TECHNICAL NOTE 66050

Screening and semi-quantitation of 212 fentanyl analog compounds by the Orbitrap Exploris 120 mass spectrometer

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Keywords: Orbitrap Exploris 120 MS, Tox Explorer Collection, Vanquish Flex UHPLC, Opioid Certified Reference Material Kit, CDC, fentanyl, fentanyl analog, reversed-phase UHPLC, toxicology

Goal

Demonstrate the screening capabilities and benefits of high-resolution accurate mass (HRAM) mass spectrometry on the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer for accurate analysis of 212 fentanyl-related compounds, including fentanyl, in urine for clinical research and forensic toxicology.

Application benefits

- Screening and semi-quantitation of 212 fentanyl analog compounds in human urine in one analytical run
- Confident identification by retention time, accurate *m/z*, isotope pattern, and MS/MS spectral matching
- Capability for retrospective analysis of unknowns in addition to targeted screening

Introduction

According to the National Forensics Laboratory Information System (NFLIS), the number of reported fentanyl-related cases increased from 945 in 2013 to 71,341 in 2017, while the case load rose minimally over this same time span[1]. Deaths attributed to synthetic fentanyl analogs rose 10% from 2017 to 2018 and are thought to be likely driven by illicitly manufactured fentanyl analogs. Laboratories have established methods for known fentanyl analogs and other clinically relevant opioids, but the list of new illicit fentanyl analogs is rapidly expanding and can be challenging

for laboratories to confirm without certified reference standards. For this reason, the Centers for Disease Control and Prevention (CDC) for in collaboration with Cayman Chemical, released a Fentanyl Analog Screening Kit (FAS Kit) and Emergent Panels (FAS V1, FAS V2, FAS V3) to provide standards to laboratories for screening of fentanyl analogs and emerging fentanyls. Here, we present a method for fentanyl screening and semi-quantitation in urine for all 212 fentanyl analog compounds contained in the FAS Kit and Emergent Panels. Sample preparation includes simple 20× dilution in water, quantitation by Full HRAM MS scan, and confident confirmation by retention time, accurate *m/z*, isotope pattern matching to calculated theoretical isotope pattern, and matching experimentally collected MS^2 spectra to an in-house MS^2 spectral library.

Experimental

Target analytes

A large panel of 212 fentanyl analog compounds was spiked into urine and diluted 20× with water for screening and semi-quantitative analysis. The 212 fentanyl analog compounds analyzed are part of the Fentanyl Analog Screening Kit and Emergent Panels shown in Figure 1. Sample preparation was kept minimal since the purpose of this technical note was to demonstrate the screening capability of the instrument. For increased sensitivity on the Orbitrap Exploris 120 mass spectrometer for fentanyl analysis, refer to Thermo Fisher Scientific Technical Note 73957 that incorporates supported liquid extraction for sample preparation[2].

Creation of an MS^2 library and targeted inclusion list

An MS^2 spectral library, with associated retention times for 213 fentanyl analog compounds, was created by analyzing the 212 fentanyl compounds contained in the FAS Kit and Emergent Panels along with carfentanil from the Opioid Certified Reference Material (CRM) Kit[3,4] by the same LC-MS/MS method described here. Carfentanil is not included in the FAS Kit; it was included here for the most complete and comprehensive library collection available. Fentanyl compounds were prepared in 100 ng/mL mixes containing 10–14 compounds each to avoid co-eluting isomers. Following analysis by LC-MS/MS, the acquired MS^2 spectra were validated for retention time and spectral quality and were used to create an MS^2 spectral library using Thermo Scientific™ mzVault™ software. This library was transferred to Thermo Scientific™ TraceFinder™ software and used to identify fentanyl compounds by matching the experimental MS^2 spectra with the library spectra.

Calibration standards

The 212 fentanyl analog compounds in the FAS Kit and Emergent Panels were divided into 22 different mixes, containing 8–12 compounds each, to avoid having co-eluting isomers in the same mix. These mixes were prepared in methanol at a final concentration of 10 µg/mL. A 100 ng/mL standard was prepared in urine for each of the 22 mixes and was diluted serially with urine down to 0.25 ng/mL to create a 9-point calibration curve. The 9-point calibration curve for each of the 22 mixes were then diluted 20× in water and analyzed by Full MS scan followed by data-dependent MS/MS scan.

Figure 1. Fentanyl Analog Screening Kit (FAS Kit) and Emergent Panels V1, V2, and V3 from Cayman Chemical. A total of 212 unique fentanyl analog compounds are present in the FAS Kit and Emergent Panels.

