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LC-MS/MS detection of fentanyl and related synthetic opioids in biological matrices

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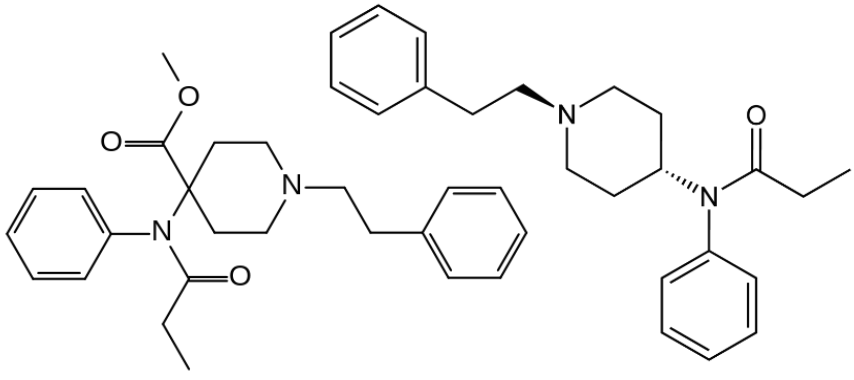
Novel Aspects

A single LC-MS/MS method was developed and optimized to identify and determine a limit of detection (LOD) for several synthetic opioids. including fentanyl , and several fentanyl analogues as well as a few of their major metabolites in blood and urine matrices.

Introduction

The potency of these compounds result in small concentrations being detected in real-world samples, thus requiring methods utilized by a forensic or clinical laboratory to be very sensitive. Hyphenated techniques such liquid chromatography-tandem mass spectrometry (LC-MS/MS) can achieve this needed sensitivity through multiple reaction monitoring (MRM). The Shimadzu LCMS-8060 features a heated dual ionization source (DUIS) coupled with a along with ultrafast MRM acquisition software and polarity switching for increased accuracy, sensitivity and robustness.

DUIS combines electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) by using the ESI source and the corona needle used in APCI. The development process involved MRM optimization, MS source optimization and finally column selection for a method that successfully separated and identified a mixture of synthetic opioids. This analytical method when used in combination with a validated solid phase extraction procedure, achieved sub-ng/mL detection limits in blood and urine samples.



Chemical Structure of Fentanyl (top) and Carfentanyl (bottom)

