (United Chemical Technologies Bristol, PA). Desired drugs were selectively eluted by maintaining the pH of reagents and column close to 6.0 through the addition of PBS buffer. Briefly, SPE columns were preconditioned and activated with 3.0 mL of methanol, washed with 3.0 mL of water, and conditioned to pH 6.0 with PBS. Slight positive pressure (~10 psi) was employed for each wash using a UCT Positive Pressure Manifold.

Calibrants, controls, and samples were loaded into the SPE columns, which were then washed with 3.0 mL of water, 1.0 mL of 1.0 M of acetic acid, and 3.0 mL of methanol to remove potential interferences. The cationic IMFs were eluted with 3.0 mL

of a v/v/v methylene chloride/isopropanol/ammonium hydroxide mixture (78:20:2). The eluate was collected and evaporated at 40 °C under a stream of air. Analytes were then reconstituted with 100.0 μL of methanol and injected into LC-MS/MS.

2.6 Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS).

Separation of fentanyl analytes was achieved with a Raptor biphenyl analytical column heated to 40 °C. Mobile phase A (MPA) consisted of 10.0 mM ammonium formate and 0.1% formic acid in water. Mobile phase B (MPB) was made of 0.1% formic acid in ACN. MPA and MPB were held for 2 min at 90/10%. MPA was gradually ramped down from 90 to 10% over 6 min, then held for 0.5 min at 10/90%, and finally returned to 90/10% in 0.1 min, and was held for the remainder of the time. A total run time of 13.5 min ensured the elution of analytes and the equilibration of the column.

Electrospray ionization in a positive ion scan mode was selected for MS measurements. Source parameters were maintained for nitrogen gas temperature (350 °C), gas flow (12.0 L min^{-1}), and capillary voltage (4000 V). Detection was accomplished by using a dynamic MRM scan function. Precursor and product ions were identified using the Optimizer software (Agilent) and manual determination (Table 1).

Table 1. Precursor ions along with their qualitative and quantitative transitions for all IMF analogs (N = 24) and internal standards (N = 3, in bold).

Peak	Analyte	Quant Transition (m/z)	Qualifier Transitions (m/z)	Fragmentor (V)	Collision Energy (V)
1	**Norfentanyl-d_5**	238.4-84.1	238.4-55.2	106	16, 44
2	Norfentanyl	233.4-84.1	233.4-94.0, 233.4-55.2	106	16, 36, 44
3	Furanyl norfentanyl	271.4-84.1	271.4-95.0	106	16, 44
4	Remifentanil acid	363.4-53.2	363.4-81.1	111	72, 44
5	Butyryl norfentanyl	247.3-84.1	247.3-94.0, 247.3-55.2	106	16, 32, 44
6	Remifentanil	377.5-317.0	377.5-345.0	25	15
7	Acetyl fentanyl	323.0-105.0	323.0-188.0	141	20, 40
8	**Acetyl Fentanyl $^{13}C_6$**	329.4-105.0	329.4-77.1	136	44, 96
9	Alfentanil	417.5-165.0	417.5-99.0, 417.5-77.1	131	36, 40, 100

14	AH-7921	329.0-95.1	329.0-284.0	111	20, 36
10	U-47700	329.0-81.0	329.0-204.0	120	36, 25
11	Acetyl fentanyl 4-methylphenethyl	337.5-119.0	337.5-91.1	136	36, 72
12	Acrylfentanyl	335.5-105.0	335.5-77.1, 335.5-51.2	141	44, 92, 140
15	**Fentanyl-d_5**	342.5-105.0	342.5-77.1	141	44, 100
13	Fentanyl	337.5-188.0	337.5-105.0	131	20, 44
17	*para*-Methoxyfentanyl	367.6-105.0	367.6-77.1, 367.6-51.2	136	44, 108, 160
16	4-ANPP	281.4-105.1	284.4-77.2, 281.4-51.3	116	36, 76, 124
18	Furanyl fentanyl	375.1-105.0	375.1-188.2	125	40, 25
19	Despropionyl *para*-Fluorofentanyl	299.4-105.0	299.4-77.1, 299.4-51.2	111	36, 88, 88
22	Carfentanil	395.2-113.0	395.2-105.0, 395.2-77.1	131	36, 56, 112

20	*(±)-cis*-3-Methyl fentanyl	351.5-202.1	351.5-105.0	150	20, 48
21	Butyryl/Isobutyryl fentanyl	351.2-188.1	351.2-105.1	146	24, 48
23	*para*-Fluorobutyryl/*para*-Fluoroisobutyryl fentanyl	369.2-105.1	369.2-188.1, 369.2-77.1	141	44, 24, 108
24	Sufentanil	387.6-111.0	387.3-238.2, 387.6-132.0	121	44, 36, 36
25	Valeryl fentanyl	365.5-105.0	365.5-77.1, 365.5-51.2	136	44, 112, 164

2.7 LC-MS/MS Assay Validation.

Validation followed method development and occurred daily over 5 days. It included a batch of seven calibrators, controls in triplicate, a negative blood blank, and a water blank. The limit of detection (LOD), limit of quantitation (LOQ), bias, precision (coefficient of variation, % CV), linearity, matrix effects, recovery, carryover, and any potential interferences were determined within the validation period.

2.8 Data Analysis.

The software used for data analysis was MassHunter Qualitative and Quantitative analysis. Data was plotted in Excel 2016 and Origin 8 software. The **development of the LC-MS/MS method** required the adaptation of the following: (1) **SPE extractions** for separation of the analytes of interest from interferences inherent in biological matrices, (2**) LC** for further improvement in sensitivity and specificity, and (3) **MS/MS** for MRM transitions specific to each IMF analogue and analogue quantification at sub ng mL^{-1} concentrations.

SPE Extractions were performed according to the United Chemical Technologies extraction method (10.5)[26] for N = 9 IMF analogues in urine (fentanyl, alfentanil, carfentanil, sufentanil, 3-methyl fentanyl, para-fluorofentanyl, α-methyl fentanyl, thianfentanil, and lofentanil). This gas chromatography−mass spectrometry (GC−MS) method was successfully adapted for the extraction of all N = 24 IMF analogues in whole

blood specimens.

2.9 LC Optimization.

First, two columns were tested: C-18 and biphenyl. The C-18 column proved inefficient for the separation of all IMF analogues, whereas the biphenyl column was found to generate an improved signal-to-noise ratio and separation. Thus, the biphenyl column was selected for method validation. Three mobile phase mixtures (methanol and formic acid, ammonium formate and formic acid in water (MPA), and formic acid in ACN (MPB)) were explored to provide the best separation of IMF analogues in the shortest amount of time.

Exploratory work deemed methanol and formic acid as unsuitable because separation of IMF analogues could not be achieved within acceptable time frames (<20 min) and corresponding chromatography exhibited poor signal-to-noise ratios under the studied conditions. Following this, MPA and MPB mixtures were deemed acceptable for a gradient method by achieving time efficient separation (13.5 min). A flow rate of 0.400 mL min^{-1} was selected to accommodate the maximum column pressure on both LC-MS/MS systems. The gradient change of mobile phases was then optimized from 90% MPA/ 10% MPB to 10% MPA/90% MPB to achieve a total run time of 13.5 min per sample (Figure S1).

2.10 MS/MS Optimization.

Electrospray ionization mode paired with tandem quadrupole mass spectrometry was employed for MRM transitions, which were optimized for high sensitivity of each IMF analogue. Briefly, precursor-ion and product-ion transitions for each IMF analogue and internal standards (Table 1) were mostly determined using the Agilent Optimizer software. Manual adjustment of the fragmentor and collision energy voltage was done when the software adjustment led to low sensitivity for the qualifier transitions ((±)-cis-3-methyl fentanyl, U-47700, and remifentanil). MRM transitions were identified by the highest sensitivity and specific discrimination between coeluting analogues (e.g., AH-7921 and U-47700 in Table 1). Separation and identification between butyryl fentanyl and isobutyryl fentanyl and para-fluorobutyryl fentanyl and 4-fluoroisobutyryl fentanyl could not be achieved under current conditions. Thus, they were classified as butyryl/ isobutyryl and para-fluoroisobutyryl fentanyl/para-fluorobutyryl fentanyl. Using this method, the isomeric IMF analogues can be detected but not distinguished from each other. Thus, Figure 2 shows only the quantitative transitions of N = 22 fentanyl analogues and N = 3 internal standards. The acquisition method report is provided in the Supporting Information.

