Analysis of Fentanyl and Designer Fentanyl Derivatives in Urine using SPE and HPLC-MS/MS

UCT Part Numbers

ASFBETA-GLUC-10
Abalonase™ +
Purified Beta- glucuronidase with enhanced sulfatase activity

CSXCE106
Clean Screen® XCEL I
130mg / 6mL SPE Cartridge

SLPFPP100ID21-3UM
Selectra® PFPP HPLC Column
100 x 2.1 mm, 3 µm

SLPFPPGDC20-3UM
Selectra® PFPP Guard Column
10 x 2.1 mm, 3 µm

SLGRDHLDR
Guard Column Holder

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Summary:

Fentanyl abuse is drastically on the rise in the United States. In addition to traditional fentanyl, modified versions of the drug are also being abused (i.e. fentanyl analogs). With the list of derivatives on the rise forensic toxicologists need the ability to rapidly identify not only fentanyl, but its modified forms as well. Generally, these compounds have varying levels of potency when compared to fentanyl, correlating to a wide range of encountered concentrations in biological fluids.

While immunoassays are typically employed as a reliable, first-step screening tool, they have proven to be inaccurate when applied to the initial detection of these novel, synthetic drugs/metabolites. On account of this, a universal extraction approach for both current compounds in addition to emerging analytes is therefore vital to both screen and confirm their presence.

Presented is a rapid, three step SPE procedure, that includes a concentration step, for the identification and quantification of fentanyl and its major urinary metabolite norfentanyl, along with eleven additional synthetic opioid compounds: acryl fentanyl, desmethyl U-47700, furanyl fentanyl, methoxyacetyl fentanyl, remifentanil, tetrahydrofuran fentanyl, U-47700, U-50488, U-51754, W-15 and W-18.

Sample Pretreatment:

1. **Hydrolysis**
    a) To 1 mL of urine sample add 1 mL of Abalonase™+ working enzyme stock solution (10,000 Fishman units/mL).
    b) Add internal standard(s).
    c) Gently mix by inversion or vortex 10 to 15 seconds prior to use.
    d) Hydrolyze for 15 minutes to one hour from room temperature to 70°C.

SPE Procedure:

1. **Sample Extraction**
    a) Apply the sample to the SPE cartridge (if required, use a low vacuum to draw the sample through at ≤3 mL/min).

2. **Wash Cartridge**
    a) 1 x 2 mL D.I. H_2O.
    b) 1 X 2 mL 75:25 D.I. H_2O: MeOH.
    c) Dry column under full vacuum or pressure for 10 minutes.

3. **Elution**
    a) Elute with 1 x 2 mL CH_2Cl_2/IPA/NH_4OH (75:20:5).
    b) Evaporate the sample to dryness under a gentle stream of nitrogen.
    c) Reconstitute in 100 µL 95:5 D.I. H_2O:Methanol and vortex for 1 minute.
    d) Transfer sample to autosampler vial containing a low volume insert.

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Compound Information		
Name	**Potency Relative to Morphine**	**Relative Information**
Acryl Fentanyl	Unknown	Schedule I drug. Nine intoxications confirmed in Sweden in 2016[1].
Desmethyl U-47700	Unknown	Major urinary metabolite of U-47700.
Fentanyl	50-100x	Schedule II drug in clinical use since the early 1960s. Overdoses first reported in 1980's.
Furanyl Fentanyl	50-100x	Synthesized and patented in 1980s. Extremely common in adulterated heroin[2].
Methoxyacetyl Fentanyl	Unknown	No reported fatalities as of August 2017.
Norfentanyl	N/A	Major urinary metabolite of fentanyl.
Remifentanil	100-200x	Can be detected up to 72 hours in urine.
Tetrahydrofuran Fentanyl	Unknown	Schedule II drug.
U-47700	7.5x	Yet to be restricted or classified by the DEA[3].
U-50488	Unknown; 1 mg/kg of naloxone unsuccessful in prohibiting its effects	First fatalities published in 2016; post mortem blood concentrations of 1460 ng/mL reported [4,5,6].
U-51754	Unknown	Investigated as an anticonvulsant in mid 1980's[6].
W-15	5x	Schedule I drug. Nine intoxications confirmed in Sweden in 2016[1].
W-18	10x	Major urinary metabolite of U-47700.