Liquid chromatography

The Thermo Scientific™ Tox Explorer™ Collection chromatographic method[5] of 15.5 minutes was used for the analysis of fentanyl and fentanyl analog compounds using a Thermo Scientific™ Vanquish Flex™ UHPLC system consisting of a binary pump, a column compartment, and an autosampler. The separation was performed on a Thermo Scientific™ Accucore™ Phenyl Hexyl column (2.6 µm, 100 × 2.1 mm, P/N 17926-102130) maintained at 40 °C. Mobile phases consisted of 2 mM ammonium formate in water with 0.1 % formic acid for mobile phase A and a mixture of 2 mM ammonium formate in methanol:acetonitrile (50:50 v:v) with 0.1% formic acid for mobile phase B. Chromatographic separation of a 15 µL injected sample was achieved by gradient elution under the conditions described in Table 1.

Table 1. LC gradient

Time (min)	Flow rate (mL/min)	% A	% B
0	0.5	99	1
1	0.5	99	1
10	0.5	1	99
11.5	0.5	1	99
11.51	0.5	99	1
15.5	0.5	99	1

Mass spectrometry

Compounds were detected on an Orbitrap Exploris 120 mass spectrometer equipped with a Thermo Scientific™ OptaMax™ NG ion source with a heated electrospray ionization probe. The mass spectrometer source and scan settings are listed in Table 2 and Table 3, respectively. Full MS scan was used for screening and semi-quantitation, while targeted MS^2 by data-dependent analysis was used for confirmation. A targeted mass inclusion list containing 212 fentanyl compounds with expected retention times and accurate *m/z* was used. Preference was given to the masses listed in the targeted inclusion list, but if no fentanyl compounds on the list were present, then the instrument deferred to the next most intense ion present in the Full MS scan for Top 4 $ddMS^2$ data acquisition. This allows for unknown identification and retrospective analysis of the data for non-fentanyl or fentanyl-related compounds not yet discovered and can be added to an in-house library once confirmed.

Table 2. Source parameters for Orbitrap Exploris 120 mass spectrometer

Source parameter	Value
Positive ion	3,500 V
Sheath gas	55
Aux gas	10
Sweep gas	1
Ion transfer tube temp.	325 °C
Vaporizer temp.	350 °C

Table 3. MS scan parameters

Scan parameter	Value
Global settings	
Scan type	Full MS / ddMSMS
RF %	80
Mass calibration	Thermo Scientific™ Easy-IC™ Ion Source
Data type	Profile
Full scan	
Resolution	60,000
Max injection time	Auto
Scan range	100–1000
ddMSMS	
Intensity threshold	1.0e5
Targeted mass list tolerance	≤5 ppm, ±0.5 min
Isolation window	1.5 *m/z*
Stepped NCE	18.75, 37.5, 56.25
First mass	40 *m/z*

Method evaluation and data analysis

Data from the 22 mixes were processed in TraceFinder 5.1 software to determine the limit of detection (LOD), limit of quantitation (LOQ), and limit of identification (LOI) for each compound and are shown in Figure 2. Screening parameters including data extraction and screening limits are listed in Table 4. LOD is defined as the lowest detectable concentration in which % RSD for peak area was <15% for three replicate injections. LOQ is defined as the lowest concentration in the calibration curve giving an average % bias between nominal and back-calculated concentration within ± 20%, a % CV below 20% for three replicate injections of calibrators, and a correlation coefficient of ≥0.99 r^2 for all compounds. LOI was defined as the lowest concentration where all three replicate injections have the correct identification with an isotope and MS^2 match score of >90 and 80%, respectively.

