

Confident Fentanyl Screening in Urine by HRAM Orbitrap Mass Spectrometer Analysis

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ABSTRACT

Purpose: Demonstrate the screening capability of a modified Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer for fast accurate analysis of fentanyl and its analogs.

Background: The United States is facing an opioid crisis that includes not only the abuse of prescription drugs but also synthetic opioids like fentanyl. According to the Centers for Disease Control and Prevention (CDC), rates of overdose deaths involving synthetic opioids other than methadone, but including fentanyl, increased 10 % from 2017 to 2018. Over 31,000 people died from overdoses involving synthetic opioids, other than methadone, in 2018. As synthetic opioid and fentanyl use continues to skyrocket so does the need for a fast, accurate method for the simultaneous analysis of fentanyl and its analogues.

Method: Screening and semi-quantitative method developed using the Fentanyl Analog Screening Kit (FAS Kit) and Emergent Panels (FAS V1, FAS V2, FAS V3) in urine samples diluted 20X in water. Compounds are identified and confirmed by a combination of retention time, accurate m/z, isotopic pattern, and spectral library matching.

Results: A fast and accurate method for the simultaneous analysis of fentanyl and its analogues was created successfully on a modified Thermo Scientific™ Orbitrap Exploris™ HRAM mass spectrometer.

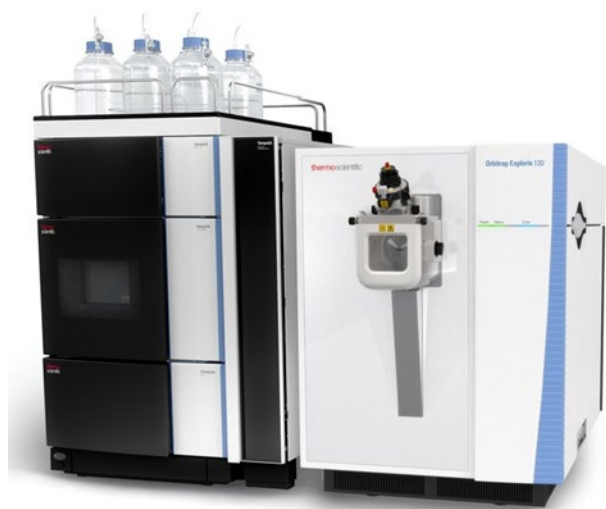


Figure 1. Thermo Scientific™ Vanquish™ Flex LC with Orbitrap Exploris 120 mass spectrometer

MATERIALS AND METHODS

Standard and Sample Preparation for Fentanyl Screening

A total of 212 fentanyl compounds from the CDC Fentanyl Analog Screening Kit shown in Figure 2 were resuspended in methanol. Standards were grouped into mixes of 8-12 compounds each to prevent from having co-eluting isomers in the same mix. There were 22 mixes in total to account for all 212 fentanyl compounds. Each of the 22 neat standard mixes were injected at a concentration of 100 ng/mL and analyzed by LC-MS/MS to obtain method specific retention times and MS/MS spectra. The acquired spectra were then used to build a Thermo Scientific™ mzVault™ library. Each mix was spiked into blank donor urine at a concentration of 100 ng/mL. A calibration curve ranging from 0.25 ng/mL to 100 ng/mL was prepared for each of the 22 mixes by serial dilution with the blank urine sample. Each standard in the urine calibration curve for each of the 22 mixes was diluted 20-fold with water prior to analysis.



Figure 2. Fentanyl Analog Screening Kit (FAS Kit) and Emergent Panels V1, V2, and V3.