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Instrumentation	
HPLC System	Shimadzu Nexera X2 LC-30AD
MS System	Shimadzu LCMS-8050
HPLC Column	UCT Selectra® PFPP, 100 x 2.1 mm, 3 µm
Guard Column	UCT Selectra® PFPP, 10 x 2.1 mm, 3 µm
Column Temperature	40°C
Flow Rate	300 µL/min
Injection Volume	5 µL

Mobile Phase Gradient		
Time (min)	**% Mobile Phase A Water + 0.1% Formic Acid**	**% Mobile Phase B Methanol + 0.1% Formic Acid**
0.0	90	10
5.0	0	100
6.0	0	100
6.1	90	10
8.0	90	10

MRM Transitions				
Compound	**tR (minutes)**	**Precursor Ion**	**Product Ion 1**	**Product Ion 2**
Norfentanyl	4.09	232.9	84.2	56.1
Norfentanyl D5	4.09	237.9	84.1	56.1
Remifentanil	4.59	377.2	113.1	317.1
Methoxyacetyl Fentanyl	4.67	354.1	188.3	105.1
Tetrahydrofuran Fentanyl	4.93	379.3	188.05	105.1
Acryl Fentanyl	4.98	335.1	188.15	105.1
Desmethyl U-47700	4.99	315.2	284.0	173.1
Furanyl Fentanyl	5.00	375.2	188.2	105.15
U-47700	5.01	329.3	284.0	173.0
Fentanyl	5.05	338.1	189.2	105.2
Fentanyl D5	5.05	342.1	188.2	105.15
U-51754	5.28	345.1	300.0	112.15
U-50488	5.41	369.3	298.05	112.3
W-15	5.76	377.0	105.1	111.15
W-18	5.83	421.8	111.1	322.05

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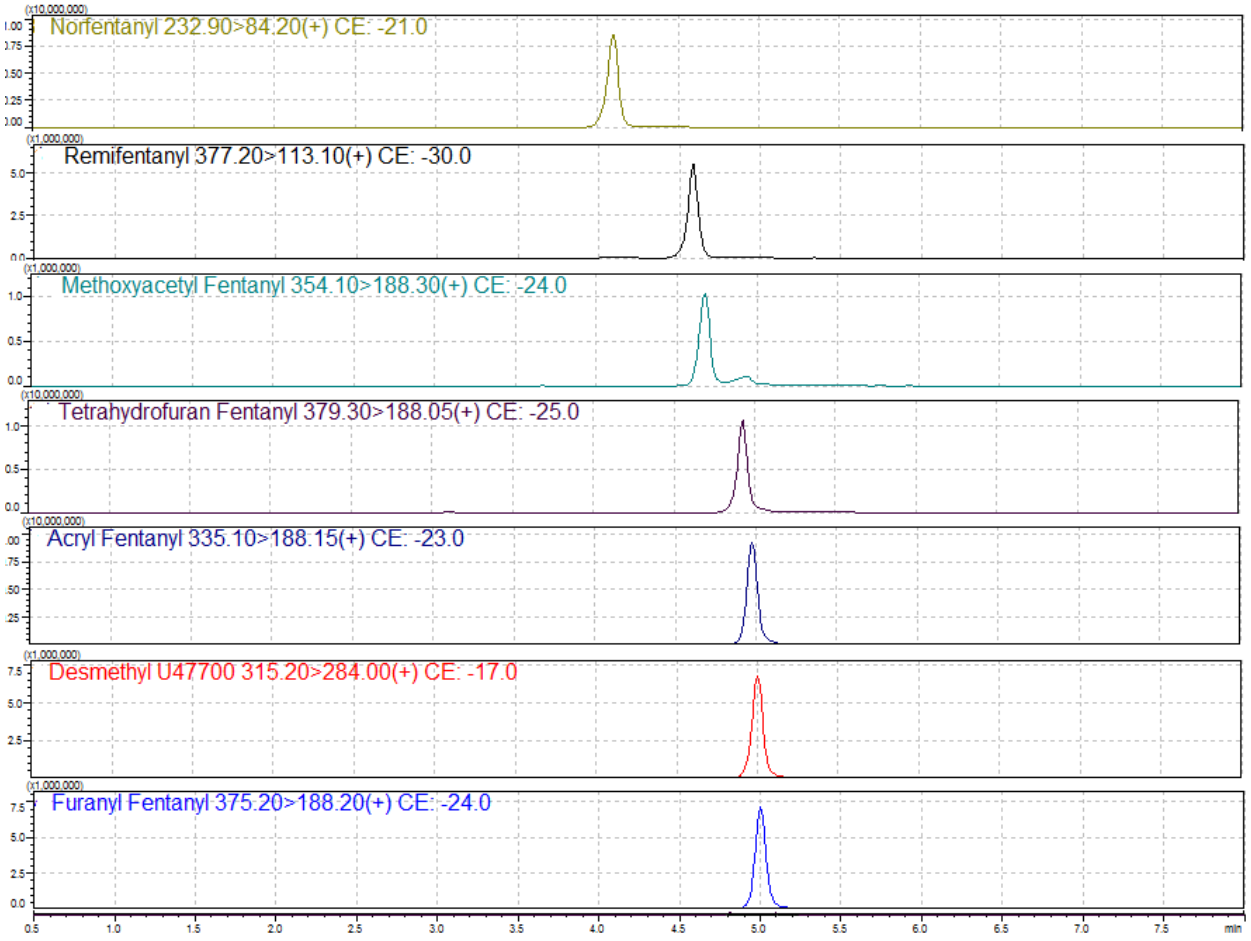
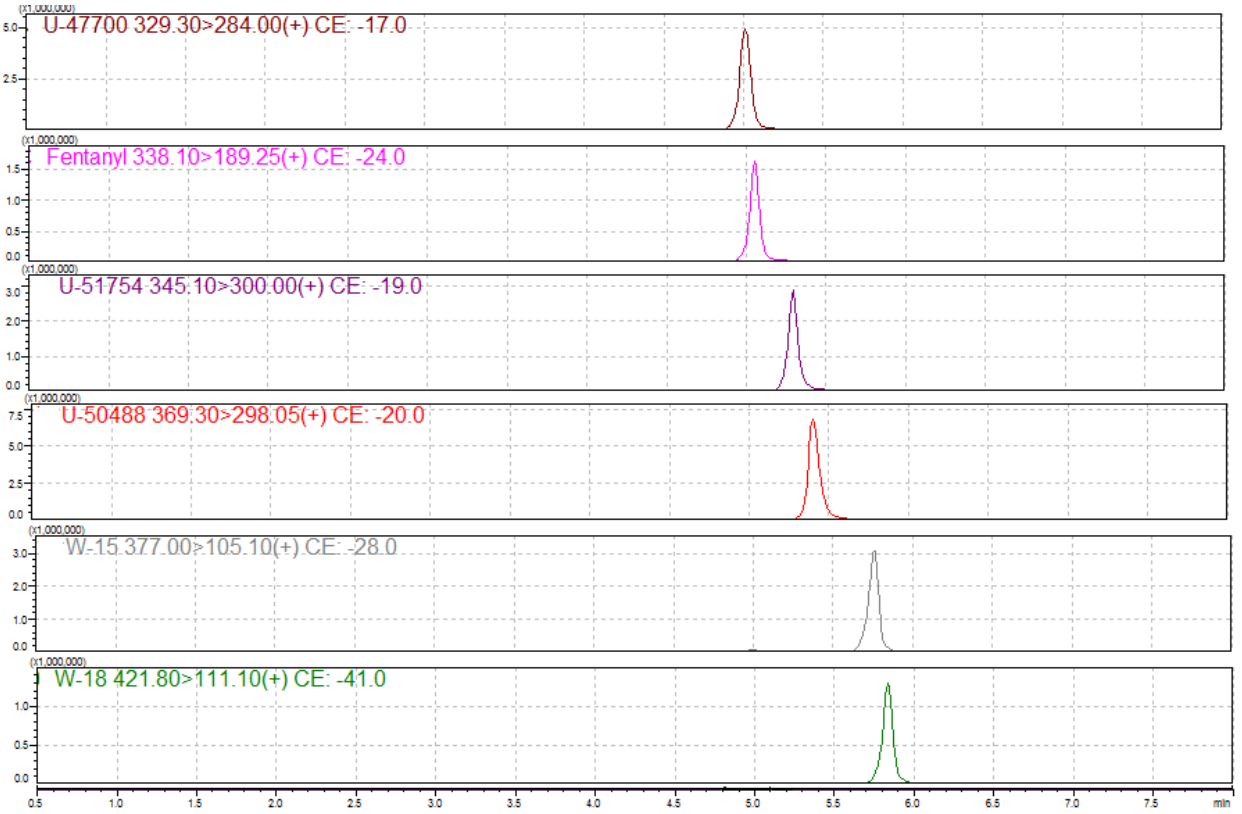
Chromatogram of a 20 ng/mL Extracted Sample