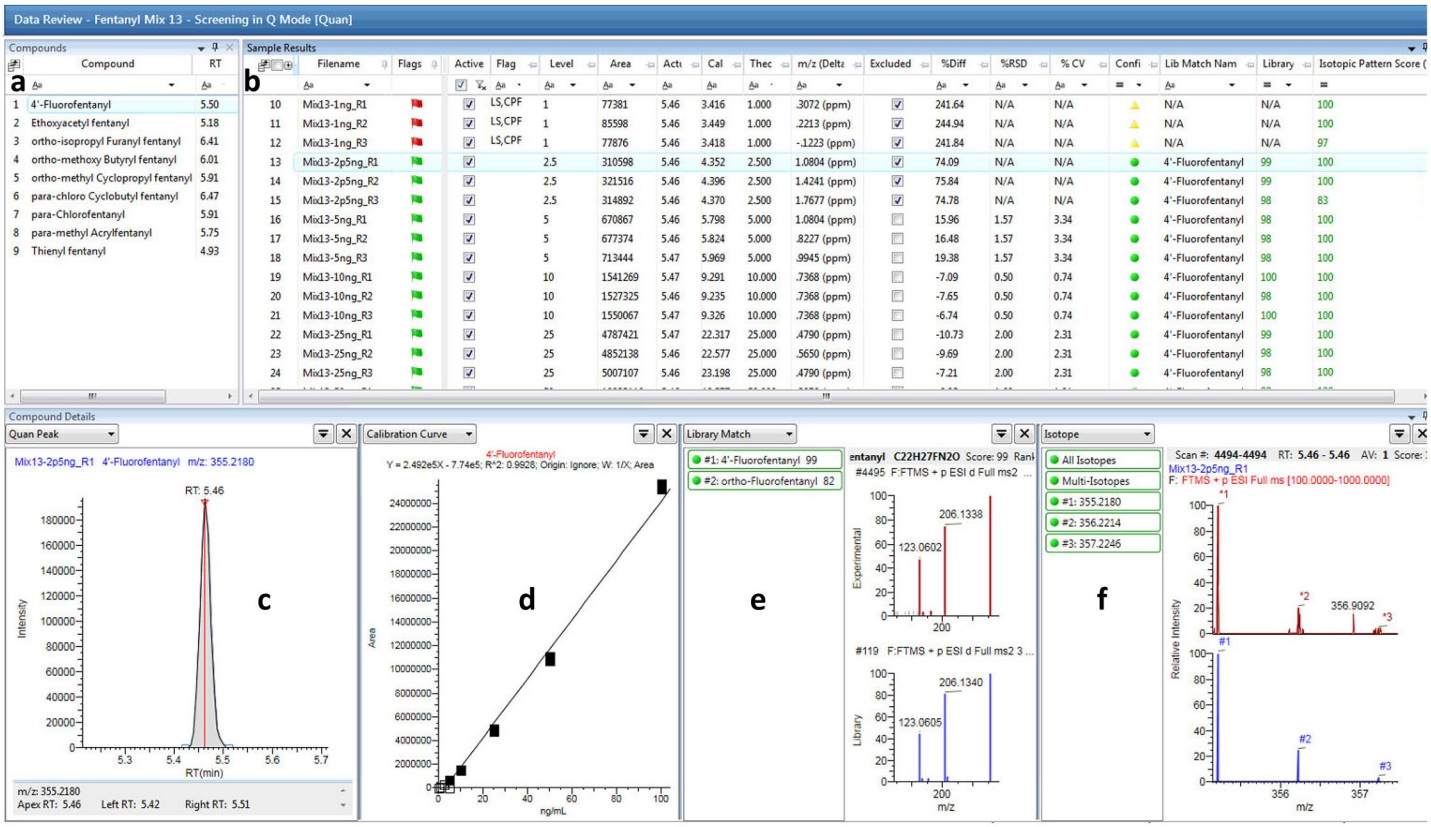

Figure 2. Quantitative results for nine fentanyl analogs in mix 13 of 22. (a) Close examination of 4'-Fluorofentanyl with LOD of 1.0 ng/mL, LOQ of 5.0 ng/mL, and LOI of 2.50 ng/mL as determined in results table (b). The quantitative peak from Full MS scan at 2.5 ng/mL (c), calibration curve (d), Library match (e), and isotope match (f) with experimental spectra highlighted in red and library spectra and theoretical isotope pattern highlighted in blue are displayed.

Table 4. Screening parameters. Parameters used for extraction and assessing calibration criteria for limit of detection (LOD), limit of quantitation (LOQ), and limit of detection (LOD) are listed.

Type	Parameter	Criteria
Data extraction criteria	*m/z* for precursor	<5 ppm
	Retention time	± 0.2 min of expected retention time
Limits defined	LOD	% RSD for peak area was <15% for three replicate injections
	LOQ	Lowest concentration in the calibration curve with an average % bias between nominal and back-calculated concentration of ≤ ±20%, % CV ≤20% for three replicate injections of calibrators, and correlation coefficient of ≥0.99 r^2
	LOI	Lowest concentration where all three replicate injections have the correct identification with an MS^2 library match score of >90% and isotope pattern score of >80%

Results and discussion

Results for retention time, LOD, LOQ, and LOI were tabulated for the 212 fentanyl analogs (Table 5). The screening limits for quantitation, detection, and identification were plotted to show the distribution of these limits (Figure 3). A majority of the fentanyl analogs had a LOD or LOQ of 2.5 ng/mL with ≥75% of the compounds having an LOQ and LOI of 10 ng/mL or better. The limit of detection for a majority of the compounds was 0.5 ng/mL with ≥75% of the compounds having an LOD of 1.0 ng/mL or better. For increased sensitivity on the Orbitrap Exploris 120 mass spectrometer for fentanyl analysis, refer to Thermo Fisher Scientific Technical Note 73957 that incorporates supported liquid extraction for sample preparation[2]. Reviewing the screening data for all 212 fentanyl analogs was simplified by designating reporting limits in the TraceFinder data processing method so any discrepancies or failures would be color coded and thus easily investigated and noted by the user.

