ANALYSIS OF DESIGNER OPIATES AND NOVEL ANALGESICS IN URINE USING SPE AND HPLC MS/MS

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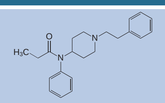
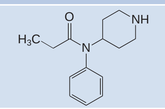
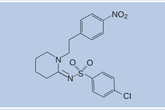
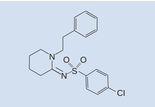
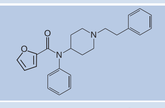
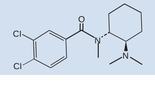
SUMMARY:

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 / designer opioid-like drugs. 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.

Name	IUPAC Name	Potency Relative to Morphine	Relative Information	Structure
Fentanyl	N-(1-(2-Phenylethyl)-4-piperidinyl)-N-phenylpropanamide	50-100x	Schedule II drug in clinical use since the early 1960s. Recreational overdoses first reported in 1980s.	
Norfentanyl	N-phenyl-N-(piperidin-4-yl) propionamide	N/A	Major urinary metabolite of fentanyl. Can be detected up to 72 hours in urine.	
W-18	4-chloro-N-[(2Z)-1-[2-(4-nitrophenyl)ethyl]piperidin -2-ylidene]benzene-1-sulfonamide	10x	Synthesized in Canada in 1980s. First report of recreational abuse: 2010	
W-15	1-Phenylethylpiperidylidene-2-(4-chlorophenyl)sulfonamide	5x	Identified as next research chemical to emerge in popularity if W-18 is to be scheduled	
Furanyl Fentanyl	(N-phenyl-N-[1-(2-phenylethyl) piperidin-4-yl]furan-2-carboxamide)	50-100x	Temporarily placed into Schedule I by DEA September 2016	
U-47700	3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide	7.5x	Placed into temporary Schedule I status as of September 2016	

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 or positive pressure 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.

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
6.5	10	90
7.0	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

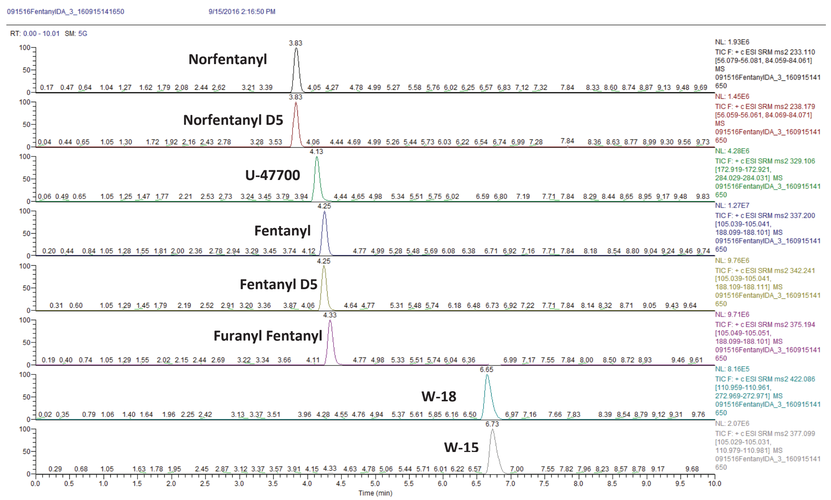


Figure 2. Chromatogram of 50 ng/mL synthetic opiate and novel analgesic standard

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. This 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. H_2O 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.

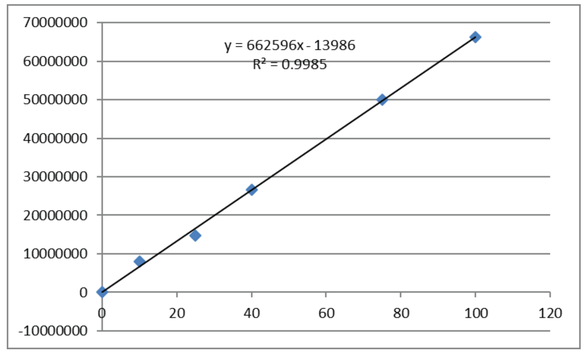


Figure 3. Linearity of Furanyl Fentanyl

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**

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 future casework.