

Introduction

During this research study, a sensitive, robust and relatively fast targeted analytical method was developed for the quantitation of 12x synthetic fentanyl opioids, 4-ANPP the synthetic precursor molecule and a similar powerful opioid-like synthetic known as W-18. Simple sample preparation routines were employed to make samples ready for analysis using an Ultivo triple quadrupole mass spectrometer LC/MS (LC/TQ) from both human serum and urine matrices.

Several separate batches were prepared and analyzed for the purpose of obtaining statistically valid analytical performance results in this research study and the resultant lower limits of quantitation, chromatographic precision, calibration linearity, range and accuracy for each synthetic opioid will be presented herein. A comparison of the analytical performance of each analyte for both urine and serum matrices will also be outlined.

Experimental

Overview:

- LC/MS analysis was performed using an Agilent 1290 UHPLC/Ultivo LC/TQ with electrospray ionization (ESI) in positive mode.
- The chromatographic column used was a Poroshell EC C18 column (2.1x50mm, 2.7 µm).
- The UHPLC mobile phases used were 0.01% formic acid and 5mM ammonium formate in water (Mobile Phase A) and 0.01% formic acid in methanol (Mobile Phase B).
- Injection volume was 5µL and the total chromatography cycle time was 7 minutes from sample to sample.
- Two MRM transitions are monitored for the analyte and a single transition for the deuterated or C13 internal standard.

The MRM transitions used and chromatographic gradient profile are outlined in tables 1 & 2, respectively.

Compound Name	Precursor Ion	Product Ion	Dwell	Fragmentor	Collision Energy
W-18 RM	422.1 m/z	174.9 m/z	10 ms	175 V	32 V
W-18 RM	422.1 m/z	110.9 m/z	10 ms	175 V	56 V
Furanyl fentanyl	375.2 m/z	188.1 m/z	10 ms	170 V	24 V
Furanyl fentanyl	375.2 m/z	105 m/z	10 ms	170 V	48 V
Valeryl fentanyl-D5	370.3 m/z	105 m/z	10 ms	180 V	48 V
para-fluorobutyrylfentanyl	369.2 m/z	188.1 m/z	10 ms	180 V	24 V
para-fluorobutyrylfentanyl	369.2 m/z	105 m/z	10 ms	180 V	52 V
Valeryl fentanyl	365.3 m/z	188.1 m/z	10 ms	180 V	24 V
Valeryl fentanyl	365.3 m/z	105 m/z	10 ms	180 V	48 V
Butyrylfentanyl-D5	356.3 m/z	188.1 m/z	10 ms	180 V	24 V
Butyrylfentanyl-D5	356.3 m/z	105 m/z	10 ms	180 V	48 V
cis-3-methyl fentanyl	351.2 m/z	202.1 m/z	10 ms	180 V	24 V
Butyrylfentanyl	351.2 m/z	188.1 m/z	10 ms	180 V	24 V
Butyrylfentanyl	351.2 m/z	105 m/z	10 ms	180 V	48 V
cis-3-methyl fentanyl	351.2 m/z	105 m/z	10 ms	180 V	48 V
Acrylfentanyl-D5	340.2 m/z	188.1 m/z	10 ms	165 V	24 V
Acrylfentanyl-D5	340.2 m/z	105 m/z	10 ms	165 V	44 V
U-47700-D6	335.2 m/z	284 m/z	10 ms	120 V	16 V
Acrylfentanyl	335.2 m/z	188.1 m/z	10 ms	165 V	24 V
Acrylfentanyl	335.2 m/z	105 m/z	10 ms	165 V	44 V
Acetylfentanyl-13C6	329.2 m/z	105 m/z	10 ms	170 V	44 V
U-47700	329.1 m/z	284 m/z	10 ms	120 V	16 V
U-47700	329.1 m/z	172.9 m/z	10 ms	120 V	36 V
Acetylfentanyl	323.2 m/z	188.1 m/z	10 ms	170 V	24 V
Acetylfentanyl	323.2 m/z	105 m/z	10 ms	170 V	44 V
N-desmethyl U-47700	315.1 m/z	284 m/z	10 ms	120 V	16 V
N-desmethyl U-47700	315.1 m/z	172.9 m/z	10 ms	120 V	36 V
Norcarfentanil	291.2 m/z	231.1 m/z	10 ms	95 V	12 V
Norcarfentanil	291.2 m/z	142 m/z	10 ms	95 V	16 V
4-ANPP-D5	286.2 m/z	105 m/z	10 ms	140 V	36 V
4-ANPP	281.2 m/z	188.1 m/z	10 ms	140 V	16 V
4-ANPP	281.2 m/z	105 m/z	10 ms	140 V	36 V
Acetyl norfentanyl-13C6	225.2 m/z	84.1 m/z	10 ms	120 V	20 V
Acetyl norfentanyl	219.1 m/z	84.1 m/z	10 ms	120 V	20 V
Acetyl norfentanyl	219.1 m/z	55.2 m/z	10 ms	120 V	44 V

Table 1 – Analytical MRM parameters used in research study.