Table 5 (part 1). Fentanyl analogs. Accurate mass of the precursor $[M+H]^+$, retention time (RT) in minutes, and LOD, LOQ, and LOD.

Compound name	$[M+H]^+$	RT (min)	LOD	LOQ	LOI
(±)-*cis*-3-methyl Fentanyl	351.2431	5.7	1.00	2.50	2.50
Acetyl fentanyl	323.2118	4.96	1.00	2.50	2.50
Acryl fentanyl	335.2118	5.33	1.00	2.50	2.50
Fentanyl	337.2274	5.42	1.00	2.50	1.00
Norfentanyl	233.1648	3.9	1.00	2.50	1.00
Remifentanil	377.2071	4.63	1.00	2.50	1.00
U-49900	357.1495	5.5	1.00	2.50	10.00
Valeryl fentanyl	365.2587	6.23	1.00	5.00	2.50
4-ANPP	281.2012	5.15	0.25	0.50	1.00
4'-methyl Acetyl fentanyl	337.2274	5.38	0.25	0.50	1.00
Benzyl fentanyl	323.2118	5.16	1.00	2.50	1.00
Methoxyacetyl fentanyl	353.2223	4.86	0.25	0.50	2.50
para-Fluorobutyryl fentanyl	369.2337	5.91	0.50	1.00	1.00
U-48800	343.1338	5.5	1.00	2.50	10.00
β-Hydroxythiofentanyl	359.1788	4.88	0.25	0.50	2.50
Butyryl fentanyl	351.2431	5.82	0.50	2.50	1.00
Cyclopropyl fentanyl	349.2274	5.64	0.50	1.00	1.00
Furanyl fentanyl	375.2067	5.54	1.00	2.50	1.00
Norcarfentanil	291.1703	4.26	0.50	1.00	2.50
para-Fluorofentanyl	355.2180	5.51	0.50	1.00	1.00
U-47700	329.1182	5.22	0.50	1.00	1.00
(±)-*cis*-3-methyl Butyryl fentanyl	365.2587	6.12	1.00	10.00	5.00
2'-fluoro *ortho*-Fluorofentanyl	373.2086	5.63	1.00	5.00	2.50
Acetyl norfentanyl	219.1492	3.21	0.25	1.00	2.50
Isobutyryl fentanyl	351.2431	5.79	1.00	2.50	2.50
ortho-Methylfentanyl	351.2431	5.73	0.25	5.00	2.50
para-fluoro Valeryl fentanyl	383.2493	6.34	2.50	10.00	10.00
para-methyl Cyclopentyl fentanyl	391.2744	6.74	25.00	25.00	25.00
para-methyl Methoxyacetyl fentanyl	367.2380	5.33	1.00	2.50	2.50
Phenylacetyl fentanyl	399.2431	6.33	2.50	5.00	10.00
Butyryl norfentanyl	247.1805	4.46	0.50	5.00	2.50
Fentanyl Methyl Carbamate	339.2067	5.25	0.25	2.50	1.00
Furanyl fentanyl 3-furancarboxamide	375.2067	5.67	1.00	5.00	2.50
Isovaleryl fentanyl	365.2587	6.17	0.25	5.00	2.50
ortho-fluoro Furanyl fentanyl	393.1973	5.62	1.00	5.00	5.00
para-Chlorobutyryl fentanyl	385.2041	6.3	0.50	10.00	10.00
para-methoxy Furanyl fentanyl	405.2173	5.67	0.25	2.50	2.50
para-Methylfentanyl	351.2431	5.84	0.50	2.50	2.50
α'-methoxy Fentanyl	367.2380	5.09	0.25	2.50	1.00
α-methyl Fentanyl	351.2431	5.57	0.50	2.50	5.00
2,2,3,3-tetramethyl-Cyclopropyl fentanyl	405.2900	7.05	5.00	25.00	25.00
2,3-seco-Fentanyl	339.2431	6.02	5.00	5.00	5.00

Table 5 (part 2). Fentanyl analogs. Accurate mass of the precursor $[M+H]^+$, retention time (RT) in minutes, and LOD, LOQ, and LOD.