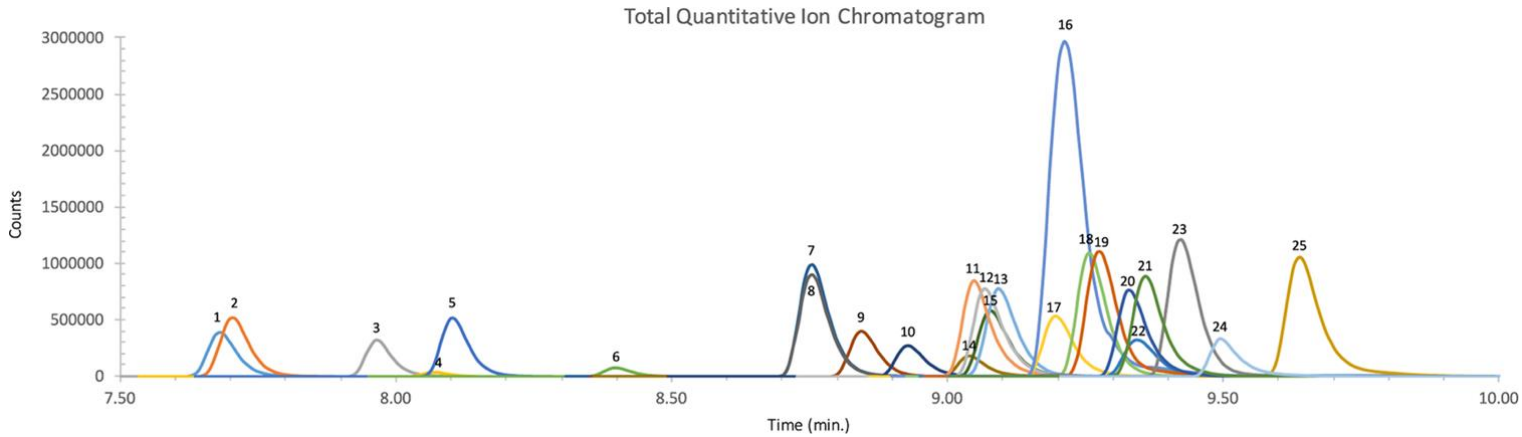

Figure 4. LC-MS/MS ion chromatogram of a high calibrator. Each peak represents the quantitative transition ion (qualitative transition ion not shown). Fentanyl analogue and internal standard peak identities: (1) norfentanyl-d_5, (2) norfentanyl, (3) furanyl norfentanyl, (4) remifentanil acid, (5) butyryl norfentanyl, (6) remifentanil, (7) acetyl fentanyl, (8) acetyl fentanyl-$^{13}C_6$, (9) alfentanil, (10) U-47700, (11) acetyl fentanyl 4-methylphenethyl, (12) acrylfentanyl, (13) fentanyl, (14) AH-7921, (15) fentanyl-d_5, (16) 4-ANPP, (17) *para*-methoxyfentanyl, (18) furanyl fentanyl, (19) despropionyl *para*-fluorofentanyl, (20) (±)-cis-3-methyl fentanyl, (21) butyryl/isobutyryl fentanyl, (22) carfentanil, (23) *para*-fluorobutyryl/ *para*-fluoroisobutyryl fentanyl, (24) sufentanil, and (25) valeryl fentanyl. Separation between butyryl/isobutyryl and *para*-fluoroisobutyryl fentanyl/ *para*-fluorobutyryl fentanyl was not achieved due to isomerism.

2.11 Method Validation.

The directed assay was validated by determining the limit of detection (LOD), limit of quantitation (LOQ), selectivity and specificity, recovery, ion suppression/ enhancement, process efficiency, bias, and precision. All analyses were performed after a 5 day validation period. LOD, lower limit of quantitation (LLOQ), upper limit of quantitation, bias, and precision were calculated over five replicates from five consecutive days.

2.12 Limit of Detection (LOD).

LODs for IMF analogues are listed in Table 2. Evaluation of LOD for most IMF analogues (excluding fentanyl) was carried out by using a linear calibration curve model. LOD was estimated using eq. 1

$$LOD = \frac{3.3\ s_y}{Avg_m} \quad (1)$$

where s_y is the standard deviation of the y-intercept and Avg_m is the average of the calibration slopes.

LOD of fentanyl (quadratic fit, 1/x) was determined by evaluating the calibration standards 1/2, 1/5, and 1/10 of the lowest calibrator (i.e., 0.05, 0.025, and 0.01 ng mL^{-1}) for the lowest fentanyl concentration with an acceptable signal-to-noise ratio. The standard that exhibited a signal five times greater than the background noise was then selected as the LOD for fentanyl.

2.13 Limit of Quantitation (LOQ).

LODs represent the lowest quantity that can be distinguished from a blank, whereas LOQs define our range of quantitation for the assay. The lowest limit of quantitation (LLOQ) was chosen to be the lowest nonzero calibrator that demonstrated acceptable bias and precision (<20, >80%), along with reproducible chromatography. The upper limit of quantitation (ULOQ) was selected as the highest calibrator within the calibration range (i.e., 10.0 ng mL^{-1} for all IMF analogues except for fentanyl and norfentanyl at 50.0 ng mL^{-1}).

Table 2. Retention Times, Limit of Detection (LOD), Lower Limit of Quantitation (LLOQ), and Linear Ranges for IMF Analogues (N = 22) along with Corresponding Internal Standards[a]

Analyte	Internal Standard	Retention Time (min)	LOD (ng mL^{-1})	LLOQ (ng mL^{-1})	Linear Range (ng mL^{-1})
Norfentanyl	Norfentanyl-d_5	7.62	0.038	0.100	0.100 – 50.0
Furanyl norfentanyl	Norfentanyl-d_5	7.90	0.058	0.250	0.250 – 10.0
Remifentanil acid	Norfentanyl-d_5	7.99	0.100	0.500	0.500 – 10.0
Butyryl norfentanyl	Norfentanyl-d_5	8.04	0.044	0.100	0.100 – 10.0
Remifentanil	Fentanyl-d_5	8.33	0.053	0.100	0.100 – 10.0
Acetyl fentanyl	AcetylFentanyl-$^{13}C_6$	8.68	0.017	0.100	0.100 – 10.0
Alfentanil	Fentanyl-d_5	8.77	0.048	0.100	0.100 – 10.0
AH-7921	Fentanyl-d_5	8.96	0.042	0.100	0.100 – 10.0
U-47700	Fentanyl-d_5	8.85	0.019	0.100	0.100 – 10.0
Acetyl fentanyl 4-methylphenethyl	Fentanyl-d_5	8.97	0.037	0.100	0.100 – 10.0
Acrylfentanyl	Fentanyl-d_5	8.99	0.034	0.100	0.100 – 10.0
Fentanyl	Fentanyl-d_5	9.01	0.050	0.100	0.100 – 50.0
para-Methoxyfentanyl	Fentanyl-d_5	9.11	0.056	0.100	0.100 – 10.0
4-ANPP	Fentanyl-d_5	9.13	0.025	0.100	0.100 – 10.0

Furanyl fentanyl	Fentanyl-d_5	9.17	0.029	0.100	0.100 – 10.0
Despropionyl *para*-Fluorofentanyl	Fentanyl-d_5	9.19	0.016	0.100	0.100 – 10.0
Carfentanil	Fentanyl-d_5	9.26	0.050	0.100	0.100 – 10.0
(±)-cis-3-Methyl fentanyl	Fentanyl-d_5	9.24	0.048	0.250	0.250 – 10.0
Butyryl/Isobutyryl fentanyl	Fentanyl-d_5	9.27	0.026	0.100	0.100 – 10.0
para-Fluorobutyryl/*para*-Fluoroisobutyryl fentanyl	Fentanyl-d_5	9.33	0.042	0.100	0.100 – 10.0
Sufentanil	Fentanyl-d_5	9.42	0.100	0.250	0.250 – 10.0
Valeryl fentanyl	Fentanyl-d_5	9.54	0.047	0.100	0.100 – 10.0

[a]N = 3

2.14 Selectivity/Specificity.

All IMF analogues were evaluated for endogenous and exogenous interferences. Endogenous interferences were evaluated (N = 5) daily with whole blood previously screening negative for targeted IMF analogues. Negative blanks were extracted daily to assess false positive results due to potential matrix interferences. All negative blanks revealed no interferences were present that could result in a false positive identification.

