Application Note

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The detection and analytical confirmation of synthetic fentanyl analogues in human urine and serum using an Agilent Ultivo LC/TQ

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Abstract

This research study develops a sensitive, robust, and relatively fast targeted analytical method for the quantitation of 12 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 used to make samples, from both human serum and urine matrices, ready for analysis using an Agilent Ultivo triple quadrupole mass spectrometer LC/MS (LC/TQ). This study outlines a comparison of the analytical performance of each analyte for both urine and serum matrices.

Introduction

In a fast and ever-changing environment of clandestine synthetic drug production, the need for new analytical methodologies to keep pace with measuring such analytes and metabolites is of utmost importance. The fentanyl-analogue opioid type are an emerging class of opioids.

A targeted analytical method was developed using Agilent Masshunter Optimizer automated software with an Agilent Ultivo Triple Quadrupole LC/MS for 12 synthetic fentanyl opioids, the synthetic precursor molecule 4-ANPP, and a similar opioid-like synthetic known as W-18 RM. Representative human sample matrices of urine and serum were prepared using simple sample preparation techniques, and the analytical research method was tested for linearity, sensitivity, and precision.

To obtain statistically valid analytical performance results, this study prepared and analyzed several separate batches. The resultant lower limits of quantitation (LLOQ), chromatographic precision, calibration linearity, range, and accuracy for each synthetic opioid are presented herein. Comparisons of the analytical performance of each analyte for both urine and serum matrices are also outlined.

Experimental

LC Configuration and parameters

Configuration	
Instruments	Agilent 1290 Infinity II high speed pump (G7120A)
	Agilent 1290 Infinity autosampler (G4226A)
	Agilent 1290 Infinity autosampler thermostat (G1330B)
	Agilent 1290 Infinity II multicolumn thermostat (G7116B)
Needle wash	100 % Methanol
Autosampler temperature	4 °C
Injection volume	5 µL
Analytical column	Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm, LC column (p/n 699775-902)
Column temperature	55 °C
Mobile phase A	0.01 % Formic acid and 5 mM ammonium formate in water
Mobile phase B	0.01 % Formic acid in methanol
Flow rate	0.4 mL/min
Gradient	Time (min) %B 0 10 0.5 15 3.0 50 5.5 95 6.0 95
Total cycle time	7 minutes

Triple quadrupole mass spectrometer configuration and parameters

Configuration	
Instrument	Agilent Ultivo Triple Quadrupole Mass Spectrometer with electrospray ionization (ESI)
MS/MS mode	MRM
Ion mode	Positive
Drying gas temperature	325 °C
Drying gas flow	9 L/min
Nebulizer pressure	35 psi
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Nozzle voltage	0 V
Capillary voltage, positive	3,500 V
MS1/MS2 resolution	0.7/0.7 Unit
Dwell time	10 ms

Figure 1. Agilent Ultivo Triple Quadrupole Mass Spectrometer.

Chemicals and reagents

Human serum and urine, used for matrix matched calibrators, were obtained from Golden West Biologicals and UTAK Laboratories (Valencia, CA), respectively. Standards and internal standards were sourced from Sigma-Aldrich (St. Louis, MO) and Cerilliant Corporation (Round Rock, TX). Sample preparation and LC solvents were from Sigma-Aldrich (St. Louis, MO) and Honeywell Riedel-de Haën (Seelze, Germany).

Sample preparation

Human serum samples (250 µL) were spiked with calibrators at various concentration levels, then cold acetonitrile (500 µL) containing the deuterated internal standard was added to affect protein precipitation, and centrifuged at 5,000 rpm. The supernatant liquid was then further diluted (1:2) with a 10:90 methanol:water solvent mixture prior to instrument injection. The resultant dilution factor was 1:6.

Negative urine was spiked with internal standards and specified calibration levels, centrifuged at 5,000 rpm at 4 °C for 10 minutes, then 100 µL of the supernatant was made up to 1 mL in the sample vial by the addition of 900 µL de-ionized water. The resultant dilution factor was 1:10. Twelve calibration levels ranged from 1 pg/mL to 500 ng/mL.

Data analysis

Data were acquired and analyzed using Agilent MassHunter software suite C.01.00 for data collection from the Ultivo. MS/MS transitions were obtained using MassHunter Optimizer software to determine optimal precursor and product ions, fragmentor voltages, and collision energies upon injection of a neat solution of each individual compound or internal standard.