Compound name	$[M+H]^+$	RT (min)	LOD	LOQ	LOI
2-fluoro MT-45	367.2544	6.5	1.00	10.00	5.00
Cyclopentenyl fentanyl	375.2431	6.09	0.50	5.00	5.00
Furanyl norfentanyl	271.1441	4.13	0.50	1.00	10.00
Isopropyl U-47700	357.1495	5.93	1.00	2.50	5.00
para-Chloroisobutyryl fentanyl	385.2041	6.27	0.50	5.00	5.00
para-fluoro Furanyl fentanyl	393.1973	5.65	1.00	2.50	2.50
para-methyl Butyryl fentanyl	365.2587	6.22	0.50	5.00	2.50
β-methyl Fentanyl	351.2431	5.71	0.25	2.50	2.50
(±)-*cis*-3-methyl Thiofentanyl	357.1995	5.51	1.00	2.50	1.00
N-(3-ethylindole) Norfentanyl	376.2383	5.64	0.50	5.00	2.50
N,N-Dimethylamido-despropionyl fentanyl	352.2383	5.53	0.25	2.50	1.00
N-methyl Cyclopropyl norfentanyl	259.1805	4.21	0.25	2.50	1.00
para-chloro Acrylfentanyl	369.1728	5.83	0.50	5.00	2.50
para-chloro Furanyl fentanyl	409.1677	6.01	1.00	5.00	2.50
para-fluoro Furanyl fentanyl 3-furancarboxamide	393.1973	5.74	0.50	5.00	2.50
para-Fluoroacetyl fentanyl	341.2024	5.05	0.50	2.50	1.00
para-methyl Isobutyryl fentanyl	365.2587	6.18	0.25	5.00	2.50
Phenyl fentanyl	385.2274	5.92	0.50	5.00	2.50
β-methyl Acetyl fentanyl	337.2274	5.27	0.25	2.50	1.00
(±)-*trans*-3-methyl Thiofentanyl	357.1995	5.47	1.00	2.50	2.50
Cyclopentyl fentanyl	377.2587	6.33	1.00	5.00	5.00
FIBF	369.2337	5.86	0.50	2.50	1.00
Norsufentanil	277.1910	4.71	1.00	1.00	5.00
ortho-fluoro Acrylfentanyl	353.2024	5.40	0.25	2.50	1.00
para-chloro Methoxyacetyl fentanyl	387.1834	5.41	0.25	2.50	50.00
para-methoxy Tetrahydrofuran fentanyl	409.2486	5.37	0.25	2.50	2.50
para-methyl Tetrahydrofuran fentanyl	393.2536	5.63	0.50	2.50	1.00
Pivaloyl fentanyl	365.2587	6.20	0.50	5.00	5.00
Thiofentanyl	343.1838	5.21	0.25	1.00	5.00
Heptanoyl fentanyl	393.2900	6.97	25.00	25.00	25.00
meta-Fluorobutyryl fentanyl	369.2337	5.91	1.00	2.50	2.50
N-methyl Norcarfentanil	305.1860	4.25	0.25	0.50	2.50
para-chloro Cyclopentyl fentanyl	411.2198	6.79	2.50	10.00	10.00
para-fluoro Acrylfentanyl	353.2024	5.40	0.25	1.00	1.00
Sufentanil	387.2101	5.99	0.25	2.50	10.00
Tetrahydrofuran fentanyl	379.2380	5.19	1.00	1.00	2.50
α'-methyl Butyryl fentanyl	365.2587	6.12	0.25	2.50	2.50
α-methyl Thiofentanyl	357.1995	5.38	1.00	1.00	5.00
β-Hydroxythioacetylfentanyl	345.1631	4.35	2.50	2.50	2.50
4'-fluoro, *para*-fluoro (±)-*trans*-3-methyl Fentanyl	387.2242	5.82	0.25	5.00	2.50
4-Phenyl fentanyl	413.2587	6.57	2.50	5.00	5.00
Benzyl Acrylfentanyl	321.1961	5.07	0.50	2.50	10.00

Table 5 (part 3). Fentanyl analogs. Accurate mass of the precursor $[M+H]^+$, retention time (RT) in minutes, and LOD, LOQ, and LOD.