Exogenous interferences were measured with solutions containing N = 70 commonly detected analytes in toxicology laboratories (Table S1). Verification of selectivity included extracting each commonly detected analyte at the concentration level specified in Table S1 and spiking with 0.35 ng mL^{-1} targeted IMF analytes in whole blood. Specificity was addressed by analyzing all N = 70 nontargeted analytes in whole blood without the addition of IMF analogues. False positives were not detected with nontargeted analytes, but large concentrations of benzodiazepine (2500 ng mL^{-1}) were found to interfere with AH-7921 and U-47700. However, these concentrations are much larger than usually seen in normal assays.

2.15 Recovery.

SPE extraction recoveries were determined by analyzing post extraction spikes against regular extractions. Recoveries were determined using LOCTRL, MEDCTRL, and HICTRL (N = 3). The average recoveries for LOCTRL, MEDCTRL, and HICTRL

were 84 ± 19, 78 ± 12, and 94 ± 4.1%, respectively, for all N = 21 nonisomeric IMF analogues (excluding butyryl/isobutyryl fentanyl and para-fluorobutyryl/ para-fluoroisobutyryl fentanyl). Recovery ranges for LOCTRL, MEDCTRL, and HICTRL were 38−140, 33−96, and 91−97%, respectively. All recovery values can be found in Table S2.

2.16 Ionization Suppression/Enhancement (ISE).

Ion suppression and enhancement (ISE) was evaluated using post extraction additions that were compared with neat standards. The signal response exhibited minor changes in most IMF analogues. Remifentanil acid exhibited the lowest ISE (<45%). The detection of each analyte was not affected by ISE. All ISE values can be found in Table S3.

2.17 Process Efficiency.

The total process efficiency was determined for each IMF analogue by comparison of neat standards against regular extractions. The process efficiency for LOCTRL, MEDCTRL, and HICTRL were 80 ± 13, 76 ± 12, and 89 ± 1.3%, respectively, for all IMF analogues. Process efficiency ranges for LOCTRL, MEDCTRL, and HICTRL were 45−104, 41−91, and 88−90%, respectively. All process efficiency values can be found in Table S4.

Statistical quantitation of each IMF analogue followed immediately after

qualitative evaluation. Structural isomers that coeluted with each other were only qualitatively determined. Quantitative determination of drugs is normally important for toxicological analyses; however, taking into account the paucity of data available on IMFs, the qualitative identification of an IMF is more important than its quantity. All other IMF analogues were evaluated for bias and precision to meet acceptable criteria.[28]

2.18 Bias and Precision.

Intra- and interday bias and precision were assessed with the help of quality control samples containing all IMF analogues (0.35, 2.5, and 25.0 ng mL^{-1} of IMF analogues). Intraday bias and precision were expressed as the largest calculated bias and precision for each of the 5 days of the validation period. All other bias and precision values fell below the maximum intraday value (Table 3). Any IMF analogue not meeting acceptable criteria (bias < 20% and precision > 80%) was defined as qualitative only.

Table 3. Intra- and Interday Bias and Precision for All IMF Analogues Excluding the Isomeric[a]

Analyte	Expected Concentration (ng mL^{-1})	Mean (ng mL^{-1})	Bias (%)---		Precision (% CV)	
			Inter-day n = 3	Intra-day *n* = 3	Inter-day n = 3	Intra-day *n* = 3
norfentanyl	0.350	0.3652 ± 0.011	8.6	4.3	97.7	97.0
	2.5	2.722 ± 0.17	18.0	8.9	94.3	93.5
	25	27.13 ± 0.67	10.0	8.5	98.3	97.4
furanyl norfentanyl	0.350	0.3179 ± 0.023	17.0	9.2	86.0	92.5
	2.5	2.351 ± 0.21	9.3	6.0	78.0	91.0
remifentanil acid	0.350	0.3211 ± 0.052	14.0	8.3	72.0	83.0
	2.5	2.327 ± 0.32	17.0	6.9	83.0	86.0
butyryl norfentanyl	0.350	0.3630 ± 0.027	17.0	3.7	94.3	92.4
	2.5	2.602 ± 0.20	18.0	4.1	94.1	92.0
remifentanil	0.350	0.3353 ± 0.017	8.7	4.2	94.1	94.7
	2.5	2.339 ± 0.17	12.0	6.4	94.0	92.6
acetyl fentanyl	0.350	0.3478 ± 0.015	6.4	0.63	97.9	95.7
	2.5	2.579 ± 0.21	17.0	3.2	93.7	91.6
alfentanil	0.350	0.3210 ± 0.019	12.0	8.3	90.9	93.8
	2.5	2.373 ± 0.15	9.4	5.1	92.9	93.6
AH-7921	0.350	0.2722 ± 0.11	65.0	22.0	-32.0	59.0
	2.5	1.761 ± 0.65	55.0	30.0	4.0	62.0
U-47700	0.350	0.3466 ± 0.038	13.0	0.97	87.0	89.0
	2.5	2.292 ± 0.15	14.0	8.3	92.0	93.3
acetyl fentanyl 4-methylphenethyl	0.350	0.3628 ± 0.015	8.8	3.7	94.7	95.8
	2.5	2.556 ± 0.15	9.0	2.2	92.7	93.9
acrylfentanyl	0.350	0.3507 ± 0.014	5.0	0.20	94.9	96.0
	2.5	2.502 ± 0.095	4.4	0.096	96.1	96.1
fentanyl	0.350	0.3326 ± 0.015	13.0	5.0	98.2	95.3
	2.5	2.458 ± 0.15	8.0	1.7	95.1	93.5

	25	24.77 ± 1.5	6.2	0.92	88.0	93.6
para-methoxyfentanyl	0.350	0.3598 ± 0.018	9.1	2.8	92.5	94.7
	2.5	2.533 ± 0.12	7.5	1.3	93.8	95.2
4-ANPP	0.350	0.3306 ± 0.043	20.0	5.5	76.0	87.0
	2.5	2.373 ± 0.32	20.0	5.1	84.0	86.0
furanyl fentanyl	0.350	0.3426 ± 0.015	4.4	2.1	94.6	95.5
	2.5	2.487 ± 0.12	5.4	0.53	95.3	95.0
despropionyl *para*-fluorofentanyl	0.350	0.2819 ± 0.044	23.0	19.0	71.0	84.0
	2.5	2.006 ± 0.33	37.0	20.0	81.0	83.0
carfentanil	0.350	0.3281 ± 0.011	9.7	6.3	96.6	96.5
	2.5	2.366 ± 0.11	9.9	5.3	91.9	95.1
(±)-cis-3-Methyl fentanyl	0.350	0.3419 ± 0.030	18.0	2.3	94.1	91.0
	2.5	2.418 ± 0.24	20.0	3.3	94.0	90.0
butyryl/isobutyryl fentanyl	0.350	0.4488 ± 0.20	86.0	28.0	26.0	54.0
	2.5	3.348 ± 1.4	95.0	34.0	31.0	55.0
para-fluorobutyryl/*para*-fluoroisobutyryl fentanyl	0.350	0.3665 ± 0.042	19.0	4.7	92.1	88.0
	2.5	2.709 ± 0.26	19.0	8.4	96.3	90.1
vufentanil	0.350	0.2903 ± 0.035	30.0	17.0	87.0	88.0
	2.5	2.074 ± 0.16	26.0	17.0	93.3	91.9
valeryl fentanyl	0.350	0.3492 ± 0.015	6.0	0.23	95.3	95.7
	2.5	2.480 ± 0.11	5.1	0.80	95.9	95.3

[a]*Isomeric IMFs include butyryl, isobutyryl fentanyl, para-fluorobutyryl fentanyl, and FIBF. Underlined IMF analogues refer to successful quantitation that met acceptable criteria.*

3. Results and Discussion

The LC-MS/MS method developed in this study allows for the multiplex detection of N = 24 IMF analytes with good sensitivity and a short sample run time (13.5 min). Quantitated IMF analogues (N = 13) passed all evaluations. These were norfentanyl, butyryl norfentanyl, remifentanil, acetyl fentanyl, alfentanil, U-47700, acetyl fentanyl 4-methylphenethyl, acrylfentanyl, fentanyl, para-methoxyfentanyl, furanyl fentanyl, carfentanil, and valeryl fentanyl. All analytes had an LOD ≤ 0.100 ng mL^{-1} and a maximum LLOQ of 0.500 ng mL^{-1} (the lowest LLOQ value being 0.100 ng mL^{-1}).

