



## FENTANYL/ NORFENTANYL ON ORAL SWABS BY LC-MS/MS OR GC-MS CLEAN SCREEN<sup>®</sup> DAU EXTRACTION COLUMN

Part #

CSDAU – CLEAN SCREEN<sup>®</sup> DAU

SLDA100ID21-5UM – Selectra<sup>®</sup> DA HPLC Column, 100 x 2.1 mm, 5 µm

### 1. PREPARE SAMPLE

#### PREPERATION OF STANDARDS:

To separate tube add 0, 1, 5, 10 ng of Fentanyl / Norfentanyl in Methanol. Evaporate off the solvent. Add 100 µL of drug free oral fluid. Vortex mix and allow to stand for 30 minutes. Take clean, dry (drug free) swab and swab up the oral fluid and allow standing for 15 minutes. Remove oral swab.

#### SAMPLE PRE TREATMENT:

To 200 µL of Methanol (pH 6) add internal standard. Insert oral swab into Methanol and mix for 1 minute, add a further 100 µL of Methanol, allow to stand for 10 minutes. Remove swab and 3 mL of 100 mM phosphate buffer (pH 6). Vortex and centrifuge as appropriate.

### 2. CONDITION CLEAN SCREEN<sup>®</sup> EXTRACTION COLUMN:

1 x 3 mL CH<sub>3</sub>OH

1 x 3 mL D.I. H<sub>2</sub>O

1 x 3 mL 100 mM phosphate buffer (pH 6.0)

**NOTE:** Aspirate at full vacuum or pressure

### 3. APPLY SAMPLE:

Load at 1 to 2 mL/minute

### 4. WASH COLUMN:

1 x 3 mL D.I. H<sub>2</sub>O

1 x 3 mL 1% acetic acid

1 x 3 mL CH<sub>3</sub>OH

Dry column (5 minutes at full vacuum or pressure)

### 5. ELUTE FENTANYL/NORFENTANYL:

1 x 3 mL Ethyl Acetate/ Acetonitrile / NH<sub>4</sub>OH (78:20:2v/v)

Collect eluate at 1 to 2 mL/minute

**NOTE:** Prepare elution solvent daily

Add IPA/ NH<sub>4</sub>OH, mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

### 6. DRY ELUATE:

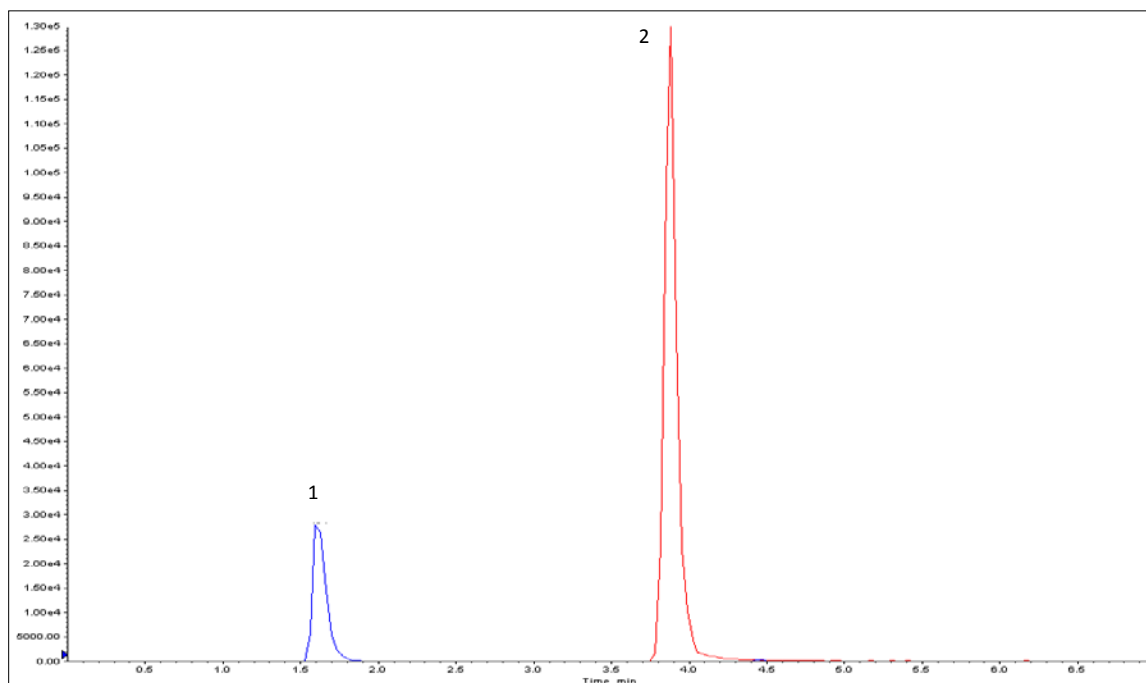
Evaporate to dryness at < 40 °C

### 7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of 0.2% Formic Acid / Methanol (50:50) Inject 10 µL.
- **GC-MS:** Dissolve residue in 100 µL of Ethyl Acetate