Compound name	$[M+H]^+$	RT (min)	LOD	LOQ	LOI
Crotonyl fentanyl	349.2274	5.65	1.00	2.50	2.50
Fentanyl Carbamate	353.2223	5.65	0.50	2.50	2.50
meta-Fluoroisobutyryl fentanyl	369.2337	5.87	0.50	2.50	1.00
N-benzyl Furanyl norfentanyl	361.1910	5.31	1.00	2.50	2.50
Tetrahydrofuran fentanyl 3-tetrahydrofurancarboxamide	379.2380	5.14	1.00	2.50	1.00
Tetrahydrothiophene fentanyl	395.2151	5.91	1.00	5.00	25.00
α-methyl Butyryl fentanyl	365.2587	5.95	0.50	2.50	1.00
Cyclobutyl fentanyl	363.2431	6.00	0.50	5.00	2.50
Furanylethyl fentanyl	327.2067	4.93	2.50	5.00	25.00
Hexanoyl fentanyl	379.2744	6.61	5.00	25.00	25.00
meta-methyl Furanyl fentanyl	389.2223	5.89	1.00	10.00	5.00
Methacrylfentanyl	349.2274	5.47	1.00	2.50	2.50
ortho-Fluorobutyryl fentanyl	369.2337	5.92	2.50	10.00	5.00
para-fluoro Crotonyl fentanyl	367.2180	5.75	1.00	5.00	2.50
para-fluoro Cyclopentyl fentanyl	395.2493	6.42	2.50	10.00	5.00
para-methoxy Acetyl fentanyl	353.2223	5.13	0.25	1.00	2.50
AH 7921	329.1182	5.35	1.00	5.00	5.00
Benzyl Carfentanil	381.2173	5.47	1.00	5.00	2.50
meta-methyl Cyclopropyl fentanyl	363.2431	6.03	1.00	5.00	2.50
ortho-Fluoroisobutyryl fentanyl	369.2337	5.89	1.00	5.00	5.00
ortho-methyl Acrylfentanyl	349.2274	5.62	0.25	5.00	2.50
ortho-methyl Furanyl fentanyl	389.2223	5.8	0.50	10.00	5.00
para-fluoro Cyclopropyl fentanyl	367.2180	5.71	1.00	5.00	2.50
para-methoxy Valeryl fentanyl	395.2693	6.33	2.50	25.00	10.00
Phenoxyacetyl fentanyl	415.2380	6.23	2.50	10.00	10.00
β-hydroxy Fentanyl	353.2223	5.08	0.50	2.50	2.50
4'-Fluorofentanyl	355.2180	5.5	1.00	5.00	2.50
Ethoxyacetyl fentanyl	367.2380	5.18	1.00	1.00	2.50
ortho-isopropyl Furanyl fentanyl	417.2536	6.41	10.00	25.00	25.00
ortho-methoxy Butyryl fentanyl	381.2536	6.01	2.50	5.00	10.00
ortho-methyl Cyclopropyl fentanyl	363.2431	5.91	1.00	5.00	2.50
para-chloro Cyclobutyl fentanyl	397.2041	6.47	2.50	25.00	10.00
para-Chlorofentanyl	371.1885	5.91	2.50	5.00	5.00
para-methyl Acrylfentanyl	349.2274	5.75	0.25	5.00	2.50
Thienyl fentanyl	329.1682	4.93	1.00	2.50	2.50
Alfentanil	417.2608	5.36	0.25	0.50	5.00
meta-fluoro Methoxyacetyl fentanyl	371.2129	4.96	0.25	0.50	1.00
meta-Fluorofentanyl	355.2180	5.5	0.50	1.00	2.50
meta-methyl Methoxyacetyl fentanyl	367.2380	5.28	0.25	1.00	2.50
MT-45	349.2638	6.3	1.00	5.00	5.00
ortho-methyl Acetyl fentanyl	337.2274	5.24	0.25	0.50	2.50
para-fluoro Tetrahydrofuran fentanyl	397.2286	5.3	0.50	0.50	2.50

Table 5 (part 4). Fentanyl analogs. Accurate mass of the precursor $[M+H]^+$, retention time (RT) in minutes, and LOD, LOQ, and LOD.

