Poster Reprint

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Analysis of Synthetic Fentanyl Opioids in Serum using Captiva EMR-Lipid Sample Preparation by LC-QTOF

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Introduction

In a rapidly changing environment with new synthetic drugs appearing almost weekly, there is an analytical need to confidently identify these analytes in a timely manner. The use of high resolution accurate mass technology coupled with a novel sample preparation using Agilent's Captiva EMR-Lipid allows for highly specific and selective identification of these emerging compounds in serum matrix. Captiva EMR–Lipid provides highly selective and efficient lipid/matrix removal without unwanted analyte loss. The novel EMR-Lipid technology removes lipids based on a combination of size exclusion and hydrophobic interaction. Effective lipid removal assures minimal ion suppression of target analytes, which significantly improves method reliability and ruggedness. This novel sample preparation approach allows for lower limits of detection and quantitation as well as better linearity across the entire dyn

Time (min)	% Mobile Phase B
0.0	40
3.0	40
4.5	95
6.0	95

Table 1. UHPLC Conditions

LC/MS analysis was performed using an Agilent 1290 UHPLC/ 6545 QTOF with electrospray ionization (ESI) in positive mode

Column	Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm
Injection Volume	1 µL
Mobile Phase A	H_2O + 5 mM Ammonium Formate + 0.01% Formic Acid
Mobile Phase B	Methanol + 0.01% Formic Acid
Needle Wash	50:20:20:10 IPA:MeOH:ACN:H2O
Autosampler Temp	5 °C
Column Temp	55 °C
Flow Rate	0.35 mL/min
Stop Time	6.0 min
Post Time	1.0 min

Table 2. 6545 AJS Source Conditions

4-ANPP *(synthetic precursor)	Acetylnorfentanyl	Carfentanil	Norcarfentanyl	U-47700
3-methylfentanyl	Acrylfentanyl	Furanylfentanyl	Para-fluorobutyrylfentanyl	W-18 RM
Acetylfentanyl	Butyrylfentanyl	N-desmethyl U-47700	Valerylfentanyl	

Table 3. List of 14 Analytes in Study

Experimental

Sample Preparation: For post-spiked samples, blank biological matrix was stripped of proteins and lipids by adding 800ul of 1% formic acid in acetonitrile and 200ul of matrix into a 1ml Captiva EMR cartridge. Mixture was allowed to stand for 5 minutes (passive mixing) and was then passed through the sorbent bed under low vacuum. A secondary elution step consisting of 200ul ACN/water (80:20) was passed through the sorbent bed and combined with the previous eluant. For the calibration curve, depleted matrix was spiked with fentanyl analogues at the following levels: 0.1 ng/ml, 1 ng/ml, 10 ng/ml, 50 ng/ml, 125 ng/ml, 250 ng/ml and 500 ng/ml. For pre-spiked samples, fentanyl analogues were added directly to matrix and allowed to equilibrate for 30 minutes. 200ul of spiked matrix were then added to 800ul of 1% formic acid in ACN inside a 1ml Captiva EM processed as described above.

Captiva EMR eluant was dried under nitrogen and reconstituted in starting gradient conditions.

Size exclusion – Unbranched hydrocarbon chains (lipids) enter the sorbent; bulky analytes do not

Sorbent chemistry – Lipid chains that enter the sorbent are trapped by hydrophobic interactions

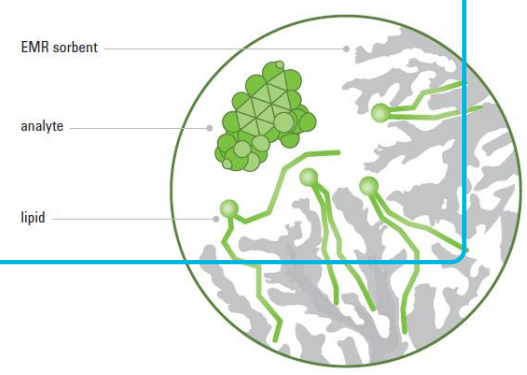

Figure 1. EMR sorbent technology effectively traps lipids through two mechanisms.