3.1 Casework

Since its development and validation in January 2017, the LC-MS/MS method was successfully utilized in the IMF analysis of N = 725 blood samples at the Montgomery County Coroner's Office (MCCO) in Dayton, Ohio. The postmortem samples were collected from accidental drug overdose death cases that occurred between February 2015 and November 2016. The MCCO laboratory provides postmortem forensic toxicology services to approximately 40 of Ohio's 88 counties. The following N = 10 IMF analogues were found to be present in the analyzed samples: (±)-cis-3-methyl fentanyl, 4-ANPP, acetyl fentanyl, carfentanil, despropionyl para-fluorofentanyl, fentanyl, furanyl fentanyl, furanyl norfentanyl, norfentanyl, and U-47700.

Table 4. Total number of times each IMF analog was detected in the N = 725 cases of unintentional drug overdose death.

IMF Analog	Number of times detected
(±)-*cis*-3-methyl fentanyl	1
4ANPP	82
Acetyl fentanyl	40
Carfentanil	22
Despropionyl fluorofentanyl	1
Fentanyl	662
Furanyl fentanyl	39
Furanyl norfentanyl	2
Norfentanyl	582
U-47700	3

Table 4 summarizes the total number of times each IMF analogue was detected across the N = 725 whole blood samples. Fentanyl (N = 662, 91%) and its metabolite, norfentanyl (N = 582, 80%), were the most commonly encountered in the examined cases. Furthermore, N = 82 cases (11%) tested positive for 4-ANPP, which is an impurity related to the synthesis of fentanyl and also a metabolite of fentanyl. There were also 40 acetyl fentanyl (6%), 39 furanyl fentanyl (5%), and 22 carfentanil (3%) positive cases.

The analysis of more recent accidental overdose cases at MCCO laboratory that occurred in between January and February 2017, identified N = 13 IMF analogues

(fentanyl, acrylfentanyl, furanyl fentanyl, carfentanil, norfentanyl, despropionyl fentanyl (4-ANPP), despropionyl para-fluorofentanyl, furanyl norfentanyl, acetyl fentanyl, butyryl/isobutyryl fentanyl, butyryl norfentanyl, fluorobutyryl/fluoroisobutyryl fentanyl, and U-47700).[29]

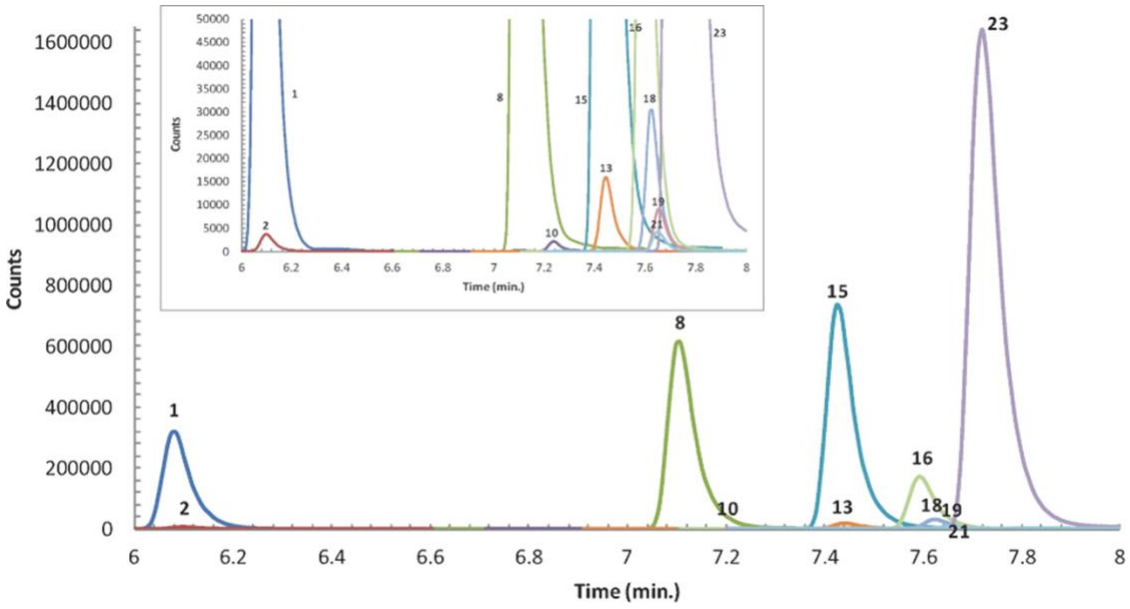

Figure 5. Quantitative ion chromatogram of an accidental overdose case from late 2016. Abbreviations are as follows: (1) norfentanyl-d_5, (2) norfentanyl, (8) acetyl fentanyl $^{13}C_6$, (10) U-47700, (13) fentanyl, (15) fentanyl-d_5, (16) 4-ANPP, (18) furanyl fentanyl, (19) despropionyl para-fluorofentanyl, (21) butyryl/isobutyryl fentanyl, and (23) para-fluorobutyryl/para-fluoroisobutyryl fentanyl. Inset shows the low-response count region of IMF analogues.

Figure 3 illustrates the recent, multiplex detection of N = 8 IMF analogues from a single whole blood sample at MCCO along with their corresponding concentrations: norfentanyl (0.47 ng mL^{-1}), U-47700 (0.50 ng mL^{-1}), fentanyl (1.2 ng mL^{-1}), and furanyl fentanyl (1.5 ng mL^{-1}). Because of cases like this, it is critical to incorporate such flexible methods into the routine toxicological analysis at forensic laboratories worldwide. A

second manuscript is in production, in which N = 725 cases will be discussed in detail.

3.2 Knowledge Gaps

Several previous studies[8−10,30−35] have already demonstrated the capabilities of the LC-MS/MS-based analytical method in detecting IMF analogues, in the 0.050−0.500 ng mL^{-1} concentration range.[10] However, the LOQ was determined to be the lowest calibrator at 0.100 ng mL^{-1}, which is equivalent to the LOQ value of this study. **To the best of our knowledge**, these LC-MS/MS studies on human blood detected at most N = 17 IMF analogues and homologues with a 35 min scan time but without quantitation.[30] Furthermore, those LC-MS/MS methods that offered quantitation did not tackle more than N = 9 IMF analogues.[35] As the frequency of opioid abuse cases is drastically increasing both in the forensic and clinical world, both the qualitative identification and quantitation of such analytes is becoming equally important.[36] The LC-MS/MS method of this study will address this deficit by facilitating both the identification (N = 22) and quantification (N = 13 for illustrative purposes) of IMF analogues and metabolites (total of N = 24 of the most commonly encountered IMFs in human blood) down to 0.100 ng mL^{-1}, i.e., the lowest LOQ reported to date according to our knowledge. Additionally, this LC-MS/ MS method can be easily adapted to accommodate newly emerging IMFs in various drug analysis settings and with the shortest screening time (13.5 min) under the studied conditions.

Other analytical methods, such as gas chromatography−mass spectrometry (GC−MS)[37] and thermal desorption direct analysis in real time mass spectrometry,[38] have also been explored. Although successful in the qualitative detection of N = 17 and quantitation of N = 4 IMF analogues and metabolites, these methods had greater LODs (0.08−0.351 ng mL^{-1}) and LLOQs (0.500 ng mL^{-1}) than the ones described in this LC-MS/MS method, namely, 0.017−0.050 and 0.100 ng mL^{-1}, respectively, for all N = 13 quantitated IMF analogues.

4. Conclusion

An LC-MS/MS-based method was developed for the multiplex detection of N = 24 IMF analogues and metabolites in postmortem blood at sub ng mL^{-1} concentrations. It was successfully implemented at the Montgomery County Coroner's Office/Miami Valley Regional Crime Laboratory in Dayton, Ohio, where it aided in the analysis of N = 725 postmortem blood samples collected from accidental drug overdose death cases. This forensic work demonstrated the cost- and time-efficiency of the newly developed IMF detection method. In addition to employing commercially available, inexpensive supplies and common forensic instrumentation, the method requires 13.5 min scan time for a single sample and 5− 10 min for quantitative and qualitative analysis. The LC-MS/MS-based protocol can be easily adapted by forensic laboratories worldwide; it is currently undergoing modifications to incorporate the addition of four new IMF analogues (β-hydroxythiofentanyl, para-fluorofentanyl, tetrahydrofuran fentanyl, and cyclopropyl

fentanyl) at the Montgomery County Coroner's Office.