MS/MS Compound information for analytes and internal standards

Compound	ISTD	Precursor ion	Product ion	Fragmentor	CAV	Collision energy
W-18 RM		422.1 *m/z*	174.9 *m/z*	175 V	9 V	32 V
W-18 RM		422.1 *m/z*	110.9 *m/z*	175 V	9 V	56 V
Furanyl fentanyl		375.2 *m/z*	188.1 *m/z*	170 V	9 V	24 V
Furanyl fentanyl		375.2 *m/z*	105.0 *m/z*	170 V	9 V	48 V
Valeryl fentanyl-D5	✓	370.3 *m/z*	105.0 *m/z*	180 V	9 V	48 V
p-fluorobutyrylfentanyl		369.2 *m/z*	188.1 *m/z*	180 V	9 V	24 V
p-fluorobutyrylfentanyl		369.2 *m/z*	105.0 *m/z*	180 V	9 V	52 V
Valeryl fentanyl		365.3 *m/z*	188.1 *m/z*	180 V	9 V	24 V
Valeryl fentanyl		365.3 *m/z*	105.0 *m/z*	180 V	9 V	48 V
Butyrylfentanyl-D5	✓	356.3 *m/z*	188.1 *m/z*	180 V	9 V	24 V
Butyrylfentanyl-D5	✓	356.3 *m/z*	105.1 *m/z*	180 V	9 V	48 V
cis-3-methyl fentanyl		351.2 *m/z*	202.1 *m/z*	180 V	9 V	24 V
Butyrylfentanyl		351.2 *m/z*	188.1 *m/z*	180 V	9 V	24 V
Butyrylfentanyl		351.2 *m/z*	105.0 *m/z*	180 V	9 V	48 V
cis-3-methyl fentanyl		351.2 *m/z*	105.0 *m/z*	180 V	9 V	48 V
Acrylfentanyl-D5	✓	340.2 *m/z*	188.1 *m/z*	165 V	9 V	24 V
Acrylfentanyl-D5	✓	340.2 *m/z*	105.0 *m/z*	165 V	9 V	44 V
U-47700-D6	✓	335.2 *m/z*	284.0 *m/z*	120 V	9 V	16 V
Acrylfentanyl		335.2 *m/z*	188.1 *m/z*	165 V	9 V	24 V
Acrylfentanyl		335.2 *m/z*	105.0 *m/z*	165 V	9 V	44 V
Acetylfentanyl-$^{13}C_6$	✓	329.2 *m/z*	105.0 *m/z*	170 V	9 V	44 V
U-47700		329.1 *m/z*	284.0 *m/z*	120 V	9 V	16 V
U-47700		329.1 *m/z*	172.9 *m/z*	120 V	9 V	36 V
Acetylfentanyl		323.2 *m/z*	188.1 *m/z*	170 V	9 V	24 V
Acetylfentanyl		323.2 *m/z*	105.1 *m/z*	170 V	9 V	44 V
N-desmethyl U-47700		315.1 *m/z*	284.0 *m/z*	120 V	9 V	16 V
N-desmethyl U-47700		315.1 *m/z*	172.9 *m/z*	120 V	9 V	36 V
Norcarfentanil		291.2 *m/z*	231.1 *m/z*	95 V	9 V	12 V
Norcarfentanil		291.2 *m/z*	142.0 *m/z*	95 V	9 V	16 V
4-ANPP-D5	✓	286.2 *m/z*	105.1 *m/z*	140 V	9 V	36 V
4-ANPP		281.2 *m/z*	188.1 *m/z*	140 V	9 V	16 V
4-ANPP		281.2 *m/z*	105.0 *m/z*	140 V	9 V	36 V
Acetyl norfentanyl-$^{13}C_6$	✓	225.2 *m/z*	84.1 *m/z*	120 V	9 V	20 V
Acetyl norfentanyl		219.1 *m/z*	84.1 *m/z*	120 V	9 V	20 V
Acetyl norfentanyl		219.1 *m/z*	55.2 *m/z*	120 V	9 V	44 V

Results and discussion

Excellent linearity and reproducibility were obtained for human serum extracts, typically within an actual concentration range from 10 or 50 pg/mL to 500 ng/mL (50/250 fg on-column to 2,500 pg on-column) for each synthetic opioid analyte with a linearity coefficient of >0.997 for three batches prepared. Precision data observed over the three batches resulted in a %RSD variation of <7 % across all calibration levels.

Results for the diluted urine samples yielded an actual concentration range from 50 or 100 pg/mL to 500 ng/mL (250/500 fg on-column to 2,500 pg on-column) for each synthetic opioid with a linearity coefficient of >0.996 over three batches prepared. Precision data observed for n = 3 batches resulted in a %RSD <9 % across all calibration levels.