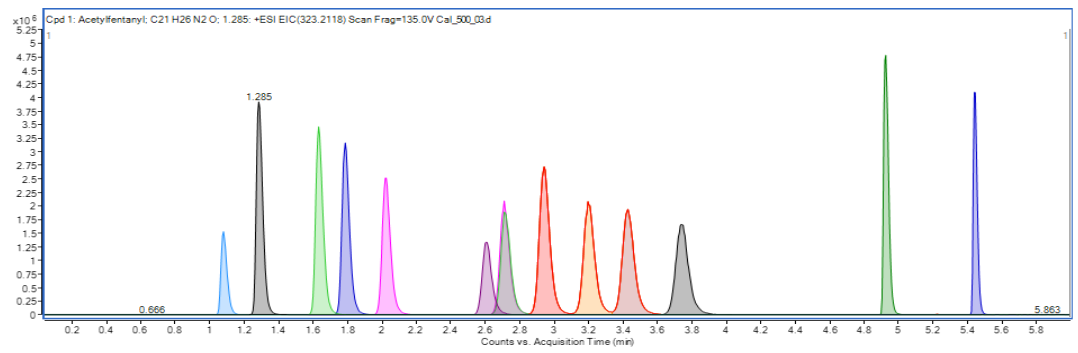

Figure 2. Overlaid EICs of the LC Separation of the 14 Analytes in Study

Experimental

Parameters	
Ion mode	AJS ESI, positive
Gas temperature	325 ºC
Drying gas flow	9 L/min
Nebulizer gas	35 psi
Sheath gas temperature	350ºC
Sheath gas flow	11 L/min
Capillary voltage	3500 V
Nozzle voltage	0 V

MS Parameters	
Acquisition Mode	MS
Fragmentor Voltage	135 V
MS Scan Rate (spectra/sec)	3

MS/MS Parameters	
Acquisition Mode	Targeted MS\MS
Isolation Width	Narrow (1.3 amu)
Fragmentor Voltage	135 V
MS Scan Rate (spectra/sec)	10
MS\MS Scan Rate (spectra/sec)	3
Fixed Collision Energies (V)	10,20,40

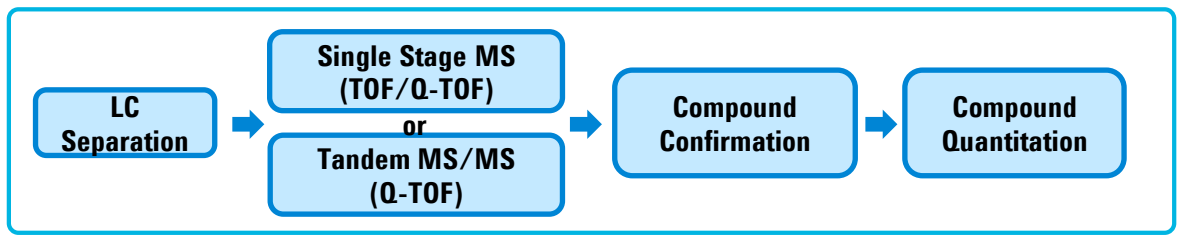

Figure 3. QTOF targeted/untargeted screening workflow which begins with an LC separation, followed by data acquisition in single stage MS or tandem MS/MS mode, followed by compound confirmation using Mass Hunter Qualitative Software in conjunction with PCDL (Personal Compound Database and Library), and compound quantitation.

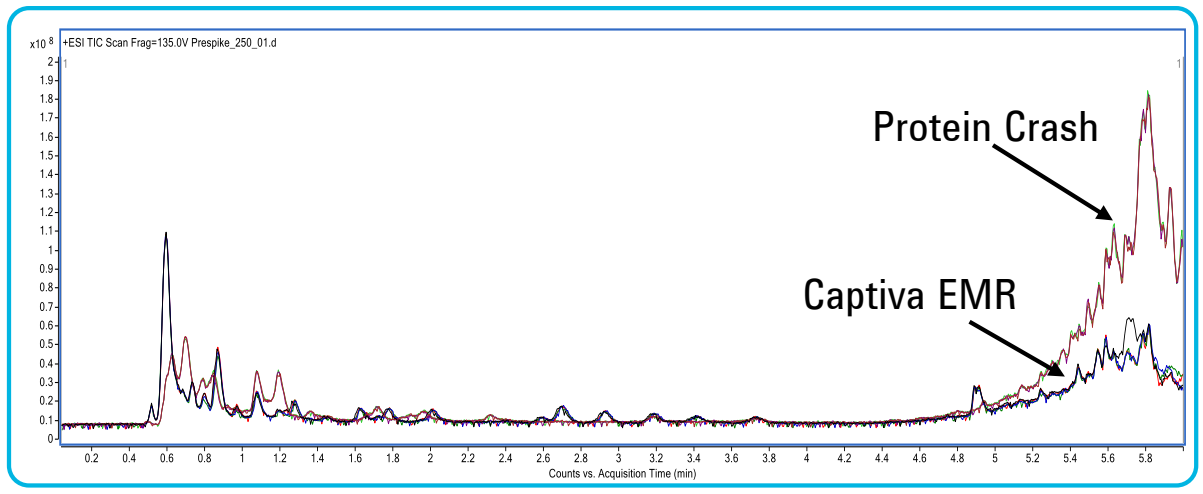

Figure 4. Overlaid TICs of a protein crash sample preparation and Captiva EMR sample preparation demonstrating the matrix removal of lipids using captiva EMR.

