CLINICAL

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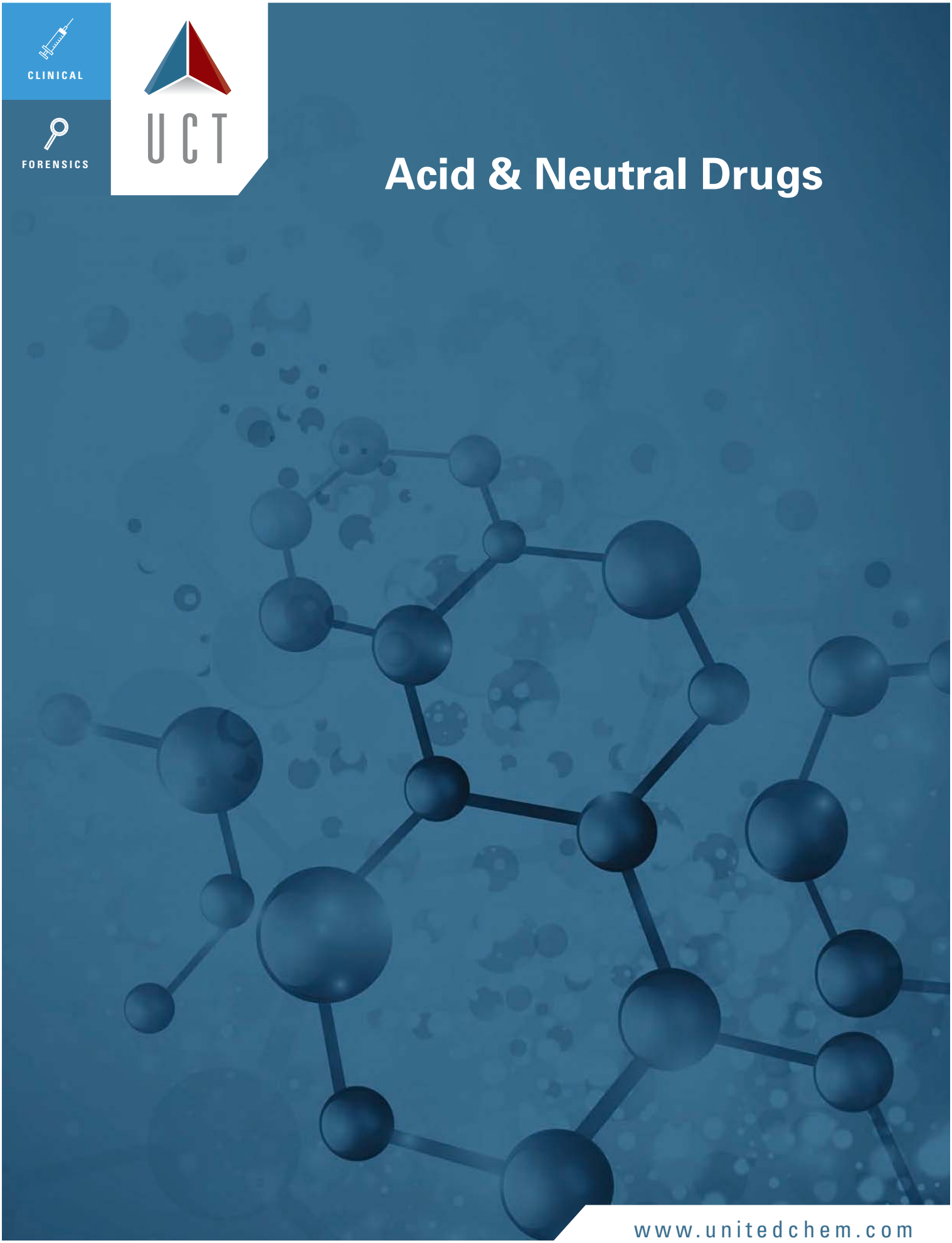
UCT

Acid & Neutral Drugs



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BARBITURATES IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

