



## CARISOPRODOL AND MEPROBAMATE IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN<sup>®</sup> DAU EXTRACTION COLUMN

Part #

CSDAU – CLEAN SCREEN<sup>®</sup> DAU

SLDA50ID21-5UM – Selectra<sup>®</sup> DA HPLC Column, 50 x 2.1 mm, 5  $\mu$ m  
or

SLC-18100ID21-3UM – Selectra<sup>®</sup> C18 HPLC Column, 100 x 2.1 mm, 3  $\mu$ m

### 1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer ( pH 6.0 ) add internal standards  
Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g ( 1:4 ) tissue homogenate  
Mix/vortex and let stand for 5 minutes  
Add 2 mL of 100 mM phosphate buffer ( pH 6.0 ). Mix/vortex  
Sample pH should be 6.0  $\pm$  0.5.  
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.  
Centrifuge for 10 minutes at 2000 rpm and discard pellet

### 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 100 mM hydrochloric acid  
Dry column (10 minutes at full vacuum or pressure)  
1 x 3 mL Hexane

### 5. ELUTE CARISOPRODOL/MEPROBAMATE:

1 x 3 Ethyl Acetate:Hexane (50:50)  
Collect eluate at 1 to 2 mL/minute

### 6. DRY ELUATE:

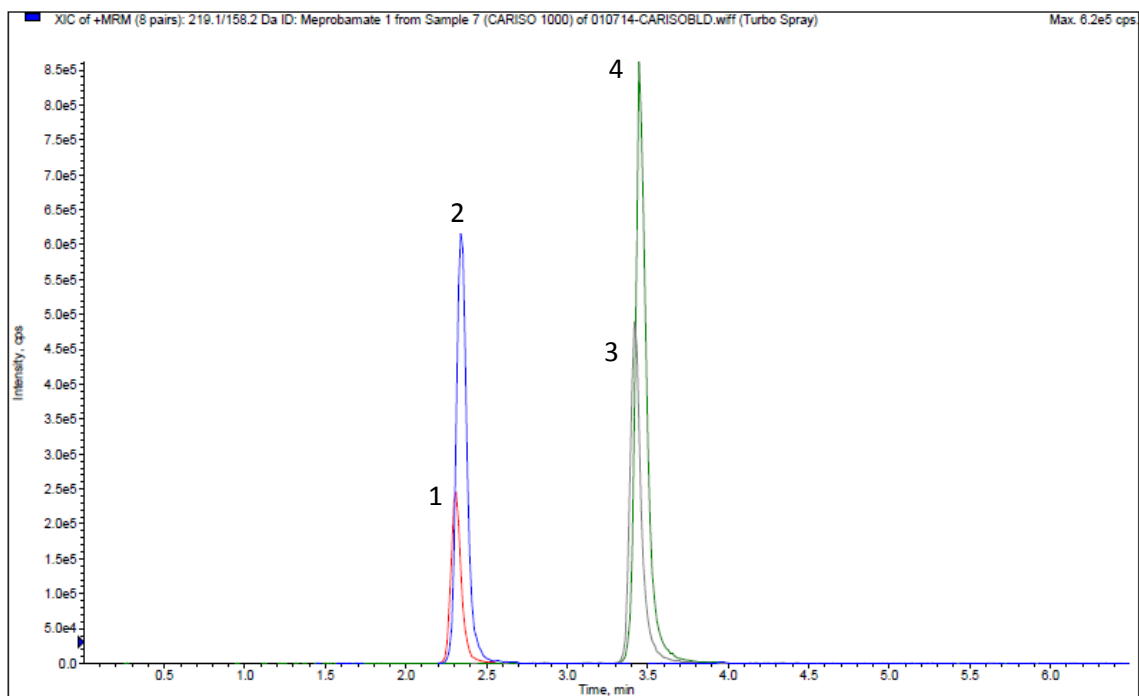
Evaporate to dryness at < 40 °C

### 7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100  $\mu$ L of mobile phase  
Inject 10-15  $\mu$ L.
- **GC-MS:** Dissolve residue in 100  $\mu$ L of Ethyl Acetate