NOTE: Laboratory findings were made possible, in part, by the Centers for Disease Control and Prevention's design and support of Traceable Opioid Material™ Kits. #tomkits

Liquid Chromatography and Mass Spectrometry Analysis

1. Chromatographic separation of the Samples were performed on a Vanquish Flex UHPLC system using the following conditions:

- Column – Accucore Phenyl Hexyl 100 x 2.1 mm (2.6 µm), maintained at 40°C
- Mobile Phase A: 2 mM ammonium formate + 0.1 % formic acid in water
- Mobile Phase B: 2 mM ammonium formate + 0.1% formic acid in acetonitrile/methanol/water (50/50/1/0.1) (v/v/v)

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	1	1
15.5	0.5	99	1

Table 1. Liquid Chromatography Gradient

2. Samples were then analyzed by Orbitrap Exploris 120 with the following source and scan parameters:

Source Parameter	Value
Positive Ion	3500 V
Sheath Gas	55
Aux Gas	10
Sweep Gas	1
Ion Transfer Tube Temp	325 ° C
Vaporizer Temp	350 ° C

Table 2. Source Parameters

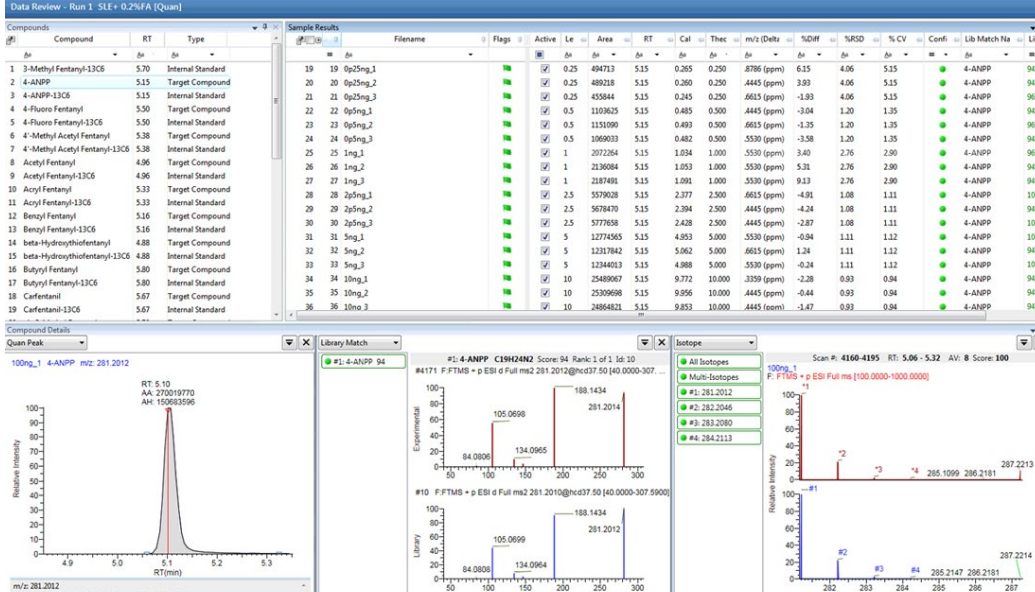
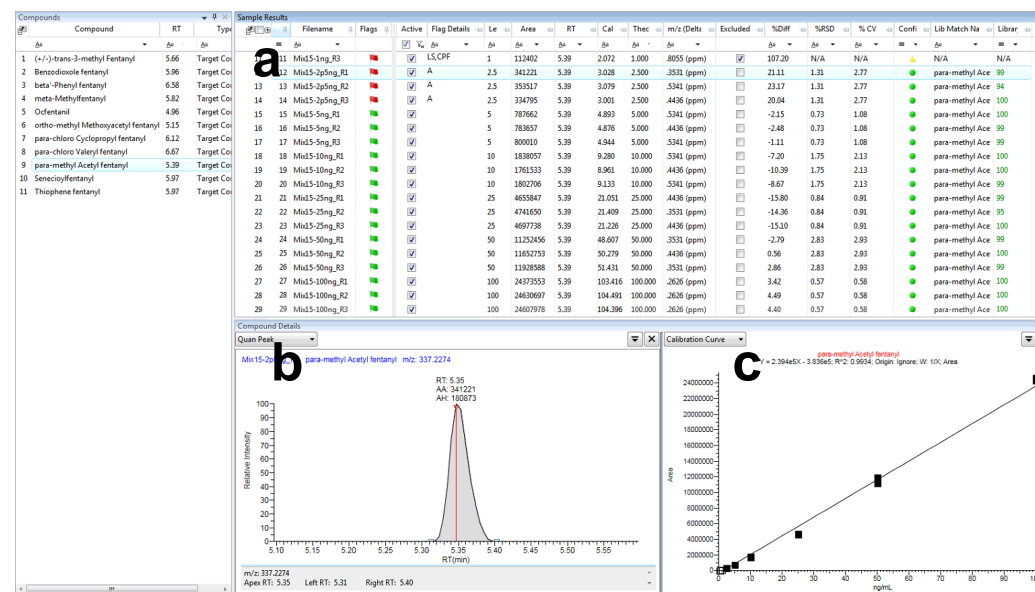
Scan Parameter	Value
GLOBAL SETTINGS	
Scan Type	Full MS / ddMSMS
RF %	80
Mass Calibration	Easy-IC™
Data Type	FULL SCAN
FULL SCAN	
Resolution	60,000
Max Injection Time	Auto
Scan Range	100-1000
ddMSMS	
Intensity Threshold	1.0e5
Targeted Mass List	≤ 5 ppm, +/- 0.5 min
Tolerance	
Isolation Window	1.5 m/z
Stepped NCE	18.75, 37.5, 56.25
First Mass	40 m/z

