Analysis of Synthetic Opiates and Novel Analgesics in Urine using SPE and HPLC-MS/MS

UCT Part Numbers

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

SPHPHO6001-5
Select pH Buffer
100 mM Phosphate pH 6.0

SLDA100ID21-3UM
Selectra® DA HPLC Column
100 X 2.1 mm, 3 µm

SLDAGDC20-3UM
Selectra® DA Guard Column
10 X 2.1 mm, 3 µm

SLGRDHLDR
Guard Column Holder

CLINICAL FORENSICS

Summary:

Opiate abuse is drastically on the rise in the United States. In addition to traditional naturally occurring opiate compounds, forensic toxicologists also need the ability to rapidly identify synthetic opioid-like drugs. Many of these compounds present as heroin-like overdoses in death investigation cases, but traditional basic drug extractions may unassumingly wash the target compounds off of the SPE column prior to elution.

Often manufactured overseas and shipped to the United States illegally, these compounds have varying levels of potency when compared to morphine (Table 1). Immunoassays are not reliable screening tools for these designer drugs/metabolites, and it's difficult for mass spectrometry libraries to keep up with their constantly changing structures.

Presented is a rapid, three step SPE procedure for the identification and quantification of fentanyl and its major urinary metabolite norfentanyl, in addition to four "designer" compounds: U-47700, W-18, W-15 and furanyl fentanyl. Due to the rapid use and abuse of fentanyl in medical and recreational settings, respectively, it is important to develop a method that would accurately extract this Schedule II drug from any other novel compounds that may be present.

Sample Pretreatment:

To 1 mL of urine sample, add 1 mL of 100 mM Phosphate Buffer (pH=6) and appropriate amount of internal standard

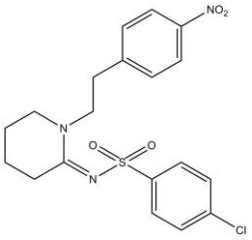
SPE Procedure:

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).

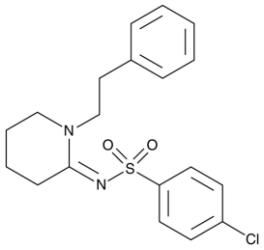
2. **Wash Cartridge**
    a) 1 x 3 mL D.I. H_2O.
    b) 1 X 3 mL 100 mM Acetic Acid.
    c) Dry column under full vacuum or pressure for 10 minutes.

3. **Elution**
    a) Elute with 1 x 3 mL Methanol containing 2% Ammonium Hydroxide (MeOH:NH_4OH, 98:2 v/v).
    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.

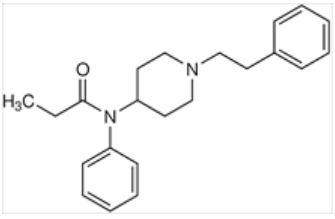
Figure 1: Structures of Designer Opiates



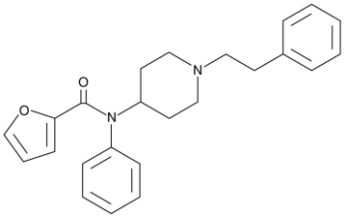
W-18



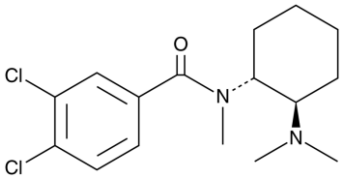
W-15



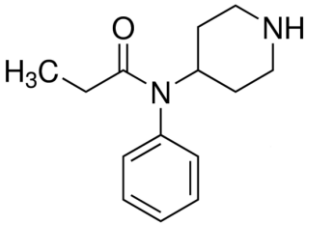
Fentanyl



Furanyl Fentanyl



U-47700



Norfentanyl

CLINICAL

FORENSICS

LC/MS-MS Parameters:

Instrumentation	
HPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000 UHPLC
MS system	Thermo Scientific™ TSQ Vantage™ (MS/MS)
HPLC column	UCT Selectra® DA, 100 × 2.1 mm, 3 µm
Guard column	UCT Selectra® DA, 10 × 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	95	5
1.0	10	90
5.0	10	90
5.2	95	5
10.0	95	5