The method used the abilities of LC/TQ to detect multiple compounds spanning a wide range of concentrations simultaneously. The calibration concentrations ranged from 12 ng/mL to 200,000 ng/mL for the various analytes. Top concentrations ranged from 1.5 to 200 µg/mL, and are given, along with curve fit parameters, in Table 1. R^2 values were all >0.997, with some compounds displaying a linear response across their concentration range, and others requiring a quadratic fit.

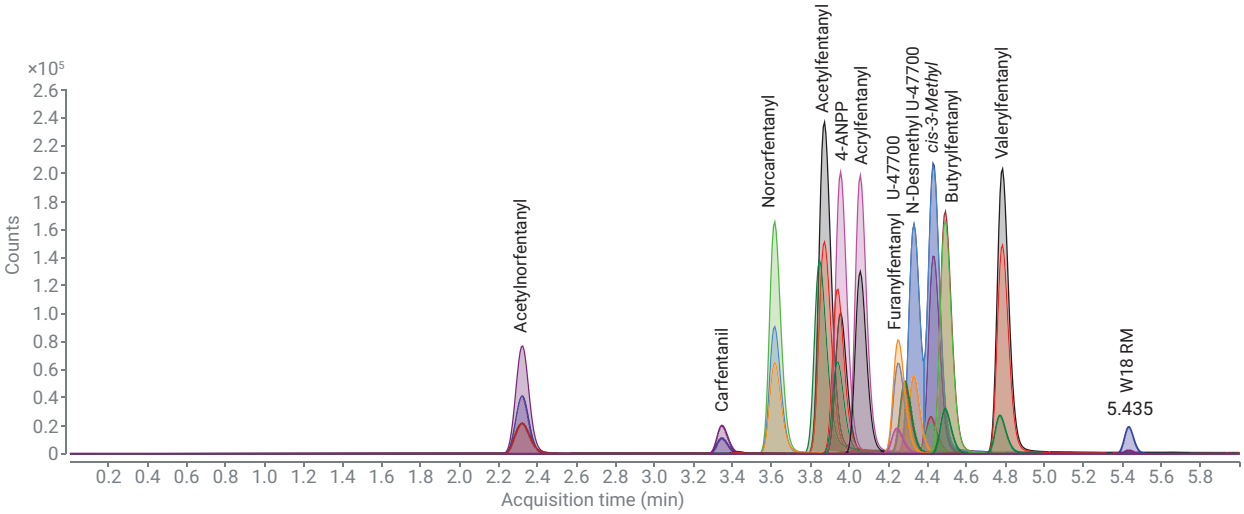

Figure 2. Example MRM chromatogram showing an overlay of the synthetic fentanyl compounds.

Table 1. LLOQ concentrations obtained from serum and urine, with the %RSD at 50 pg/mL and 100 pg/mL in serum and urine, respectively.

Analyte	Actual LLOQ (Serum) (pg/mL)	Analyte CV at 50 pg/mL in serum (n = 3)	Actual LLOQ (Urine) (pg/mL)	Analyte CV at 100 pg/mL in urine (n = 3)
4-ANPP	10	1.94 %	100	15.33 %
3-methylfentanyl	50	4.92 %	100	2.94 %
Acetylfentanyl	10	5.42 %	50	6.07 %
Acetylnorfentanyl	50	6.35 %	100	3.59 %
Acrylfentanyl	10	4.21 %	50	15.32 %
Butyrylfentanyl	50	3.33 %	100	9.61 %
Carfentanil	10	4.36 %	50	7.31 %
Furanylfentanyl	50	5.97 %	50	5.82 %
Para-fluorobutyrylfentanyl	50	7.96 %	50	11.62 %
Norcarfentanyl	50	14.94 %	100	2.06 %
N-desmethyl U-47700	50	11.56 %	100	17.70 %
Valerylfentanyl	50	6.29 %	100	3.79 %
U-47700	50	4.31 %	100	6.74 %
W-18 RM	50	6.33 %	50	8.30 %