Supporting Information

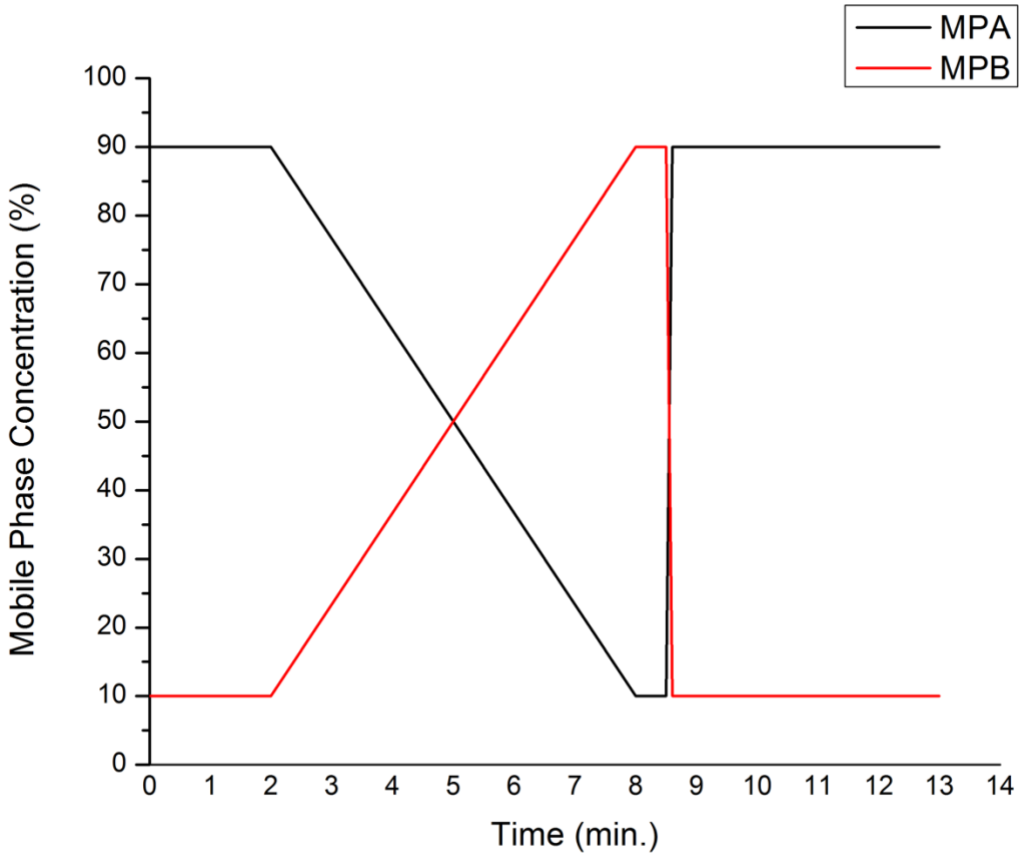

Figure S1. MPA and MPB gradient concentration change over a 13.5 minute per sample run period.

Table S1. Drugs commonly detected in forensic toxicology casework (N = 70) and tested in this study for exogenous interferences.

Injection Mix	Drugs included in the interference study test mix	Concentration ($ng\ mL^{-1}$)
Benzodiazepines	7-Aminoclonazepam, 7-Aminoflunitrazepam, Alprazolam, Chlordiazepoxide, Clonazepam, Diazepam, Flunitrazepam, Flurazepam, Lorazepam, Midazolam, Nordiazepam, Oxazepam, Phenazepam, Temazepam, Triazolam	2,500
Opiates	Hydrocodone, Hydromorphone*, Oxycodone, Morphine, Oxymorphone*, 6-Acetylmorphine*, Codeine	2,500* 10,000
Stimulants	Cocaine, Cocaethylene, Ecgonine methyl ester, Benzoylecgonine, Amphetamine, Phenylpropanolamine, Phentermine, Methamphetamine, Pseudoephedrine, Methylenedioxyamphetamine, Benzyl piperazine, Methylenedioxymethamphetamine, Methylenedioxyethylamphetamine, Methylphenidate	1,250
THC	11-nor-9-carboxy Tetrahydrocannabinol*, Tetrahydrocannabinol	1,250* 500
Bases	Chlorpheniramine, Citalopram, Dextromethorphan, Doxylamine, Lidocaine, Methadone, Mirtazapine, Tramadol, Trazodone, Verapamil, Zolpidem	1,250
AD	Venlafaxine, Desvenlafaxine, Quetiapine, Paroxetine, Desipramine, Norfluoxetine, Imipramine, Fluoxetine, Nortriptyline, Cyclobenzaprine, Amitriptyline, Sertraline, Duloxetine	1,250
AE	Gabapentin, Lamotrigine, Levetiracetam, Pregabalin, Topiramate, Zonisamide	5,000
GHB	Gamma hydroxybutyric acid	50,000
Rx	Naloxone	10,000

Table S2. Recovery percentages for N =22 illicitly manufactured fentanyl (IMF) analogs and metabolites at low (0.35 ng mL^{-1}), medium (2.5 ng mL^{-1}), and high control concentrations (25.0 ng mL^{-1}). Set #1 and #2 refer to post extraction spiked and regular extraction samples, respectively.

Norfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High**		**Low**	**Med**	**High**
1	17631.48	140730.77	1000604.93		13814.81	108589.51	894437.11
2	18047.00	137202.97	829527.44		14056.68	89730.02	960464.26
3	18557.65	130331.00	1175213.13		16278.87	101081.64	872363.61
Average	18078.71	136088.25	1001781.83		14716.78	99800.39	909088.33
Recovery (%)	81.40	73.34	90.75				
Furanyl norfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	4590.15	31704.02	230975.70		2561.18	25373.77	203161.69
2	5487.52	28719.55	212752.81		2868.62	15808.62	254305.58
3	5446.13	27056.84	304847.16		3643.37	17184.88	197428.08
Average	5174.60	29160.14	249525.22		3024.39	19455.76	218298.45
Recovery (%)	58.45	66.72	87.49*				
Remifentanil acid							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	2089.06	16561.54	167171.23		888.60	5835.51	50776.07
2	2334.54	16266.17	157158.90		800.58	4657.90	52331.12
3	2339.62	15863.99	155740.28		885.15	5417.15	49659.41
Average	2254.40	16230.56	160023.47		858.11	5303.52	50922.20
Recovery (%)	38.06	32.68	31.82*				
Butyryl norfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	19330.65	177026.12	1153778.73		18740.57	135942.06	1035652.82
2	21813.09	175292.19	934465.45		17835.67	118133.80	1076581.04
3	20551.76	168768.33	1307357.39		21253.04	129095.32	992372.29
Average	20565.17	173695.55	1131867.19		19276.43	127723.73	1034868.72

Recovery (%)	93.73	73.53	91.43*				
Remifentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	3398.58	25879.27	247196.84		2769.84	19518.17	206515.06
2	3631.82	26098.03	251472.03		2732.97	16166.15	214967.39
3	3855.28	23094.96	262547.87		2863.09	20413.23	191769.16
Average	3628.56	25024.09	253738.91		2788.64	18699.18	204417.20
Recovery (%)	76.85	74.72	80.56*				
Acetyl fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	39163.01	307276.32	2737509.47		32202.32	242612.47	2319607.20
2	39599.84	295551.80	2686140.35		31485.04	208628.84	2443187.71
3	41336.33	279207.70	2690796.28		35728.32	246516.70	2248135.27
Average	40033.06	294011.94	2704815.37		33138.56	232586.00	2336976.72
Recovery (%)	82.78	79.11	86.40*				
Alfentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	16613.52	111052.10	1069888.51		9410.82	83578.37	829356.47
2	16148.05	106762.53	1091804.94		10494.02	68625.62	847084.67
3	16345.64	96063.01	1030880.31		11468.14	85460.45	800546.70
Average	16369.07	104625.88	1064191.25		10457.66	79221.48	825662.61
Recovery (%)	63.89	75.72	77.59				
AH-7921*							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	5425.16	41519.09	171578.91		4987.49	30028.21	268991.42
2	5448.88	38275.23	187181.59		4626.44	29397.38	299962.16
3	5587.03	35003.78	317460.45		4828.00	33563.29	248723.39
Average	5487.02	38266.03	225406.98		4813.98	30996.29	272558.99
Recovery (%)	87.73	81.00	120.92*				
U-47700							
	Set #1				**Set 2**		