Compound name	$[M+H]^+$	RT (min)	LOD	LOQ	LOI
para-methoxy Butyryl fentanyl	381.2536	5.94	1.00	5.00	5.00
para-methyl Cyclopropyl fentanyl	363.2431	6.04	0.25	5.00	5.00
para-methyl Furanyl fentanyl	389.2223	5.9	2.50	5.00	5.00
(±)-*trans*-3-methyl Fentanyl	351.2431	5.66	0.50	2.50	2.50
Benzodioxole fentanyl	429.2173	5.96	1.00	10.00	10.00
meta-Methylfentanyl	351.2431	5.82	0.50	5.00	2.50
Ocfentanil	371.2129	4.96	1.00	1.00	2.50
ortho-methyl Methoxyacetyl fentanyl	367.2380	5.15	0.50	1.00	2.50
para-chloro Cyclopropyl fentanyl	383.1885	6.12	1.00	10.00	5.00
β'-Phenyl fentanyl	413.2587	6.58	5.00	25.00	25.00
Benzodioxole fentanyl	429.2173	5.96	1.00	25.00	25.00
para-methyl Acetyl fentanyl	337.2274	5.39	0.50	2.50	2.50
Senecioylfentanyl	363.2431	5.97	1.00	5.00	5.00
Thiophene fentanyl	391.1838	6.58	1.00	10.00	10.00
4'-methyl Fentanyl	351.2431	5.8	0.50	5.00	2.50
Cyclohexyl fentanyl	391.2744	6.59	5.00	25.00	10.00
ortho-Fluorofentanyl	355.2180	5.53	0.50	2.50	2.50
ortho-methoxy Furanyl fentanyl	405.2173	5.67	0.50	5.00	2.50
ortho-methyl Phenyl fentanyl	399.2431	6.17	2.50	10.00	10.00
para-fluoro Methoxyacetyl fentanyl	371.2129	4.97	0.50	2.50	1.00
para-methoxy Acrylfentanyl	365.2223	5.49	0.50	2.50	2.50
para-methoxy Methoxyacetyl fentanyl	383.2329	5.05	0.50	2.50	5.00
para-Methoxyfentanyl	367.2380	5.56	0.25	2.50	5.00
α-methyl Acetyl fentanyl	337.2274	5.13	0.25	2.50	2.50
(±)-*cis*-3-methyl Norfentanyl	247.1805	4.22	0.25	0.50	1.00
N-(2C-I) Fentanyl	523.1452	6.5	5.00	25.00	25.00
N-(2C-N) Fentanyl	442.2336	5.81	0.50	5.00	5.00
N-(2C-T-4) Fentanyl	471.2676	6.74	25.00	25.00	50.00
N-(6-APB) Fentanyl	391.2380	5.91	0.50	5.00	5.00
N-(DOM) Fentanyl	425.2799	6.35	2.50	5.00	5.00
N,N-didesmethyl U-47700	301.0869	5.14	5.00	5.00	10.00
N-benzyl *para*-fluoro Cyclopropyl norfentanyl	353.2024	5.48	0.50	2.50	2.50
N-methyl U-47931E	339.1066	4.78	0.50	1.00	2.50
para-Toluoyl fentanyl	399.2431	6.27	2.50	25.00	10.00
U-48753E (maleate)	329.1182	5.64	0.50	2.50	5.00
3,4-Ethylenedioxy U-51754	333.2173	4.54	0.50	1.00	2.50
3,4-Methylenedioxy U-47700	305.1860	4.1	0.50	0.50	1.00
N-(2-APB) Fentanyl	391.2380	6.07	1.00	25.00	10.00
N-(2C-C) Fentanyl	431.2096	6.19	2.50	10.00	10.00
N-(2C-D) Fentanyl	411.2642	6.21	2.50	10.00	5.00
N-(2C-T) Fentanyl	443.2363	6.18	5.00	10.00	25.00
N-(2C-T-7) Fentanyl	471.2676	6.82	10.00	25.00	50.00

Table 5 (part 5). Fentanyl analogs. Accurate mass of the precursor $[M+H]^+$, retention time (RT) in minutes, and LOD, LOQ, and LOD.

Compound name	$[M+H]^+$	RT (min)	LOD	LOQ	LOI
N-(DOI) Fentanyl	537.1608	6.59	5.00	25.00	10.00
N-benzyl *para*-fluoro Norfentanyl	341.2024	5.25	0.25	5.00	1.00
N-methyl Norfentanyl	247.1805	3.9	0.25	1.00	10.00
Propyl U-47700	357.1495	6.04	0.50	10.00	5.00
meta-fluoro Furanyl fentanyl	393.1973	5.64	1.00	5.00	5.00
meta-methyl Acetyl fentanyl	337.2274	5.37	0.25	2.50	1.00
N-(2,5-DMA) Fentanyl	411.2642	5.9	0.25	5.00	1.00
N-(2C-B) Fentanyl	475.1591	6.3	1.00	25.00	100.00
N-(2C-iP) Fentanyl	439.2955	6.9	10.00	25.00	25.00
N-(DOC) Fentanyl	445.2252	6.3	0.50	10.00	10.00
N-desmethyl U-47700	315.1025	5.18	0.50	2.50	5.00
para-fluoro 4-ANBP	285.1761	4.93	0.25	2.50	1.00
Remifentanil Acid	363.1914	4.37	0.50	0.50	0.00
U-51754	343.1338	5.64	0.25	2.50	10.00
3'-methyl Acetyl fentanyl	337.2274	5.38	1.00	1.00	5.00
4-phenyl U-51754	351.2431	6.05	2.50	10.00	5.00
Despropionyl 2'-fluoro *ortho*-Fluorofentanyl	317.1824	5.55	2.50	5.00	5.00
Despropionyl *meta*-Methylfentanyl	295.2169	5.55	2.50	5.00	2.50
N-(2C-T-2) Fentanyl	457.2519	6.47	10.00	10.00	25.00
N-(6-APDB) Fentanyl	393.2536	5.71	2.50	2.50	10.00
N-(DOB) Fentanyl	489.1747	6.41	5.00	10.00	0.00
N-(DOET) Fentanyl	439.2955	6.71	5.00	25.00	10.00
N-(Phentermine) Fentanyl	365.2587	5.71	2.50	2.50	2.50
para-Bromofentanyl	415.1379	6.03	2.50	10.00	0.00
2'-methyl Acetyl fentanyl	337.2274	5.28	0.25	2.50	2.50
2'-methyl Fentanyl	351.2431	5.71	0.25	5.00	2.50
3,4-Ethylenedioxy U-47700	319.2016	4.26	0.25	0.25	1.00
N-(2C-B-fly) Fentanyl	499.1591	6.33	5.00	25.00	25.00
N-(2C-E) Fentanyl	425.2799	6.58	5.00	25.00	25.00
N-(2C-P) Fentanyl	439.2955	6.92	10.00	25.00	50.00
N-(2C-TFM) Fentanyl	465.2359	6.55	10.00	25.00	25.00
N-(MDA) Fentanyl	395.2329	5.59	0.50	2.50	5.00
U-48520	295.1572	4.61	2.50	2.50	2.50
U-50488	369.1495	5.83	0.50	2.50	5.00
(±)-*cis*-Isofentanyl	337.2274	5.42	0.50	5.00	2.50
2',5'-dimethoxy Fentanyl	397.2486	5.76	0.50	5.00	5.00
3'-methyl Fentanyl	351.2431	5.8	0.25	5.00	2.50
Despropionyl *para*-Fluorofentanyl	299.1918	5.27	0.50	2.50	2.50
N-(2C-G) Fentanyl	425.2799	6.43	5.00	10.00	10.00
N-(3,4,5-TMA) Fentanyl	441.2748	5.49	0.50	2.50	2.50
N-(3C-B-fly) Fentanyl	513.1747	6.44	10.00	25.00	100.00
N-(DOBU) Fentanyl	467.3268	7.36	25.00	25.00	50.00
N-Benzyl phenyl norfentanyl	371.2118	5.7	0.50	5.00	5.00
U-47931E	325.0910	4.73	0.50	1.00	5.00