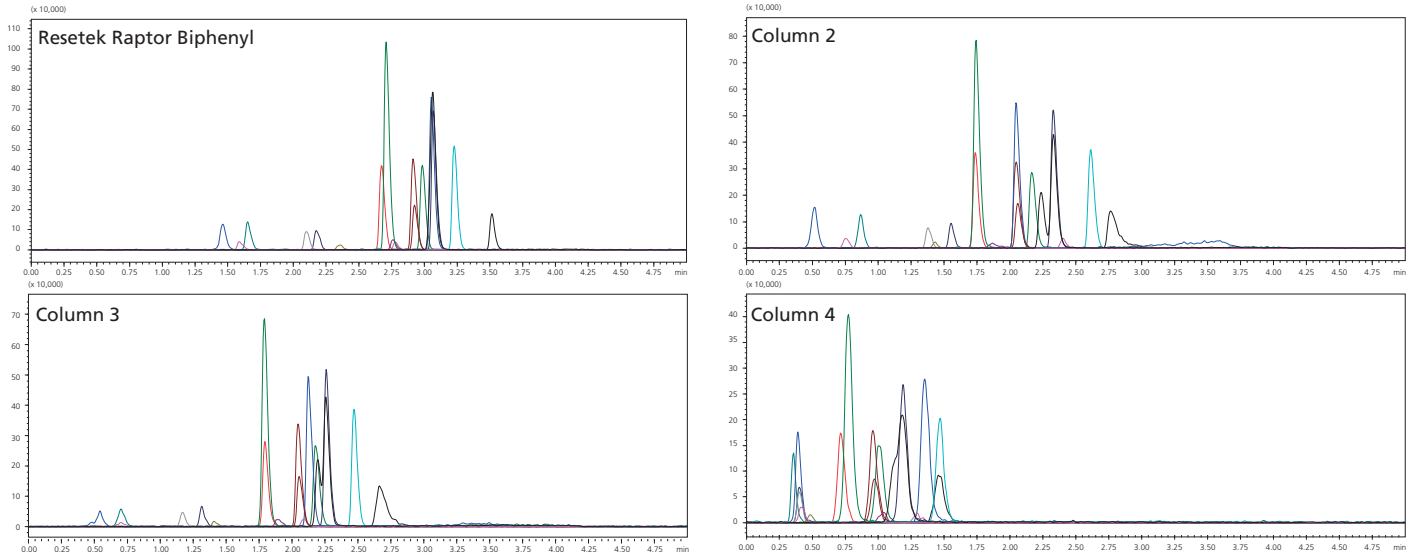
Method

Method development began with optimizing the compounds using flow injection analysis and LabSolutions MRM Optimization Wizard. The MRM transitions were fully optimized to enhance sensitivity of all of the compounds in the mixture.
The LC flow rate was set at 0.5 mL/min with a 50:50 mixture of mobile phase A and B. The mass spectrometer source parameters and LC conditions are listed below. The optimized MRM transitions for each compound are listed in results section.
Following the MRM optimization, four LC columns were analyzed using a five minute LC gradient starting at 20% mobile phase B increasing to 80%.

Chromatography Parameters	
Column	: Restek Raptor Biphenyl
Column Temp	: 40 °C
Autosampler Temp	: 15 °C
Injection Volume	: 30 µl
Mobile Phase A	: H2O: MeOH w/ Formic Acid
Mobile Phase B	: MeOH w/ Formic Acid
Injection Solvent	: 80 MPA: 20 MPB
Flow Rate	: 0.5 mL/min
LCMS-8060 Parameters	
Nebulizing Gas	: 1.5 L/min
Interface Temp	: 400 °C
DL Temp	: 200 °C
Heat Block Temp	: 250 °C
Drying Gas Flow	: 5 L/min
Heating Gas Flow	: 15 L/min
Interface Temp	: 400 °C
Ion Source	: DUIS

Serial dilutions (1:5) of a 1000 pg/mL solution were used to create a 6 point calibration curve in certified blank urine and certified blank whole blood. Fentanyl-d5 was used as the internal standard.
The samples were extracted for analysis using a simple solid phase extraction. The sample was added to the cartridge after the SPE cartridge was conditioned. The wash solutions were added to the cartridge and the compounds were eluted off using a mixture of DCM/IPA/NH_4OH. Once eluted the samples were dried down and reconstituted in a 80:20 Water:MeOH mixture. The calibration curve was prepared in triplicate to along with two blank samples; a certified matrix blank spiked with internal standard and a solvent blank, and a single unknown blood sample. The case sample was run against both matrix curves.