Trial no.	Low	Med	High*		Low	Med	High*
1	8789.37	69172.60	663875.37		7419.27	55748.60	525822.70
2	7814.93	65002.70	675335.12		7911.76	51056.55	554450.77
3	9579.90	60507.01	611724.71		8857.27	53830.68	501931.10
Average	8728.06	64894.10	650311.73		8062.76	53545.28	527401.53
Recovery (%)	92.38	82.51	81.10*				
Acetyl fentanyl 4-methylphenethyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	43670.09	362857.36	3004272.27		39273.94	294742.30	2564883.21
2	49639.28	358218.25	2896594.04		40145.21	270667.02	2671448.69
3	52373.23	325855.55	2865642.66		46585.53	276548.83	2533831.52
Average	48560.87	348977.05	2922169.66		42001.56	280652.71	2590054.47
Recovery (%)	86.49	80.42	88.63*				
Acrylfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	46580.17	341583.14	2646397.46		37202.88	281712.87	2428913.55
2	46952.36	337096.36	2475688.79		37972.97	242770.34	2537661.55
3	48849.41	310714.29	2705321.61		43752.84	287579.53	2351749.58
Average	47460.65	329797.93	2609135.95		39642.89	270687.58	2439441.56
Recovery (%)	83.53	82.08	93.50*				
Fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High**		**Low**	**Med**	**High**
1	36202.96	270883.19	2119693.09		29709.08	227320.04	2041829.06
2	35742.90	270642.79	2000594.92		29167.22	198024.25	2134377.46
3	36708.57	245358.38	2271119.18		35764.60	218804.82	1996871.66
Average	36218.14	262294.79	2130469.06		31546.97	214716.37	2057692.73
Recovery (%)	87.10	81.86	96.58				
***para*-Methoxyfentanyl**							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	27904.20	216676.89	1614255.82		22788.33	171129.57	1444155.49
2	28721.90	201953.30	1627239.48		22972.15	154837.11	1453530.64

3	27416.76	187643.49	1650187.28		28583.31	181306.70	1446219.28
Average	28014.29	202091.22	1630560.86		24781.26	169091.13	1447968.47
Recovery (%)	88.46	83.67	88.80*				
4-ANPP							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	166174.74	1261945.01	8907584.39		190178.80	1229445.02	8369358.55
2	164486.85	1336291.01	7977401.72		180041.98	1076604.71	8607365.57
3	153677.98	1223611.13	9025869.40		202286.59	1208246.04	7867551.53
Average	161446.52	1273949.05	8636951.83		190835.79	1171431.92	8281425.22
Recovery (%)	118.20	91.95	95.88*				
Furanyl fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	52810.26	387507.42	2941574.99		43119.60	311988.90	2601610.85
2	55872.20	383238.32	2976957.89		42896.47	273618.10	2713430.81
3	54758.43	355667.52	3015865.39		49315.64	319827.23	2468265.83
Average	54480.30	375471.09	2978132.76		45110.57	301811.41	2594435.83
Recovery (%)	82.80	80.38	87.12*				
Despropionyl *para*-fluorofentanyl							
	Set #1				**Set #2**		
Trial no.	**Low:**	**Med**	**High***		**Low**	**Med**	**High***
1	32789.88	314597.61	2611783.53		44611.95	298602.77	2379969.31
2	32015.33	308942.79	2020791.13		42323.78	262062.45	2461242.98
3	32208.91	279852.22	2524003.09		48160.70	302515.48	2219544.67
Average	32338.04	301130.88	2385525.92		45032.14	287726.90	2353585.65
Recovery (%)	139.25	95.55	98.66*				
(±)-*cis*-3-Methyl fentanyl							
	Set 1				**Set 2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	29995.09	191506.88	1699602.14		20491.67	156986.46	1478382.82
2	27567.04	188660.85	1677071.23		21452.07	141056.37	1509950.96

3	30375.57	168344.98	1696079.64		23994.77	155435.24	1401345.31
Average	29312.57	182837.57	1690917.67		21979.50	151159.36	1463226.36
Recovery (%)	74.98	82.67	86.53*				
Butyryl/Isobutyrl fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	43402.19	351851.45	2988080.64		39780.73	286850.10	2561000.97
2	45696.44	347785.05	2928941.15		40474.67	250218.59	2732200.06
3	45562.36	310423.46	2915792.27		41938.47	284702.64	2492138.07
Average	44887.00	336686.65	2944271.35		40731.29	273923.77	2595113.03
Recovery (%)	90.74	81.36	88.14*				
Carfentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	26402.18	190896.65	1252684.53		20082.97	141783.79	1156826.21
2	26772.94	193793.45	1293548.17		19522.51	125922.13	1232173.90
3	27415.09	164202.38	1352347.09		21596.45	150266.11	1127003.16
Average	26863.40	182964.16	1299526.60		20400.64	139324.01	1172001.09
Recovery (%)	75.94	76.15	90.19*				
***para*-Fluorobutyryl fentanyl/FIBF**							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	56583.98	381385.01	3052026.12		40598.62	312539.77	2654829.38
2	58804.88	378836.24	2952835.55		39133.46	276976.56	2748411.38
3	55929.45	348080.56	3027438.83		46283.60	320551.42	2534921.27
Average	57106.10	369433.94	3010766.83		42005.23	303355.92	2646054.01
Recovery (%)	73.56	82.11	87.89*				
Sufentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	23138.46	154831.15	1361946.43		16366.94	120199.93	1242166.50
2	23670.37	148236.77	1328720.78		15683.01	110473.97	1269157.94
3	24917.06	133487.14	1383698.29		19433.81	121634.57	1138826.22
Average	23908.63	145518.35	1358121.83		17161.25	117436.15	1216716.88
Recovery (%)	71.78	80.70	89.59*				

Valeryl fentanyl							
	Set 1				**Set 2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	56843.33	462938.62	3830025.05		51047.18	370795.17	3328155.43
2	61663.39	453705.57	3921682.44		50457.26	327512.59	3497367.43
3	59012.31	424183.71	3828719.32		58040.14	369396.01	3238100.13
Average	59173.01	446942.63	3860142.27		53181.53	355901.26	3354541.00
Recovery (%)	89.87	79.63	86.90*				

*Recovery percentages for all IMF analogs except for fentanyl and norfentanyl were estimated only theoretically at the high control concentration, where the control concentration exceeded the calibration range.

Table S3. Ionization suppression and enhancement percentages for N = 22 illicitly manufactured fentanyl (IMFs) analogs at low (0.35 ng mL^{-1}), medium (2.5 ng mL^{-1}), and high control concentrations (25.0 ng mL^{-1}). Set #1 and #2 refer to regular and neat quality standards, respectively.

Norfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High**		**Low**	**Med**	**High**
1	16183.09	112346.21	1040696.84		17631.48	140730.77	1000604.93
2	16400.53	110543.30	1001399.58		18047.00	137202.97	829527.44
3	15576.46	120348.17	1069657.21		18557.65	130331.00	1175213.13
Average	16053.36	114412.56	1037251.21		18078.71	136088.25	1001781.83
Matrix Effect (%)	112.62	118.95	96.58				
Furanyl norfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	4565.76	26311.47	282170.68		4590.15	31704.02	230975.70
2	4934.86	27258.58	196048.07		5487.52	28719.55	212752.81
3	4379.86	27359.57	295672.89		5446.13	27056.84	304847.16
Average	4626.83	26976.54	257963.88		5174.60	29160.14	249525.22
Matrix Effect (%)	111.84	108.09	96.73*				
Remifentanil acid							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	1912.22	13115.17	148736.10		2089.06	16561.54	167171.23
2	2044.45	12727.17	158545.18		2334.54	16266.17	157158.90
3	1799.16	13111.16	146491.63		2339.62	15863.99	155740.28
Average	1918.61	12984.50	151257.64		2254.40	16230.56	160023.47
Matrix Effect (%)	117.50	125.00	105.80*				
Butyryl norfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	17944.00	133950.12	1168174.85		19330.65	177026.12	1153778.73

2	19068.89	136111.96	1178733.62		21813.09	175292.19	934465.45
3	18347.13	146972.56	1186049.58		20551.76	168768.33	1307357.39
Average	18453.34	139011.55	1177652.68		20565.17	173695.55	1131867.19
Matrix Effect (%)	111.44	124.95	96.11*				
Remifentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	3698.61	24148.82	245580.98		3398.58	25879.27	247196.84
2	4110.52	31187.58	258044.21		3631.82	26098.03	251472.03
3	3395.64	30361.85	270170.79		3855.28	23094.96	262547.87
Average	3734.92	28566.08	257931.99		3628.56	25024.09	253738.91
Matrix Effect (%)	97.15	87.60	98.37*				
Acetyl Fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	32900.81	272480.22	2696875.16		39163.01	307276.32	2737509.47
2	38733.20	274894.98	2701173.97		39599.84	295551.80	2686140.35
3	38518.67	280970.95	2735233.43		41336.33	279207.70	2690796.28
Average	36717.56	276115.38	2711094.19		40033.06	294011.94	2704815.37
Matrix Effect (%)	109.03	106.48	99.77*				
Alfentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	16619.67	112384.98	1045263.57		16613.52	111052.10	1069888.51
2	18109.61	118183.23	1046701.52		16148.05	106762.53	1091804.94
3	17756.09	119055.84	1092001.13		16345.64	96063.01	1030880.31
Average	17495.12	116541.35	1061322.07		16369.07	104625.88	1064191.25
Matrix Effect (%)	93.56	89.78	100.27*				
AH7921							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***