Qualitative Results

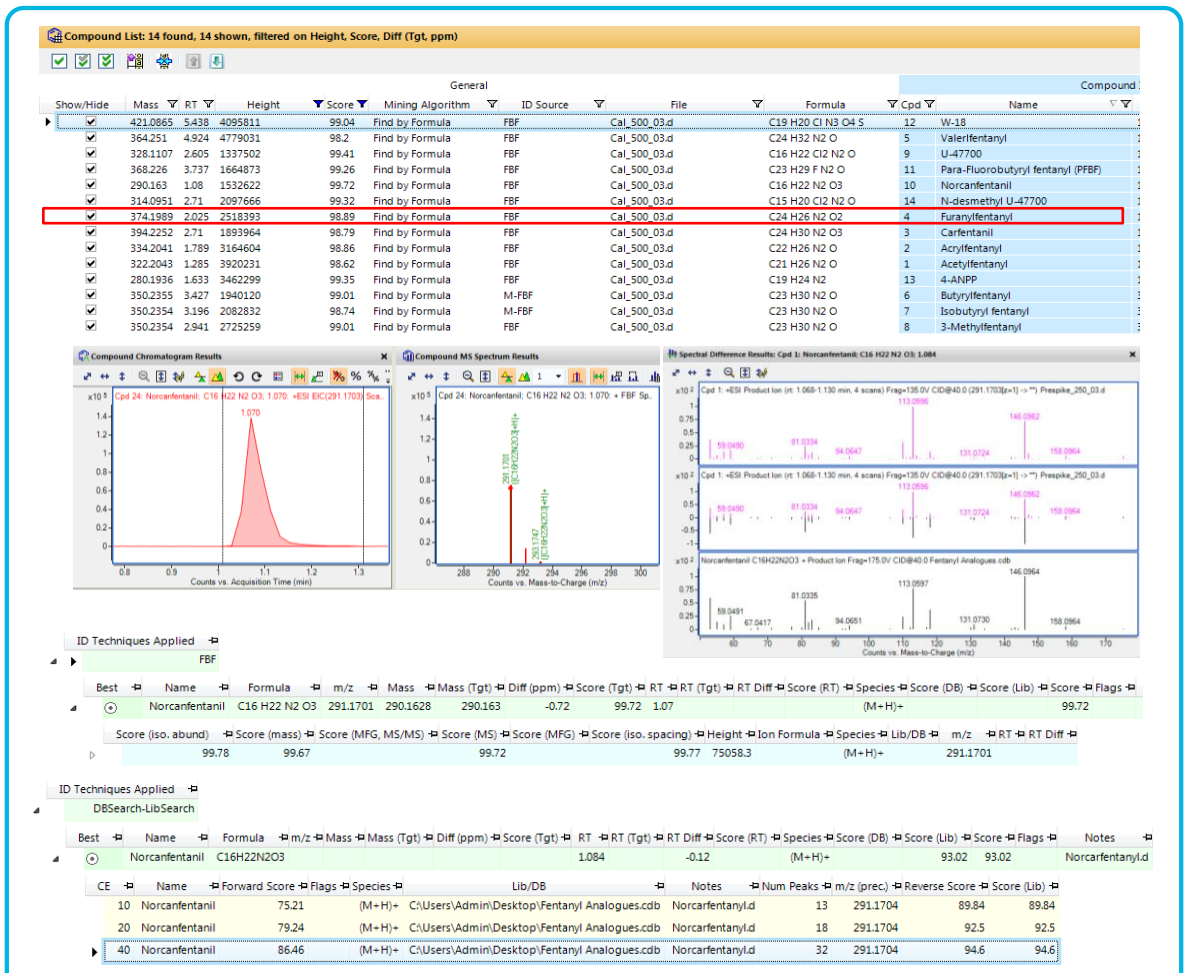

Figure 5. Example of Mass Hunter Qualitative results for a screening workflow demonstrating good hits for all 14 analytes of interest. Using norcarfentanil as an example, the combined results and subsequent scoring that warrant a confident hit are accurate mass, retention time, isotopic spacing and abundance, MS/MS fragmentation pattern match to a library.

Results and Discussion

High resolution accurate mass LC-QTOF-MS technology was used to identify, quantify and characterize 14 emerging fentanyl analogues in serum. Employing a novel sample preparation technique of Captiva EMR-Lipid for optimal sample cleanup in conjunction with the quadrupole-time-of-flight mass spectrometer's (Q-TOF) quadrupole, the combined fragmentation pattern with accurate mass, retention time and isotopic fidelity.

