

# APPLICATIONS

## Extraction and Analysis of Fentanyl and Analogs from Whole Blood Using a Kinetex<sup>®</sup> F5 LC Column

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### Introduction

Fentanyl and fentanyl analogs are high potency synthetic opioids that are 80-100x more powerful than morphine. Although fentanyl use is strictly regulated, new fentanyl analogs keep emerging with molecular structures that are closely related, making separation, identification, and quantitation difficult for analysts. This method explores the identification of 26 compounds and quantitation of 24 compounds using multiple sample preparation techniques to clean-up the whole blood matrix. A core-shell LC column was utilized for its high efficiency and selective properties and the F5 phase was chosen for separating geometric and structural isomers.

### Methods

#### Sample Pre-treatment

500  $\mu$ L of a whole blood sample was lysed osmotically with 500  $\mu$ L of DI Water and internal standard was added. The samples were vortexed for 7 seconds and they sat for 10 minutes. Protein precipitation was accomplished by drop-wise addition of 1.5 mL of ice cold methanol/acetonitrile (10:90) while vortexing. The samples were then incubated for 10 min in the freezer and then centrifuged at 3,500 rpm (2,800 xg) for 10 min. The supernatants were poured into new 16 x 125 mm test tubes followed by the addition of 3 mL DI water and vortexed for 7 sec.

### SPE Conditions

- Cartridge:** Strata<sup>®</sup> Screen C (55  $\mu$ m, 70  $\text{\AA}$ ), 200 mg/6 mL  
**Part No.:** [8B-S016-FCH](#)  
**Condition:** 3 mL of Methanol  
**Equilibrate:** 2 mL of DI water  
**Load:** Pre-treated samples were loaded onto the cartridge under 2 psi  
**Wash 1:** 2 mL of DI water (All 3 wash steps were eluted under 5 psi positive pressure)  
**Wash 2:** 1.5 mL of 1.0 M Acetic acid  
**Wash 3:** 2 mL of Methanol  
**Dry:** Cartridge for 10 minutes  
**Elute:** 2x 1.5 mL of Dichloromethane/2-propanol/Ammonium hydroxide (78:20:2) under 5 psi. Full flow was applied at the end of each elution step for 30 sec.  
**Dry Down:** Evaporated to dryness  
**Reconstitute:** 40  $\mu$ L of initial mobile phase. The samples were then vortexed for 7 sec and centrifuged at 2,800 xg for 5 min. The supernatants were transferred to conical inserts for UHPLC-MS/MS analysis.

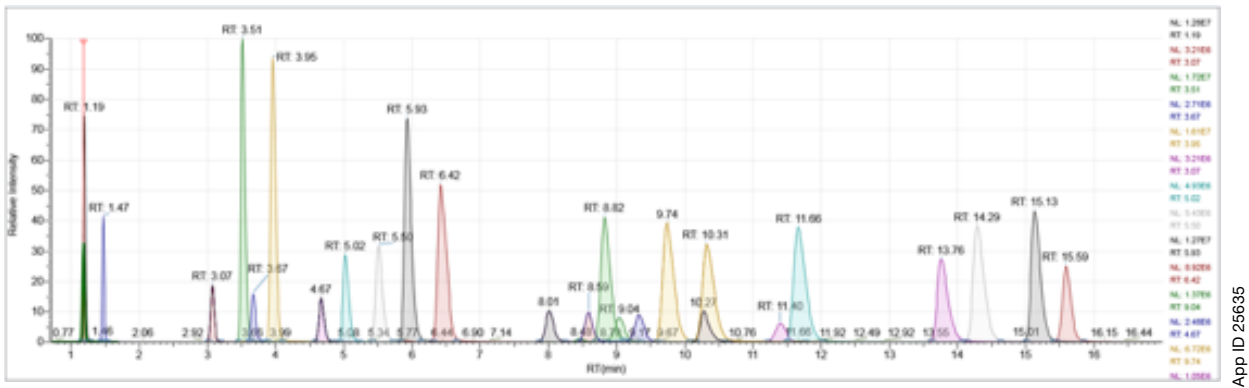
### LC Conditions

- Column:** Kinetex<sup>®</sup> 2.6  $\mu$ m F5  
**Dimensions:** 150 x 2.1 mm  
**Part No.:** [00F-4723-AN](#)  
**Mobile Phase:** A: 0.1% Formic acid in Water  
 B: 0.1% Formic acid in Acetonitrile  
**Gradient:**
- | Time (min) | % B |
|------------|-----|
| 0          | 20  |
| 17         | 25  |
| 17.25      | 95  |
| 18.75      | 95  |
| 19.5       | 20  |
| 21         | 20  |
- Flow Rate:** 0.5 mL/min  
**Column Oven Temperature:** 40  $^{\circ}$ C  
**Autosampler Temperature:** 5  $^{\circ}$ C  
**Detector:** MS/MS (Thermo TSQ Quantis Triple Quad Tandem)