Results- Columns and LOD



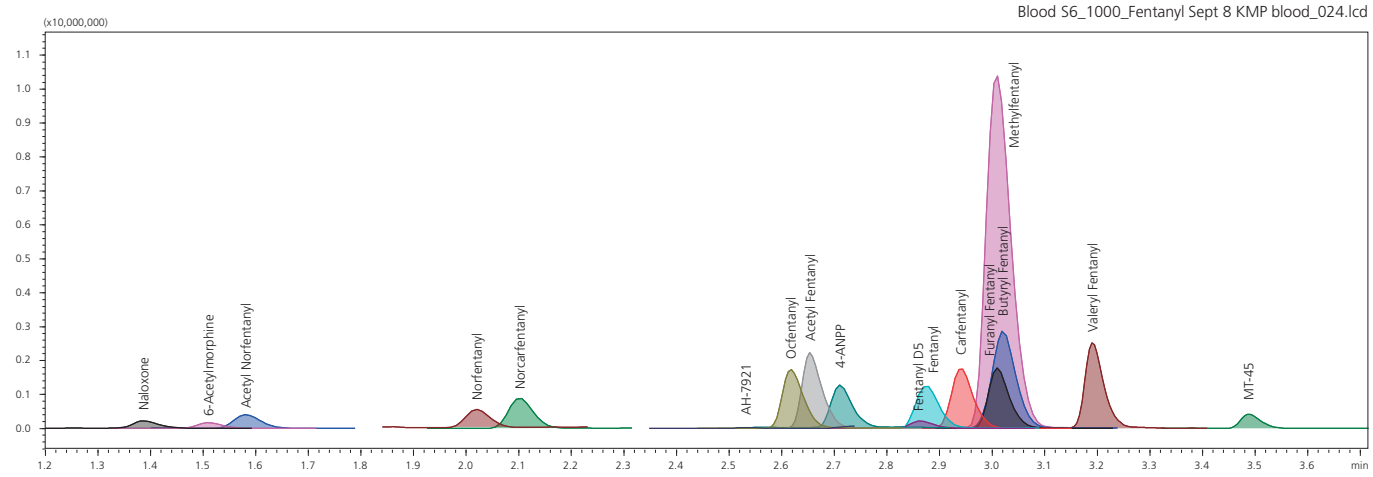
The Restek Raptor Biphenyl was chosen for the study after a comparison between peak shape, peak separation, and sensitivity on column was made. The overall chromatograms for the four columns are represented above.

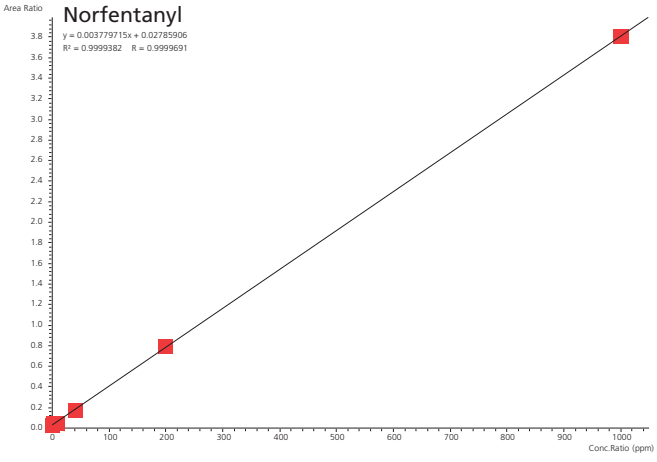
Compound	Quant Ion	Qual Ion	Blood LOD (pg/mL)	Urine LOD (pg/mL)
Methylfentanyl	351.10>105.10	351.10>202.25	1.6	40
6-Acetylmorphine	328.00>181.10	328.00>165.10	1.6	40
Naloxone	328.00>310.20	328.00>212.10	200	8
Fentanyl	337.30>188.20	337.30>105.10	8	8
Acetyl Fentanyl	323.10>188.20	323.10>105.15	1.6	40
Butyryl Fentanyl	351.10>105.25	351.10>188.20	8	40
Norfentanyl	233.00>84.05	233.00>55.20	0.32	8
4-ANPP	281.10>188.20	281.10>105.10	8	8
Valeryl Fentanyl	365.10>188.20	365.10>105.05	8	8
Ocfentanyl	371.10>188.20	371.10>355.15	1.6	1.6
MT-45	349.30>181.15	349.30>166.10	1.6	40
Furanyl Fentanyl	375.10>188.20	375.10>105.15	1.6	8
Carfentanyl	395.10>335.30	395.10>113.25	1.6	8
Norcarfentanyl	291.10>231.30	291.10>146.25	0.32	40
Fentanyl D5	342.10>188.20	342.10>105.25		