ZSDAU020 CLEAN SCREEN® DAU 200 mg, 10 mL Tube

STMPAH-0-1 – SELECTRA-SIL® TMPAH

SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

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 ± 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® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   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_2O
   1 x 1 mL 100 mM Acetic Acid
   Dry column (5 minutes at full vacuum or pressure)
   1 x 2 mL hexane

5. **ELUTE BARBITURATES:**
   1 x 3 mL Ethyl Acetate: Hexane (50:50)
   collect eluate at 1 to 2 mL/minute

6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

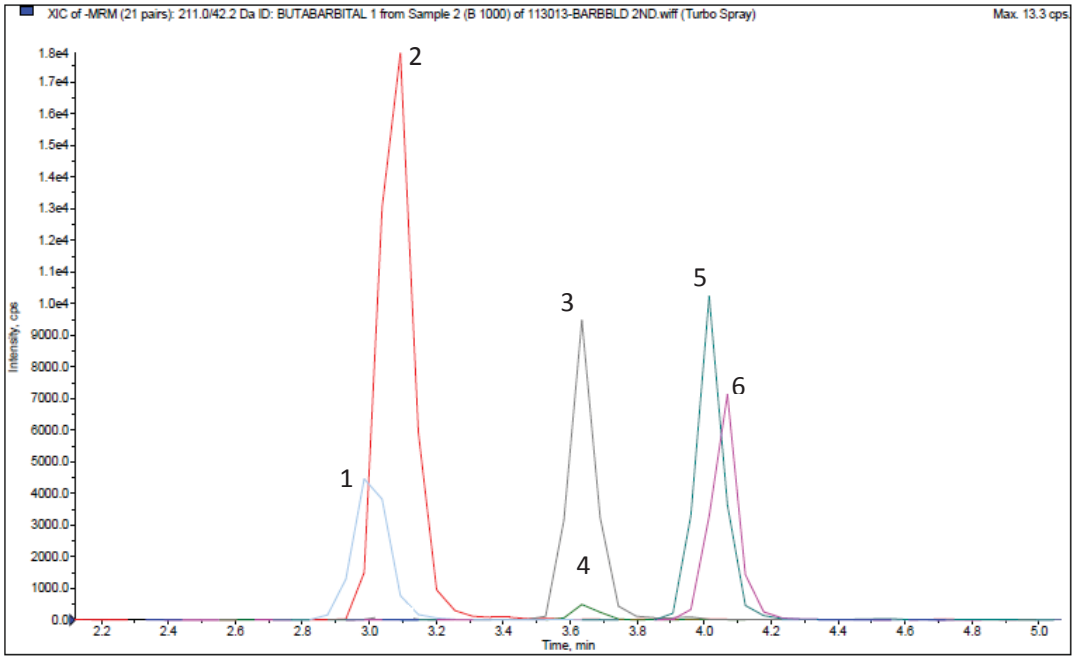
7. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL.
   - **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

   Alternate Derivatization
   Add 25 µL of 0.2 M TMPAH
   Reaction occurs in injection port

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Phenobarbital	230.8	42.0	3.0
2. Butalbital	223.0	42.1	3.1
3. Amobarbital	225.0	42.0	3.6
4. Pentobarbital	225.0	42.1	3.6
5. Secobarbital D5	242.1	42.0	4.0
6. Secobarbital	237.0	42.0	4.1

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O **Mobile Phase B:** 0.1% Formic Acid in Methanol