MRM transitions (ESI^+)				
Compound	**t_R (min)**	**Precursor ion**	**Product ion 1**	**Product ion 2**
Norfentanyl	3.8	233.1	56.0	84.0
Norfentanyl D5	3.8	238.1	56.0	84.0
U-47700	4.1	329.1	172.9	284.0
Fentanyl	4.2	337.2	105.0	188.1
Fentanyl D5	4.2	342.2	105.0	188.1
Furanyl Fentanyl	4.3	375.1	105.0	188.1
W-18	6.6	422.0	110.9	272.9
W-15	6.7	377.0	105.0	110.9

CLINICAL

FORENSICS

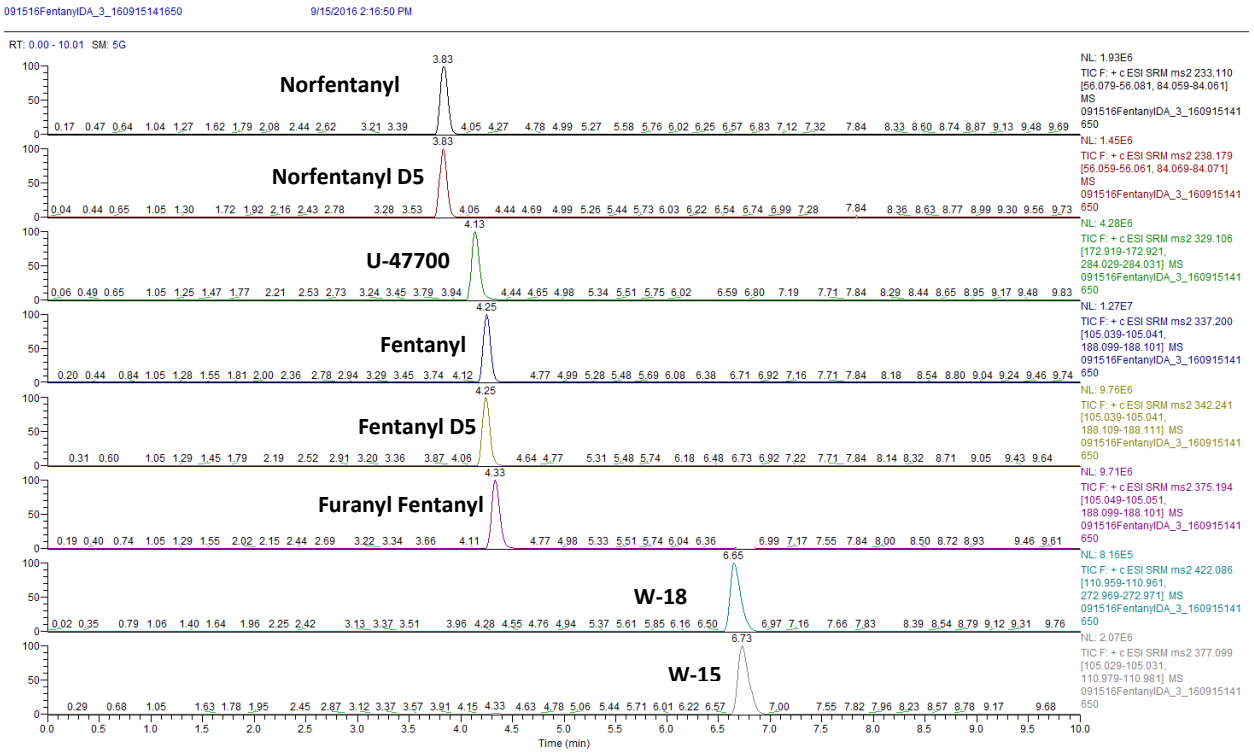


Figure 2. Chromatogram of 50 ng/mL synthetic opiate and novel analgesic standard

Results/Discussion:

Due to the large variation in fentanyl-like drugs mentioned in recent case reports, a panel of four emerging drugs of abuse were selected along with fentanyl and its major urinary metabolite norfentanyl for extraction and analysis. The universal SPE methodology was designed keeping in mind the continuously evolving target of designer opiate-like drugs and other novel analgesics. Executing a tandem wash scheme utilizing D.I. H2O followed by Acetic Acid provided removal of unwanted matrix components in addition to protonation of basic compounds, respectively, without the loss of hydrophobic analytes. A reduction in wash steps and elimination of column conditioning allows for an overall savings on both analyst time and solvent usage.