## INSTRUMENT CONDITIONS (LC-MS/MS):

### CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Norfentanyl	233.2	84.1	1.61
Norfentanyl D <sub>5</sub>	238.3	84.1	-
2. Fentanyl	337.2	188.3	3.88
Fentanyl D <sub>5</sub>	342.2	188.2	-

### PARAMETERS

**Mobile Phase A:** 0.2% Formic Acid in D.I. H<sub>2</sub>O

**Mobile Phase B:** 02% Formic Acid in Acetonitrile

**Flow Rate:** 0.6 mL/min

**Polarity:** Positive

**Injection Volume:** 10 µL

**LC Column:** Selectra<sup>®</sup> DA HPLC Column 100 x 2.1 mm 5 µm

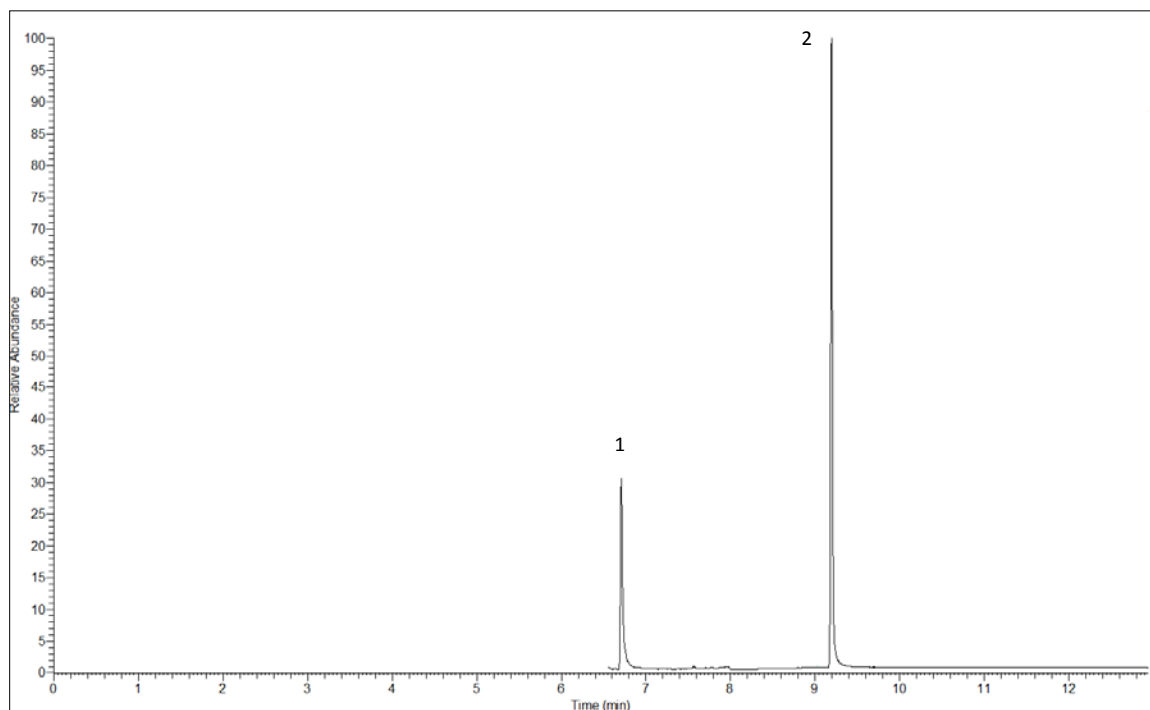
**Instrument:** API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

#### Gradient:

Time	%A	%B
0.00	70	30
6.00	10	90
6.01	70	30
7.00	STOP	

## INSTRUMENT CONDITIONS (GC-MS):

### CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Norfentanyl	93	159	175	6.72
Norfentanyl D <sub>5</sub>	98	164	180	6.69
2. Fentanyl	245	146	189	9.20
Fentanyl D <sub>5</sub>	250	151	194	9.18

### PARAMETERS

**GC/MS:** Thermo ISQ Trace 1300

**GC capillary column:** 30 m x 0.25 mm (0.25 µm) TG-1MS

**Injector:** 1 µL Splitless, 250 °C

**Oven temperature program:** 100 °C (0.5minutes) to 320 °C (30 °C/ minute): hold ( 5 minutes)

**Carrier gas:** Helium (1.2 mL/ minute)

**MSD condition:** Aux temperature: 280 °C, MS Source: 350 °C, MS Quad: 150 °C