	Analyte Name	Retention Time (min)
1.	Norfentanyl	1.19
2.	N-methyl norfentanyl	1.19
3.	Norcarfentanil	1.47
4.	Methoxyacetyl fentanyl	3.07
5.	Acetyl fentanyl	3.51
6.	$\beta$ -hydroxy fentanyl	3.67
7.	Benzyl fentanyl	3.95
8.	<i>para</i> -methoxyacetyl fentanyl	4.67
9.	Alfentanil	5.02
10.	4-ANPP	5.50
11.	Acryl fentanyl	5.93
12.	Fentanyl	6.42
13.	<i>para</i> -fluoroacryl fentanyl	8.01
14.	Cyclopropyl fentanyl	8.59
15.	<i>para</i> -fluorofentanyl	8.82
16.	2-furanyl fentanyl	9.04
17.	(E)-crotonyl fentanyl	9.35
18.	( $\pm$ )-trans-3-methylfentanyl	9.74
19.	Fentanyl carbamate	10.27
20.	( $\pm$ )-cis-3-methylfentanyl	10.31
21.	Carfentanil	11.40
22.	Butyryl fentanyl	11.66
23.	Sufentanil	13.76
24.	<i>para</i> -fluoroisobutyryl fentanyl	14.29
25.	Phenyl fentanyl	15.13
26.	Cyclopentenyl fentanyl	15.59

## Results

**Figure 1.**  
Separation and Analysis of Fentanyl and Metabolites Using Kinetex<sup>®</sup> F5 LC Column



App ID 25635

## Conclusion

Using whole blood as a matrix requires pre-treatment and sample preparation to clean-up prior to analysis. Employing a solid phase extraction method allows for matrix clean-up and the sample is concentrated which leads to more sensitive LC-MS/MS results. The Kinetex F5 LC column utilized a core-shell media to help which has 5 modes of selectivity: hydrophobic, aromatic, electrostatic, steric, and hydrogen bonding. This makes the phase extremely useful for isomeric analysis, and a great choice for fentanyl analog analysis.

## References

1. Szabolcs Sofalvi, Eric S Lavins, Ian T Brooker, Claire K Kaspar, John Kucmanic, Carrie D Mazzola, Christie L Mitchell-Mata, Cassandra L Clyde, Rindi N Rico, Luigino G Apollonio, Charissa Goggin, Brittany Marshall, Danielle Moore, Thomas P Gilson, (2019). Unique Structural/Stereo-Isomer and Isobar Analysis of Novel Fentanyl Analogues in Postmortem and DUID Whole Blood by UHPLC-MS-MS, *Journal of Analytical Toxicology*, Volume 43, Issue 9, bkz056, <https://doi.org/10.1093/jat/bkz056>

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## Ordering Information

### Kinetex® LC Columns

5 µm Minibore Columns (mm)				SecurityGuard™ ULTRA Cartridges†
Phases	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
F5	<a href="#">00B-4724-AN</a>	<a href="#">00D-4724-AN</a>	<a href="#">00F-4724-AN</a>	<a href="#">AJ0-9322</a>

for 2.1 mm ID

5 µm MidBore™ Columns (mm)			SecurityGuard ULTRA Cartridges†
Phases	100 x 3.0	150 x 3.0	3/pk
F5	<a href="#">00D-4724-Y0</a>	<a href="#">00F-4724-Y0</a>	<a href="#">AJ0-9321</a>

for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges†
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
F5	<a href="#">00B-4724-E0</a>	<a href="#">00D-4724-E0</a>	<a href="#">00F-4724-E0</a>	<a href="#">00G-4724-E0</a>	<a href="#">AJ0-9320</a>

for 4.6 mm ID

2.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
F5	<a href="#">00A-4723-AN</a>	<a href="#">00B-4723-AN</a>	<a href="#">00D-4723-AN</a>	<a href="#">00F-4723-AN</a>	<a href="#">AJ0-9322</a>

for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges†
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
F5	<a href="#">00B-4723-Y0</a>	<a href="#">00D-4723-Y0</a>	<a href="#">00F-4723-Y0</a>	<a href="#">AJ0-9321</a>

for 3.0 mm ID

2.6 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges†
Phases	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
F5	<a href="#">00B-4723-E0</a>	<a href="#">00D-4723-E0</a>	<a href="#">00F-4723-E0</a>	<a href="#">AJ0-9320</a>


for 4.6 mm ID

1.7 µm Minibore Columns (mm)				SecurityGuard ULTRA Cartridges†
Phases	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
F5	<a href="#">00B-4722-AN</a>	<a href="#">00D-4722-AN</a>	<a href="#">00F-4722-AN</a>	<a href="#">AJ0-9322</a>

for 2.1 mm ID

† SecurityGuard ULTRA Cartridges require holder, Part No.: [AJ0-9000](#).

### Strata® Screen C SPE

Format	Sorbent Mass	Part Number	Unit
	100 mg	<a href="#">8B-S016-EAK</a>	1 mL (100/box)
	100 mg	<a href="#">8B-S016-EBJ</a>	3 mL (50/box)
	150 mg	<a href="#">8B-S016-SBJ</a>	3 mL (50/box)
	150 mg	<a href="#">8B-S016-SCH</a>	6 mL (30/box)
	200 mg	<a href="#">8B-S016-FBJ</a>	3 mL (50/box)
	200 mg	<a href="#">8B-S016-FCH</a>	6 mL (30/box)
	300 mg	<a href="#">8B-S016-RBJ</a>	3 mL (50/box)
	300 mg	<a href="#">8B-S016-RCH</a>	6 mL (30/box)
	500 mg	<a href="#">8B-S016-HCH</a>	6 mL (30/box)

96-Well Plate			
	50 mg	<a href="#">8E-S016-DGB</a>	2 Plates/Box

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