Quantitation was performed against a 6-point matrix-matched calibration curve. Spiked samples were then analyzed for overall recovery at 3 varying concentration levels. All samples were run in replicates of 5 for reproducibility studies. For all compounds, the absolute recovery was greater than 70% for all three target concentrations. The mean recoveries were 82.9%, 92.2% and 103.1% at concentrations of 5 ng/mL, 20 ng/mL and 50 ng/mL, respectively (Table 2). Calibration range was from 5-100 ng/mL, with an average correlation coefficient of 0.9951 across all compounds.

CLINICAL

FORENSICS

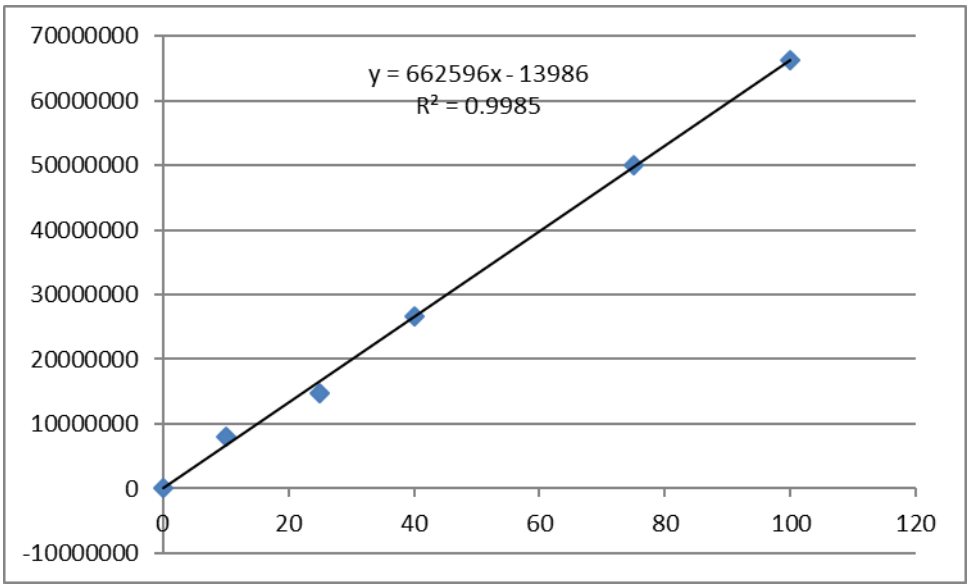


Figure 3. Linearity of Furanyl Fentanyl

Table 1: Compound Potency Relative to Morphine	
Compound	**Potency**
Fentanyl	50-100x
Norfentanyl	N/A
U-47700	7.5x
Furanyl Fentanyl	50-100x
W-15	5x
W-18	10x

Table 2: Absolute Percent Recovery			
Compound	**5 ng/mL (n=5)**	**20 ng/mL (n=5)**	**50 ng/mL (n=5)**
Fentanyl	73.8	96.8	110.1
Norfentanyl	72.9	78.6	103.5
Furanyl Fentanyl	73.8	87.3	114.6
U-47700	112.5	115.5	125.3
W-15	78.8	83.9	80.0
W-18	86.1	91.4	85.2
Mean Recovery (%)	**82.9**	**92.2**	**103.1**

CLINICAL

FORENSICS

Conclusion:

A fast and effective method was developed for the determination of six designer opiates 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® DA HPLC column which allowed for improved separation of furanyl fentanyl and fentanyl, when compared to other column phases. Absolute recoveries ranged from 72.9-125.3% 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 casework.

CLINICAL

FORENSICS

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255 www.unitedchem.com Email: methods@unitedchem.com