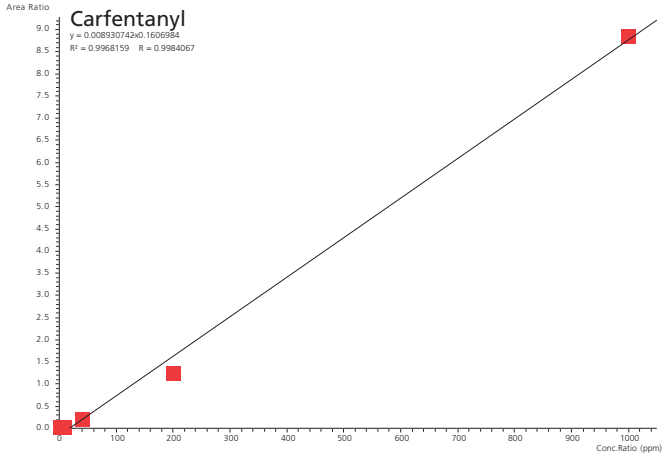
MRM optimization resulted in the above transitions. These transitions were used for both the urine and blood testing. The limit of detection (LOD) for each compound was determined by the lowest injection level that had a signal to noise calculation of greater than 3. The LOD of the compounds ranged from 0.32 pg/mL to 8 pg/mL in blood with the exception of Naloxone which was 200 pg/mL. The LODs in urine were slightly higher than in blood and ranged from 1.6 to 40 pg/mL.

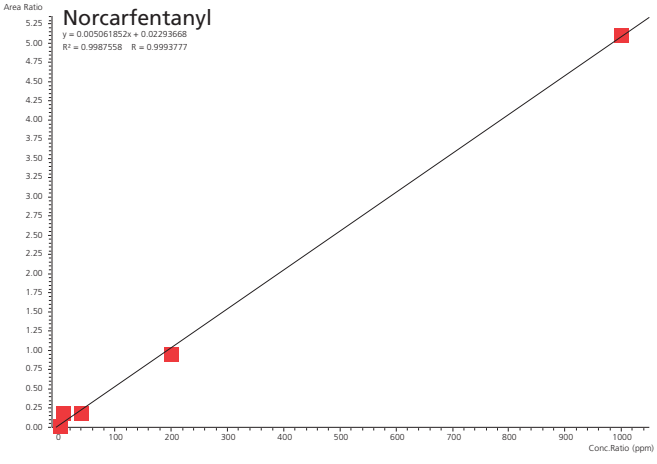
Results- Calibration Curve

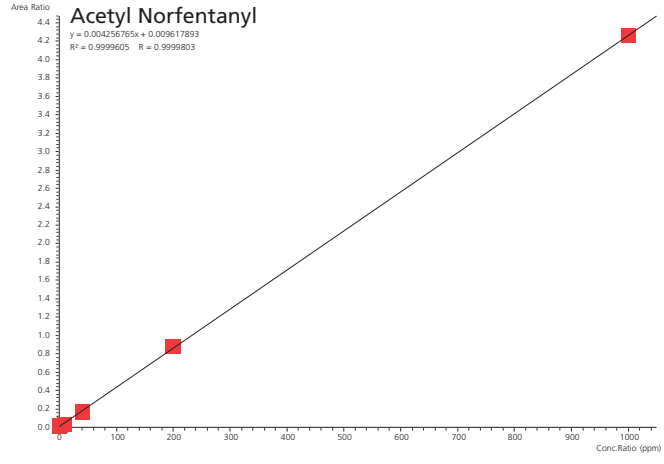
The below chromatogram is of the highest calibration point (1000 pg/mL) in the extracted matrix curve. Below are the chromatogram are four out of fourteen calibration curves in urine. All of the compounds were linear over the range of 0.32 to 1000 pg/mL with an R2 value greater than 0.998.











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Conclusion

The analytical conditions shown in this application note have demonstrated the ability of the LCMS-8060 to detect Fentanyl, Fentanyl analogs and metabolites at picogram per milliliter levels. The continuation of this study will include linear ranges, limit of quantitation of the compounds as well as precision and accuracy of the method and extraction.

For research purposes only. Not for use in diagnostic procedures.

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