Figure 1. Agilent Ultivo TQ.

Time (min)	% Mobile Phase A	% Mobile Phase B
0.50 min	85.00	15.00
3.00 min	50.00	50.00
5.50 min	5.00	95.00
6.00 min	5.00	95.00

Table 2 – Chromatographic gradient profile in research study.

Sample Preparation

Human Serum Matrix:

Human serum samples (250µL) were spiked with calibrators at various concentration levels, cold acetonitrile (500µL) containing the deuterated internal standard was added to affect protein precipitation and centrifuged at 5000rpm. The supernatant liquid was then further diluted (1:2) with a 10:90 methanol:water solvent mixture prior to instrument injection. A resultant dilution factor of 1:6.

Human Urine Matrix:

Negative urine (UTAK) was spiked with internal standards and specified calibration levels, centrifuged at 5000rpm (4°C) for 10 minutes, then 100µL of the supernatant was made up to 1mL in the sample vial by the addition of 900µL de-ionized water. A resultant dilution factor of 1:10.

Calibration Concentrations in both Serum & Urine Matrices:

Cal Level / Concentration	Cal Level / Concentration
L1 / 1pg/mL	L7 / 1ng/mL
L2 / 5pg/mL	L8 / 5ng/mL
L3 / 10pg/mL	L9 / 10ng/mL
L4 / 50pg/mL	L10 / 50ng/mL
L5 / 100pg/mL	L11 / 100ng/mL
L6 / 500pg/mL	L12 / 500ng/mL

Table 3 – Cal Level concentrations used in research study.

Results and Discussion

Human Serum:

Excellent linearity and reproducibility were obtained for human serum extracts typically within an actual concentration range from 10 or 50pg/ml to 500ng/ml (50/250fg on-column to 2500pg on-column) for each synthetic opioid analyte with a linearity coefficient of >0.997 for three batches prepared for this research study. Precision data observed over the three batches resulted with a %RSD variation of < 7% across all calibration levels in this research study.

Human Urine:

Results for the diluted urine samples yielded an actual concentration range from 50 or 100pg/ml to 500ng/ml (250/500fg on-column to 2500pg on-column) for each synthetic opioid with a linearity coefficient of >0.996 over three batches prepared for this research study. Precision data observed for n=3 batches resulted with a %RSD < 9% across all calibration levels.

Results

Analyte	Actual LLOQ (Urine) pg/ml	Actual LLOQ (Serum) pg/ml
4-ANPP	100	10
3-methylfentanyl	100	50
Acetylfentanyl	50	10
Acetylnorfentanyl	100	50
Acrylfentanyl	50	10
Butyrylfentanyl	100	50
Carfentanil	50	10
Furanyl fentanyl	50	50
Para-fluorobutyrylfentanyl	50	50
Norcarfentanil	100	50
N-desmethyl U-47700	100	50
Valeryl fentanyl	100	50
U-47700	100	50
W-18 RM	50	50

Table 4 – LLOQ concentrations obtained from serum & urine.