Flow Rate: 0.6 mL/minute **Polarity**: Positive

Reconstitute: 100 µL **Injection Volume**: 10 µL

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

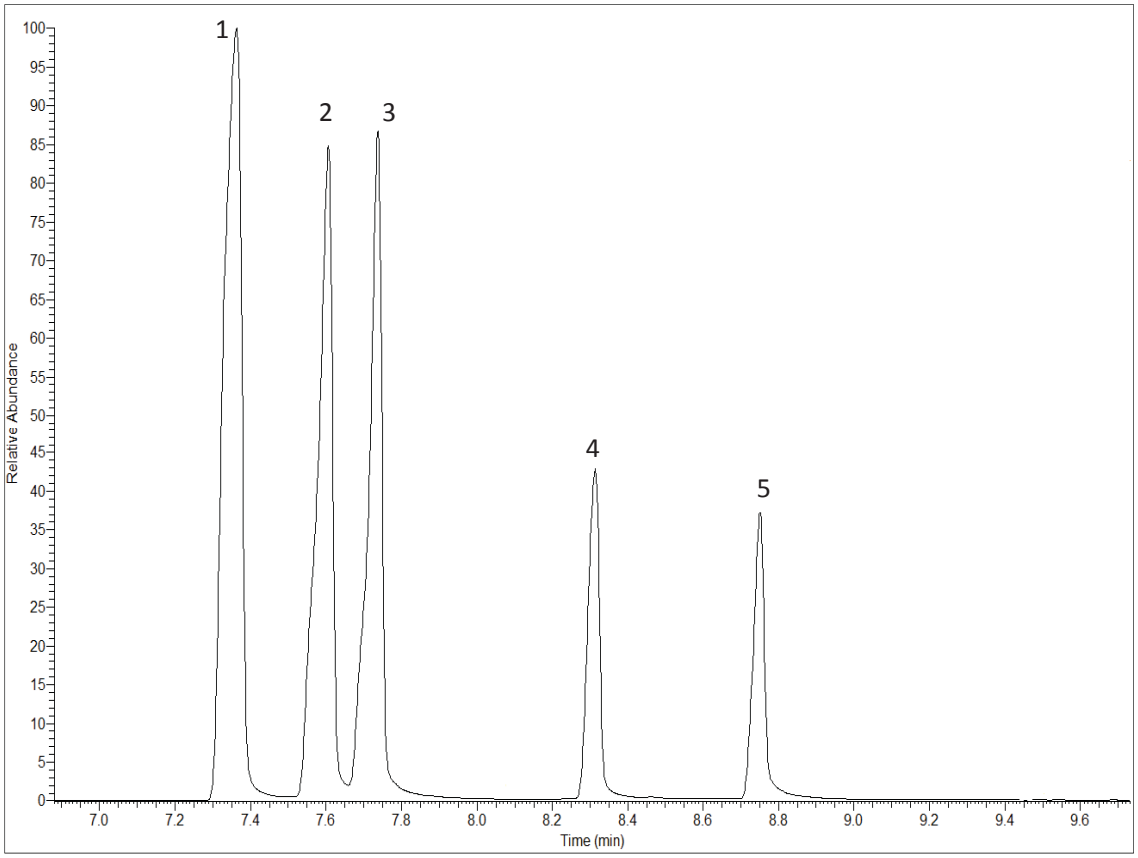
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	90	10
6.00	50	50
6.01	10	90
7.00	90	10
7.50	STOP	

INSTRUMENT CONDITIONS (GC-MS):