# INSTRUMENT CONDITIONS (LC-MS/MS):

## CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.MEPROBAMATE D <sub>7</sub>	226.2	165.1	2.32
2.MEPROBAMATE	219.1	158.2	2.34
3.CARISOPRODOL D <sub>7</sub>	268.2	183.2	3.38
4.CARISOPRODOL	261.1	176.1	3.40

### PARAMETERS

**Mobile Phase A:** 0.1% Formic Acid in D.I. H<sub>2</sub>O

**Mobile Phase B:** 0.1% Formic Acid in Methanol

**Flow Rate:** 0.8 mL/minute

**Polarity:** Positive

**Reconstitute:** 100 µL

**Injection Volume:** 15 µL

**LC Column:** Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

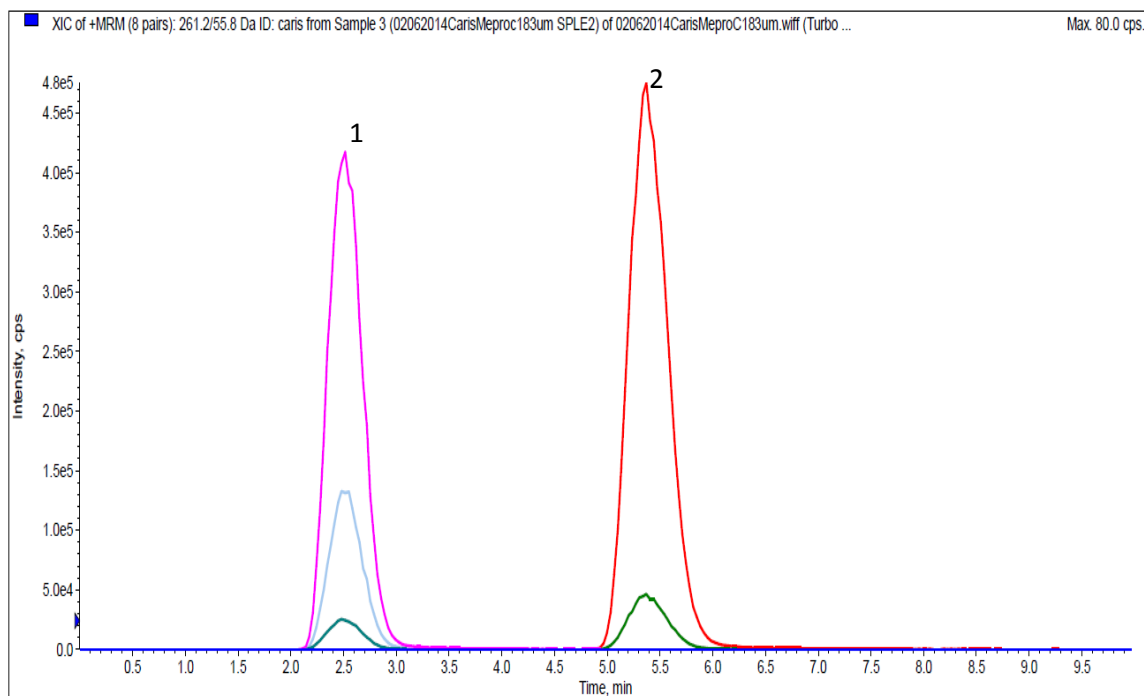
**Instrument:** API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

### Gradient:

Time	%A	%B
0.00	95	5
6.00	35	65
6.01	95	5
6.50	STOP	

# INSTRUMENT CONDITIONS (LC-MS/MS):

## CHROMATOGRAM 2 SELECTRA® C18 HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.MEPROBAMATE	219.1	158.2	2.50
MEPROBAMATE D <sub>7</sub>	226.2	165.4	
2.CARISOPRODOL	261.1	176.1	5.36
CARISOPRODOL D <sub>7</sub>	268.2	183.2	