1	6566.28	48615.06	476009.13		5425.16	41519.09	171578.91
2	7633.34	48108.20	473702.01		5448.88	38275.23	187181.59
3	6878.39	47187.37	467335.92		5587.03	35003.78	317460.45
Average	7026.00	47970.21	472349.02		5487.02	38266.03	225406.98
Matrix Effect (%)	78.10	79.77	47.72*				
U-47700							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	9035.57	60676.63	598626.36		8789.37	69172.60	663875.37
2	8841.10	60748.85	589120.64		7814.93	65002.70	675335.12
3	9052.78	61807.71	555878.02		9579.90	60507.01	611724.71
Average	8976.48	61077.73	581208.34		8728.06	64894.10	650311.73
Matrix Effect (%)	97.23	106.25	111.89*				
Acetyl fentanyl 4-methylphenethyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	48878.21	329056.56	2764066.69		43670.09	362857.36	3004272.27
2	49041.99	317459.70	2771548.48		49639.28	358218.25	2896594.04
3	46270.71	329783.06	2784287.37		52373.23	325855.55	2865642.66
Average	48063.64	325433.11	2773300.84		48560.87	348977.05	2922169.66
Matrix Effect (%)	101.03	107.23	105.37*				
Acrylfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	43882.58	308992.39	2536934.84		46580.17	341583.14	2646397.46
2	46517.72	293356.95	2596363.31		46952.36	337096.36	2475688.79
3	44189.23	305741.89	2595711.68		48849.41	310714.29	2705321.61
Average	44863.18	302697.08	2576336.61		47460.65	329797.93	2609135.95
Matrix Effect (%)	105.79	108.95	101.27*				
Fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High**		**Low**	**Med**	**High**

1	35121.93	243862.09	2277257.74		36202.96	270883.19	2119693.09
2	36338.33	272959.43	2236877.77		35742.90	270642.79	2000594.92
3	37012.04	266771.73	2381041.33		36708.57	245358.38	2271119.18
Average	36157.43	261197.75	2298392.28		36218.14	262294.79	2130469.06
Matrix Effect (%)	100.17	100.42	92.69				
para-Methoxyfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	27910.62	182350.93	1495982.69		27904.20	216676.89	1614255.82
2	28307.99	187967.00	1560701.40		28721.90	201953.30	1627239.48
3	27175.06	188179.96	1535007.01		27416.76	187643.49	1650187.28
Average	27797.89	186165.96	1530563.70		28014.29	202091.22	1630560.86
Matrix Effect (%)	100.78	108.55	106.53*				
4ANPP							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	250705.82	1588320.59	10328356.33		166174.74	1261945.01	8907584.39
2	258303.65	1581159.69	10153768.64		164486.85	1336291.01	7977401.72
3	243397.40	1581703.72	10159658.74		153677.98	1223611.13	9025869.40
Average	250802.29	1583728.00	10213927.90		161446.52	1273949.05	8636951.83
Matrix Effect (%)	64.37	80.44	84.56*				
Furanyl Fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	49598.48	349942.60	2901533.55		52810.26	387507.42	2941574.99
2	53154.12	346954.45	2836640.95		55872.20	383238.32	2976957.89
3	51906.40	357217.84	2887911.90		54758.43	355667.52	3015865.39
Average	51553.00	351371.63	2875362.13		54480.30	375471.09	2978132.76
Matrix Effect (%)	105.68	106.86	103.57*				
Despropionyl *para*-fluorofentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***

1	55310.89	410215.90	3346009.48		32789.88	314597.61	2611783.53
2	59539.77	417208.83	3306663.71		32015.33	308942.79	2020791.13
3	54462.41	429930.16	3259141.25		32208.91	279852.22	2524003.09
Average	56437.69	419118.30	3303938.15		32338.04	301130.88	2385525.92
Matrix Effect (%)	57.30	71.85	72.20*				
(±)-*cis*-3-Methyl fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	24054.35	166202.47	1583431.31		29995.09	191506.88	1699602.14
2	27032.87	169935.48	1605989.18		27567.04	188660.85	1677071.23
3	28262.03	170303.84	1617468.23		30375.57	168344.98	1696079.64
Average	26449.75	168813.93	1602296.24		29312.57	182837.57	1690917.67
Matrix Effect (%)	110.82	108.31	105.53*				
Butyryl/Isobutyrl Fent							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	45422.09	314101.40	2930769.08		43402.19	351851.45	2988080.64
2	43811.81	330407.44	2863257.61		45696.44	347785.05	2928941.15
3	43617.99	318274.99	2892301.46		45562.36	310423.46	2915792.27
Average	44283.97	320927.94	2895442.71		44887.00	336686.65	2944271.35
Matrix Effect (%)	101.36	104.91	101.69*				
Carfentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	29581.26	190158.75	1361256.73		26402.18	190896.65	1252684.53
2	28035.22	185363.84	1327128.00		26772.94	193793.45	1293548.17
3	28947.85	186650.17	1340755.27		27415.09	164202.38	1352347.09
Average	28854.78	187390.92	1343046.67		26863.40	182964.16	1299526.60
Matrix Effect (%)	93.10	97.64	96.76*				
para-Fluorobutyryl/FIBF							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***

1	54002.16	342912.34	2955695.73		56583.98	381385.01	3052026.12
2	56611.46	339053.46	2918068.58		58804.88	378836.24	2952835.55
3	58760.62	353647.81	2923275.93		55929.45	348080.56	3027438.83
Average	56458.08	345204.54	2932346.74		57106.10	369433.94	3010766.83
Matrix Effect (%)	101.15	107.02	102.67*				
Sufentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	21378.68	141092.61	1353710.93		23138.46	154831.15	1361946.43
2	23467.13	133429.58	1365799.90		23670.37	148236.77	1328720.78
3	21426.18	137804.06	1362923.38		24917.06	133487.14	1383698.29
Average	22090.66	137442.08	1360811.40		23908.63	145518.35	1358121.83
Matrix Effect (%)	108.23	105.88	99.80*				
Valeryl Fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	59939.19	373667.31	3834026.52		56843.33	462938.62	3830025.05
2	62192.36	419395.23	3638907.11		61663.39	453705.57	3921682.44
3	60773.11	434846.52	3925964.13		59012.31	424183.71	3828719.32
Average	60968.22	409303.02	3799632.58		59173.01	446942.63	3860142.27
Matrix Effect (%)	97.06	109.20	101.59*				

*Ionization suppression and enhancement percentages for all IMF analogs except for fentanyl and norfentanyl were estimated only theoretically at the high control concentration, where the control concentration exceeded the calibration range.

Table S4. Process efficiency percentages for N = 22 illicitly manufactured fentanyl (IMFs) analogs at low (0.35 ng mL^{-1}), medium (2.5 ng mL^{-1}), and high control concentrations (25.0 ng mL^{-1}). Set #1 and #2 refer to neat quality standards and regular extractions, respectively.

Norfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High**		**Low**	**Med**	**High**
1	16183.09	112346.21	1040696.84		13814.81	108589.51	894437.11
2	16400.53	110543.30	1001399.58		14056.68	89730.02	960464.26
3	15576.46	120348.17	1069657.21		16278.87	101081.64	872363.61
Average	16053.36	114412.56	1037251.21		14716.78	99800.39	909088.33
Process Efficiency (%)	91.67	87.23	87.64				
Furanyl norfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	4565.76	26311.47	282170.68		2561.18	25373.77	203161.69
2	4934.86	27258.58	196048.07		2868.62	15808.62	254305.58
3	4379.86	27359.57	295672.89		3643.37	17184.88	197428.08
Average	4626.83	26976.54	257963.88		3024.39	19455.76	218298.45
Process Efficiency (%)	65.37	72.12	84.62*				
Remifentanil acid							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	1912.22	13115.17	148736.10		888.60	5835.51	50776.07
2	2044.45	12727.17	158545.18		800.58	4657.90	52331.12
3	1799.16	13111.16	146491.63		885.15	5417.15	49659.41
Average	1918.61	12984.50	151257.64		858.11	5303.52	50922.20
Process Efficiency (%)	44.73	40.84	33.67*				
Butyryl norfentanyl							
	Set #1				**Set #2**		

Trial no.	Low	Med	High*		Low	Med	High*
1	17944.00	133950.12	1168174.85		18740.57	135942.06	1035652.82
2	19068.89	136111.96	1178733.62		17835.67	118133.80	1076581.04
3	18347.13	146972.56	1186049.58		21253.04	129095.32	992372.29
Average	18453.34	139011.55	1177652.68		19276.43	127723.73	1034868.72
Process Efficiency (%)	104.46	91.88	87.88*				
Remifentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	3698.61	24148.82	245580.98		2769.84	19518.17	206515.06
2	4110.52	31187.58	258044.21		2732.97	16166.15	214967.39
3	3395.64	30361.85	270170.79		2863.09	20413.23	191769.16
Average	3734.92	28566.08	257931.99		2788.64	18699.18	204417.20
Process Efficiency (%)	74.66	65.46	79.25*				
Acetyl Fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	32900.81	272480.22	2696875.16		32202.32	242612.47	2319607.20
2	38733.20	274894.98	2701173.97		31485.04	208628.84	2443187.71
3	38518.67	280970.95	2735233.43		35728.32	246516.70	2248135.27
Average	36717.56	276115.38	2711094.19		33138.56	232586.00	2336976.72
Process Efficiency (%)	90.25	84.24	86.20*				
Alfentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	16619.67	112384.98	1045263.57		9410.82	83578.37	829356.47
2	18109.61	118183.23	1046701.52		10494.02	68625.62	847084.67
3	17756.09	119055.84	1092001.13		11468.14	85460.45	800546.70
Average	17495.12	116541.35	1061322.07		10457.66	79221.48	825662.61

Process Efficiency (%)	59.77	67.98	77.80*				
AH7921							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	6566.28	48615.06	476009.13		4987.49	30028.21	268991.42
2	7633.34	48108.20	473702.01		4626.44	29397.38	299962.16
3	6878.39	47187.37	467335.92		4828.00	33563.29	248723.39
Average	7026.00	47970.21	472349.02		4813.98	30996.29	272558.99
Process Efficiency (%)	68.52	64.62	57.70*				
U-47700							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	9035.57	60676.63	598626.36		7419.27	55748.60	525822.70
2	8841.10	60748.85	589120.64		7911.76	51056.55	554450.77
3	9052.78	61807.71	555878.02		8857.27	53830.68	501931.10
Average	8976.48	61077.73	581208.34		8062.76	53545.28	527401.53
Process Efficiency (%)	89.82	87.67	90.74*				
Acetyl fentanyl 4-methylphenethyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	48878.21	329056.56	2764066.69		39273.94	294742.30	2564883.21
2	49041.99	317459.70	2771548.48		40145.21	270667.02	2671448.69
3	46270.71	329783.06	2784287.37		46585.53	276548.83	2533831.52
Average ----->	48063.64	325433.11	2773300.84		42001.56	280652.71	2590054.47
Process Efficiency (%)	87.39	86.24	93.39*				
Acrylfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***

1	43882.58	308992.39	2536934.84		37202.88	281712.87	2428913.55
2	46517.72	293356.95	2596363.31		37972.97	242770.34	2537661.55
3	44189.23	305741.89	2595711.68		43752.84	287579.53	2351749.58
Average	44863.18	302697.08	2576336.61		39642.89	270687.58	2439441.56
Process Efficiency (%)	88.36	89.43	94.69*				
Fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High**		**Low**	**Med**	**High**
1	35121.93	243862.09	2277257.74		29709.08	227320.04	2041829.06
2	36338.33	272959.43	2236877.77		29167.22	198024.25	2134377.46
3	37012.04	266771.73	2381041.33		35764.60	218804.82	1996871.66
Average	36157.43	261197.75	2298392.28		31546.97	214716.37	2057692.73
Process Efficiency (%)	87.25	82.20	89.53				
para-Methoxyfentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	27910.62	182350.93	1495982.69		22788.33	171129.57	1444155.49
2	28307.99	187967.00	1560701.40		22972.15	154837.11	1453530.64
3	27175.06	188179.96	1535007.01		28583.31	181306.70	1446219.28
Average	27797.89	186165.96	1530563.70		24781.26	169091.13	1447968.47
Process Efficiency (%)	89.15	90.83	94.60*				
4ANPP							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	250705.82	1588320.59	10328356.33		190178.80	1229445.02	8369358.55
2	258303.65	1581159.69	10153768.64		180041.98	1076604.71	8607365.57
3	243397.40	1581703.72	10159658.74		202286.59	1208246.04	7867551.53
Average	250802.29	1583728.00	10213927.90		190835.79	1171431.92	8281425.22

Process Efficiency (%)	76.09	73.97	81.08*				
Furanyl Fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	49598.48	349942.60	2901533.55		43119.60	311988.90	2601610.85
2	53154.12	346954.45	2836640.95		42896.47	273618.10	2713430.81
3	51906.40	357217.84	2887911.90		49315.64	319827.23	2468265.83
Average	51553.00	351371.63	2875362.13		45110.57	301811.41	2594435.83
Process Efficiency (%)	87.50	85.90	90.23*				
Despropionyl *para*-fluorofentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	55310.89	410215.90	3346009.48		44611.95	298602.77	2379969.31
2	59539.77	417208.83	3306663.71		42323.78	262062.45	2461242.98
3	54462.41	429930.16	3259141.25		48160.70	302515.48	2219544.67
Average	56437.69	419118.30	3303938.15		45032.14	287726.90	2353585.65
Process Efficiency (%)	79.79	68.65	71.24*				
(±)-*cis*-3-Methyl fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	24054.35	166202.47	1583431.31		20491.67	156986.46	1478382.82
2	27032.87	169935.48	1605989.18		21452.07	141056.37	1509950.96
3	28262.03	170303.84	1617468.23		23994.77	155435.24	1401345.31
Average	26449.75	168813.93	1602296.24		21979.50	151159.36	1463226.36
Process Efficiency (%)	83.10	89.54	91.32*				
Butyryl/Isobutyrl Fent							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***

1	45422.09	314101.40	2930769.08		39780.73	286850.10	2561000.97
2	43811.81	330407.44	2863257.61		40474.67	250218.59	2732200.06
3	43617.99	318274.99	2892301.46		41938.47	284702.64	2492138.07
Average	44283.97	320927.94	2895442.71		40731.29	273923.77	2595113.03
Process Efficiency (%)	91.98	85.35	89.63*				
Carfentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	29581.26	190158.75	1361256.73		20082.97	141783.79	1156826.21
2	28035.22	185363.84	1327128.00		19522.51	125922.13	1232173.90
3	28947.85	186650.17	1340755.27		21596.45	150266.11	1127003.16
Average	28854.78	187390.92	1343046.67		20400.64	139324.01	1172001.09
Process Efficiency (%)	70.70	74.35	87.26*				
para-Fluorobutyryl/FIBF							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	54002.16	342912.34	2955695.73		40598.62	312539.77	2654829.38
2	56611.46	339053.46	2918068.58		39133.46	276976.56	2748411.38
3	58760.62	353647.81	2923275.93		46283.60	320551.42	2534921.27
Average	56458.08	345204.54	2932346.74		42005.23	303355.92	2646054.01
Process Efficiency (%)	74.40	87.88	90.24*				
Sufentanil							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	21378.68	141092.61	1353710.93		16366.94	120199.93	1242166.50
2	23467.13	133429.58	1365799.90		15683.01	110473.97	1269157.94
3	21426.18	137804.06	1362923.38		19433.81	121634.57	1138826.22
Average	22090.66	137442.08	1360811.40		17161.25	117436.15	1216716.88

Process Efficiency (%)	77.69	85.44	89.41*				
Valeryl Fentanyl							
	Set #1				**Set #2**		
Trial no.	**Low**	**Med**	**High***		**Low**	**Med**	**High***
1	59939.19	373667.31	3834026.52		51047.18	370795.17	3328155.43
2	62192.36	419395.23	3638907.11		50457.26	327512.59	3497367.43
3	60773.11	434846.52	3925964.13		58040.14	369396.01	3238100.13
Average	60968.22	409303.02	3799632.58		53181.53	355901.26	3354541.00
Process Efficiency (%)	87.23	86.95	88.29*				

*Process efficiency percentages for all IMF analogs except for fentanyl and norfentanyl were estimated only theoretically at the high control concentration, where the control concentration exceeded the calibration range.

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