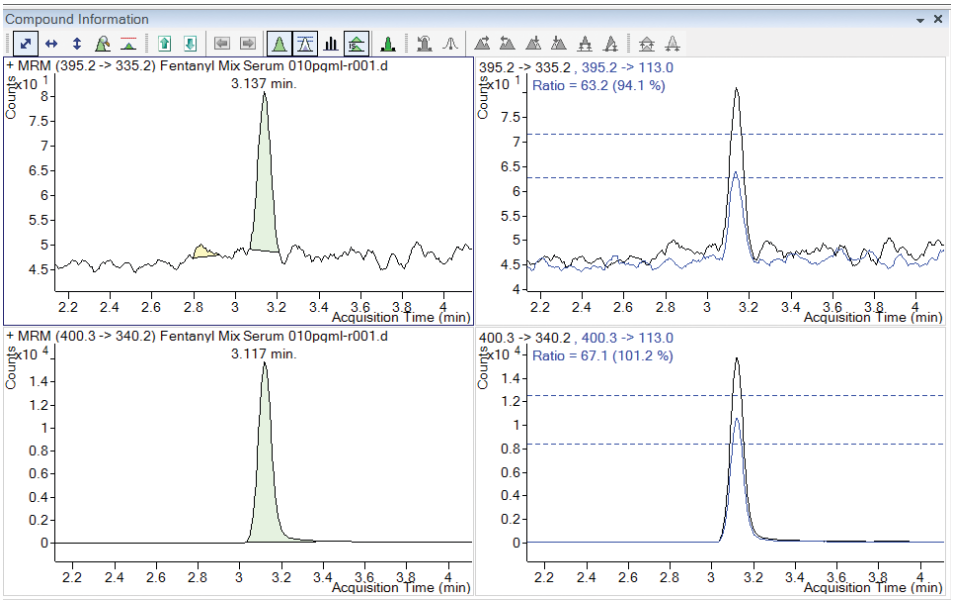

Figure 3. Carfentanyl LLOQ in serum at 10 pg/mL.

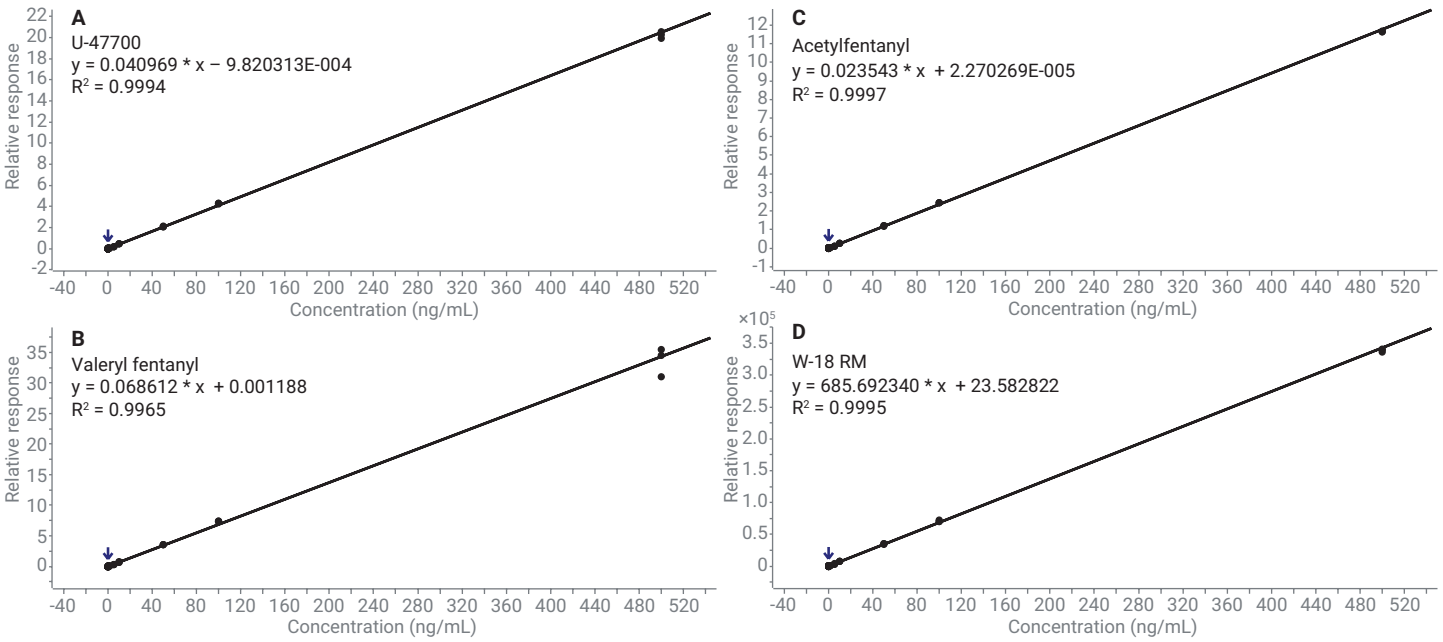

Figure 4. Example calibration curves of compounds in serum.

Conclusion

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

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

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