Table 3. Global MS Settings and Scan Parameters

DATA PROCESSING

All of the data were processed in Thermo Scientific™ TraceFinder™ software, version 5.1. to determine Limit of Detection (LOD), Limit of Quantitation (LOQ) and Limit of Identification (LOI). 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% and a % CV below 20% for 3 replicate injections of calibrators. LOI is lowest concentration where all three replicate injections have an isotope and MSMS match.

Figure 3. Quantitative results for nine fentanyl analogs in mix 13 of 22 (top) and close examination of para-methyl Acetyl fentanyl with LOD (b) and calibration curve (c) shown.



SCREENING RESULTS

Compound Name	[M+H] ⁺	Retention Time (min)	LOD (ng/mL)	LOQ (ng/mL)	LOI (ng/mL)
(±)-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
Remifentanyl	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-Fluorobutyl 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
Norcalfentanil	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 Butyl 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
Isobutyl 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-Chlorobutyl 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
2,2,3-seco-Fentanyl	339.2431	6.02	5.00	5.00	5.00
2-fluoro MT-45	367.2544	6.5	1.00	10.00	5.00
Cyclopentyl fentanyl	375.2431	6.09	0.50	5.00	5.00
ortho-norfenyl	271.1441	4.13	0.50	1.00	10.00
Isopropyl U-47700	357.1495	5.93	1.00	2.50	5.00
para-Chloroisobutyl 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 Isobutyl 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	5.4	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	6.2	0.50	5.00	5.00
Thiofentanyl	343.1838	5.21	0.25	1.00	5.00
Heptanyl fentanyl	393.2900	6.97	25.00	25.00	25.00
meta-Fluorobutyl fentanyl	369.2337	5.91	1.00	2.50	2.50
N-methyl Norcalfentanil	305.1860	4.25	0.25	0.50	2.50
para-chloro Cyclopentyl fentanyl	411.2198	6.79	2.50	10.00	10.00

Compound Name	[M+H] ⁺	Retention Time (min)	LOD (ng/mL)	LOQ (ng/mL)	LOI (ng/mL)
para-fluoro Acrylfentanyl	353.2024	5.4	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 Butyl fentanyl	365.2587	6.12	0.25	2.50	2.50
β-methyl Thiofentanyl	357.1995	5.38	1.00	1.00	5.00
4-Hydroxythioacetyl fentanyl	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
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-Fluoroisobutyl 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-tetrahydrofuran carboxamide	379.2380	5.14	1.00	2.50	1.00
Tetrahydrothiophene fentanyl	395.2151	5.91	1.00	5.00	25.00
α-methyl Butyl fentanyl	365.2587	5.95	0.50	2.50	1.00
Cyclobutyl fentanyl	363.2431	6	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-Fluorobutyl 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-Fluoroisobutyl 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
4-phenyl U-51754	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 Butyl 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-methyl Acetyl fentanyl	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
para-methoxy Butyl 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</		