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Relative % Recovery (n=5)			
Compound	**1 ng/mL**	**5 ng/mL**	**15 ng/mL**
Acryl Fentanyl	92	82	94
Desmethyl U-47700	95	89	82
Fentanyl	99	100	83
Furanyl Fentanyl	97	80	91
Methoxyacetyl Fentanyl	94	91	97
Norfentanyl	93	88	88
Remifentanil	90	92	91
Tetrahydrofuran Fentanyl	100	94	85
U-47700	94	85	94
U-50488	99	96	75
U-51754	96	93	83
W-15	82	98	95
W-18	82	98	90

Conclusion:

A fast and effective method was developed for the determination of fentanyl and other synthethic opiate compounds in urine samples. All analytes of interest were extracted using a Clean Screen XCEL® I column. Analysis of the samples was performed by LC-MS/MS utilizing a Selectra® PFPP HPLC column which allowed for improved separation of furanyl fentanyl and fentanyl, when compared to other column phases. Absolute recoveries ranged from 75-100% for all three control levels tested. With the unfortunate (and often unaware) abuse of synthetic opiates throughout the United States, it is critical that forensic laboratories have accurate and rapid SPE methods for the identification of this class of compounds. The proceeding method will be of great use as drugs with similar structures start to be found in future casework.

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