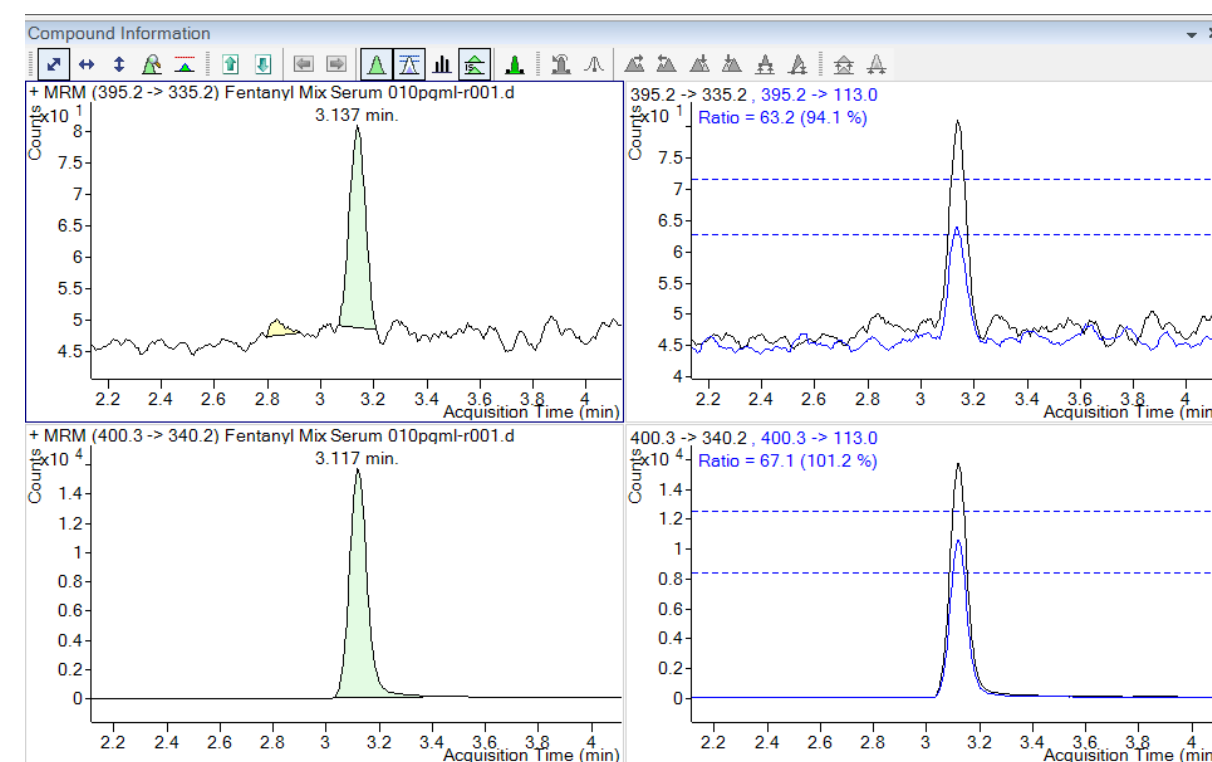


Figure 2 – Carfentanil LLOQ in Serum @ 10pg/mL.

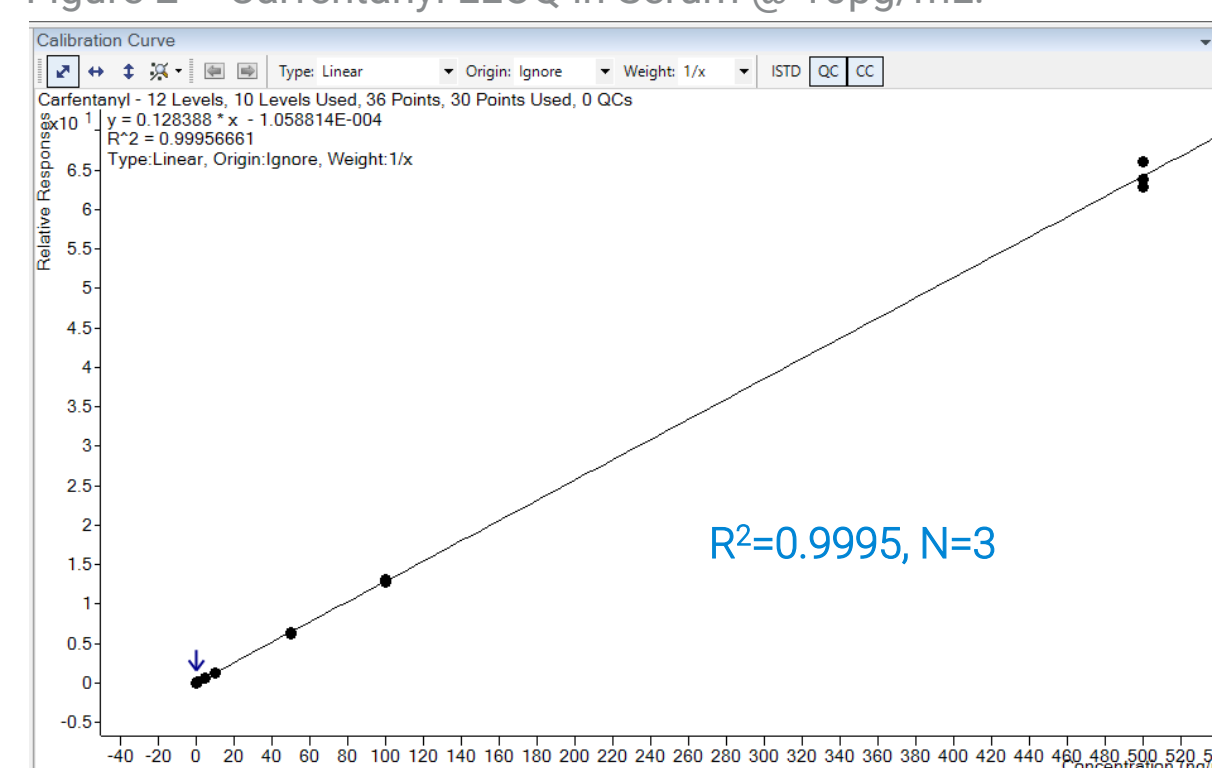


Figure 3 – Carfentanil calibration range 10pg/mL to 500ng/mL.

Conclusions

This research project demonstrates that the performance of the novel Ultivo LC/TQ with the analytical methodology described herein is producing excellent linearity, precision and analytical sensitivity across the range of 10 or 50pg/ml through 500ng/ml for each respective synthetic opioid in human serum and analytical sensitivity across the range of 50 or 100pg/ml through 500ng/ml for the respective synthetic opioid in human urine.

Future work would be needed to eliminate potential matrix or drug interferences to this analytical method and for additional synthetics to be included as and when they are discovered and standards made available.