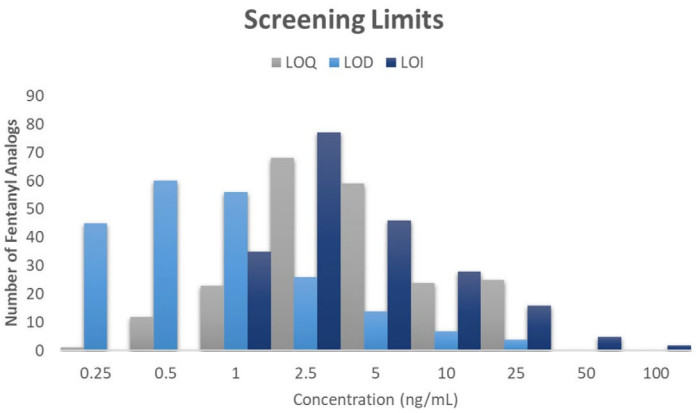

Figure 3. Screening limit distribution for quantitation (LOQ), detection (LOD), and identification (LOI) for 212 fentanyl compounds in the FAS Kit and Emergent Panels v1-v3. For a majority of the compounds, the LOQ and LOI were 2.5 ng/mL with >75% of the compounds having an LOQ and LOI of 10 ng/mL or better. A majority of the compounds had an LOD of 0.5 ng/mL with >75% of the compounds having a LOD of 2.5 ng/mL or better.

Conclusions

A large panel screening method for 212 fentanyl analogs in urine was developed on the Vanquish Flex UHPLC system and Orbitrap Exploris 120 mass spectrometer. Sample preparation and analysis included a simple 20× dilution in water followed by a 15-minute UHPLC gradient separation and HRAM detection. Screening and semi-quantitative analysis was performed and tabulated for the simple 20× diluted samples with ≥75% of the fentanyl compounds reporting a LOD of 2.5 ng/mL. Sensitivity could be improved by more selective sample preparation methods, such as supported liquid extraction. However, the method reported here offers sufficient limits of detection for screening and demonstrates how the Orbitrap Exploris 120 mass spectrometer offers confident compound identification by confirmation of retention time, accurate *m/z*, isotopic pattern matching to calculated theoretical isotopic pattern, and matching experimentally collected MS^2 spectra to an in-house MS^2 library of 213 fentanyl analog compounds. All of the MS^1 and MS^2 spectra collected by the Orbitrap Exploris 120 mass spectrometer are quality spectra that do not need to be averaged to achieve <5 ppm mass accuracy. While the method described here offers a comprehensive targeted screening method for fentanyl analogs, unknown, retrospective analysis may also be performed as an added benefit, allowing the end-user a broader picture of the evolving and rapidly changing illicit drug landscape.

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Laboratory findings were made possible, in part, by the Centers for Disease Control and Prevention's design and support of Traceable Opioid Material Kits. TRACEABLE OPIOID MATERIAL, TOM KITS, and the TOM KITS logo are marks of the U.S. Department of Health and Human Services.

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