### PARAMETERS

**Mobile Phase A:** 0.1% Formic Acid in D.I. H<sub>2</sub>O

**Mobile Phase B:** 0.1% Formic Acid in Methanol

**Flow Rate:** 0.3 mL/minute

**Polarity:** Positive

**Reconstitute:** 100 µL

**Injection Volume:** 10 µL

**LC Column:** Selectra® C18 HPLC Column 100 x 2.1 mm 3 µm

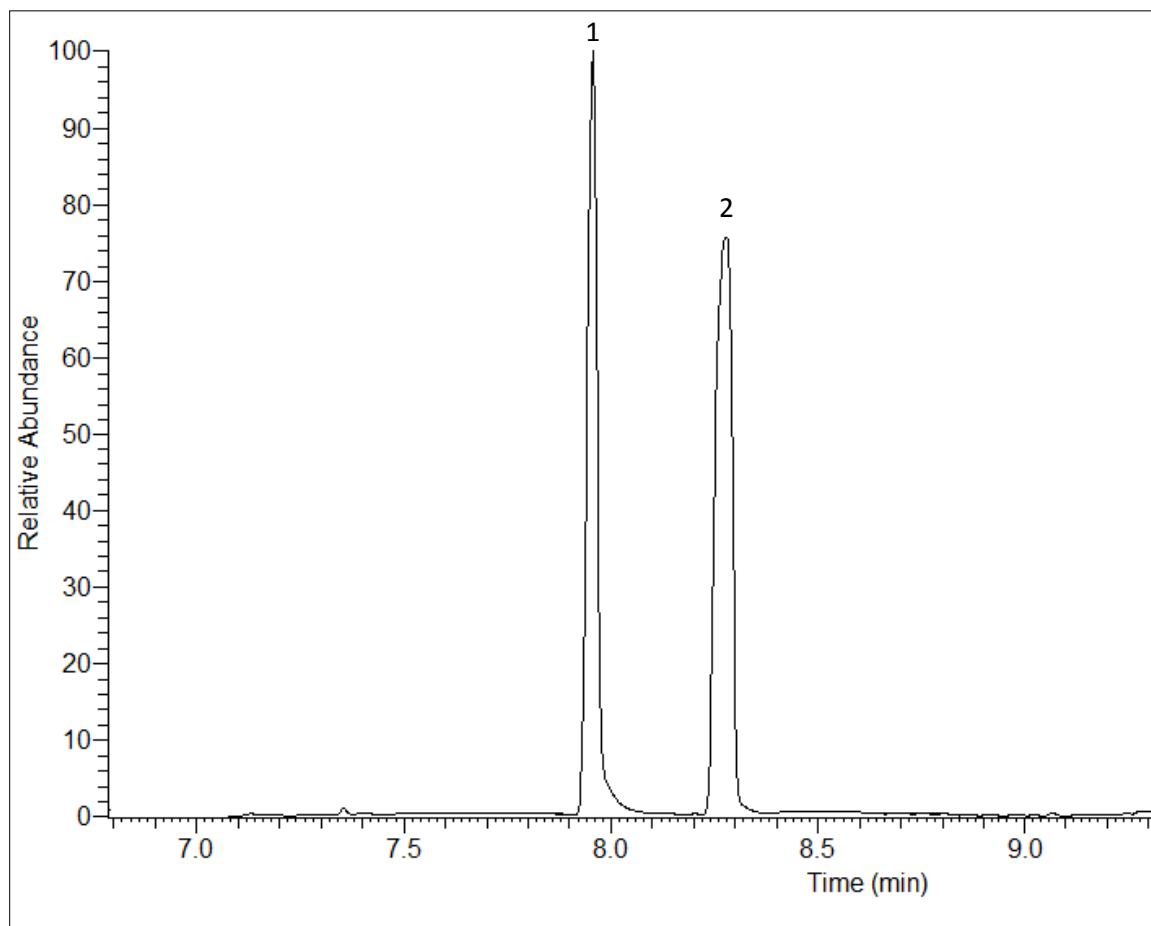
**Instrument:** API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

**Isocratic:**

Time	%A	%B
0.00	50	50
10.00	STOP	

## INSTRUMENT CONDITIONS (GC-MS):

### CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. MEPROBAMATE	83	114	144	7.96
MEPROBAMATE D <sub>7</sub>	90	121	151	-
2. CARISOPRODOL	158	184	245	8.28

### PARAMETERS

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

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

**Injector:** 1 µL Splitless, 250 °C

**Oven temperature program:** 70 °C (0.5) to 320 °C (25 °C/minute): hold ( 2 minutes)

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

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