Quantitative Results

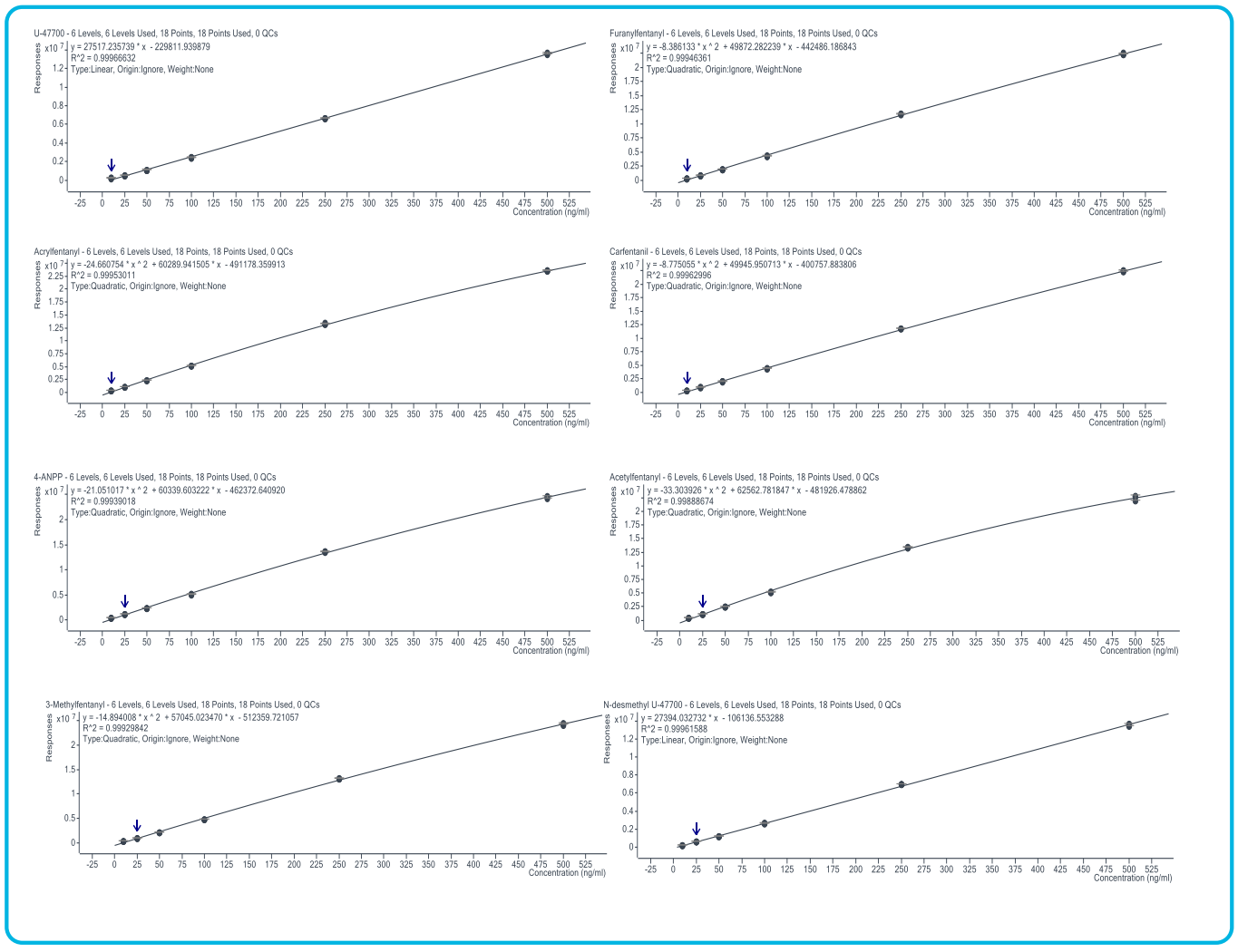

Figure 6. Examples of Quantitative Calibration Curves

Calibration Curves

Majority of calibration curves were linear across the dynamic range from 10 to 500 ng/mL, for few of analytes quartic fit was used and R^2 values >0.99. Examples of calibration curves are shown in Figure 6.

Conclusions

During this research study, a qualitative and quantitative, robust, highly sensitive, specific and relatively fast LC/MS/MS analytical screening method was developed for the identification of 12x synthetic fentanyl opioids, 4-ANPP the synthetic precursor molecule and a similar powerful opioid-like synthetic known as W-18..This project demonstrates the unique capabilities of QTOF technology as an analytical technique as described herein to produce accurate identification of arising fentanyl opioids in serum matrix using the unique Captiva EMR-Lipid sample preparation technique for highly sensitive and accurate quantitation.

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