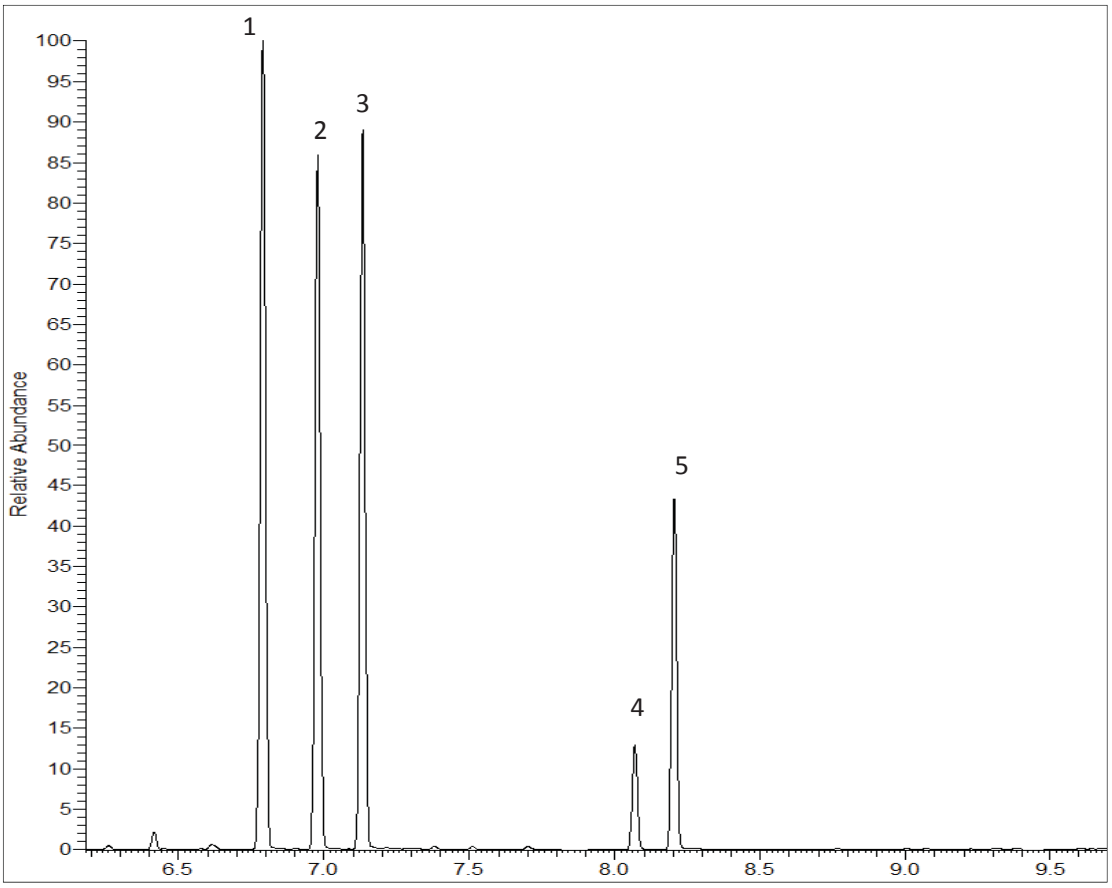
CHROMATOGRAM 1 (UNDERIVATIZED)



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1.Butabarbital	156	141	157	7.36
2.Amobarbital	156	141	157	7.61
3.Pentobarbital	156	141	197	7.74
4.Hexobarbital*	221	157	236	8.31
5.Phenobarbital	204	232	117	8.75

*Suggested internal standard for GC/MS

CHROMATOGRAM 2 (TMPAH)



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1.Butabarbital	169	184	211	6.79
2.Amobarbital	169	184	185	6.98
3.Pentobarbital	169	184	112	7.13
4.Hexobarbital	235	251	171	8.07
5.Phenobarbital	232	146	175	8.21
Phenobarbital D_5	237	151	-	

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: Helium (1.2 mL/minute)

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

CAFFEINE, THEOPHYLLINE AND THEOBROMINE IN BLOOD, PLASMA/SERUM, AND URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #: ZSDAU020

LC-PDA

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM acetic acid add internal standard.*
   Add 1 mL Blood, Serum/ Plasma, or Urine. Add 2 mL of 100 mM acetic acid.
   Vortex and centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM acetic acid.
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM acetic acid.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE CAFFEINE/THEOBROMINE/THEOPHYLLINE:**
   1 x 3 mL Ethyl Acetate : Methanol (90:10)
   Collect eluate at 1-2 mL / minute.

6. **EVAPORATION:**
   Combine eluates
   Evaporate eluates under a gentle stream of nitrogen < 40 °C.

7. **RECONSITITUE: sample in 1000 µL of 0.1 % Formic Acid (aq).**
   Inject 20 µL.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) Gold C_{18} (Thermofisher)

Mobile phase: Acetonitrile: 0.1% Formic Acid aqueous (10:90).

Flowrate: 0.1 mL/ minute

Column Temperature: ambient

Detector: Diode Array (200-350 nm)

CHROMATOGRAM OF SHOWING:

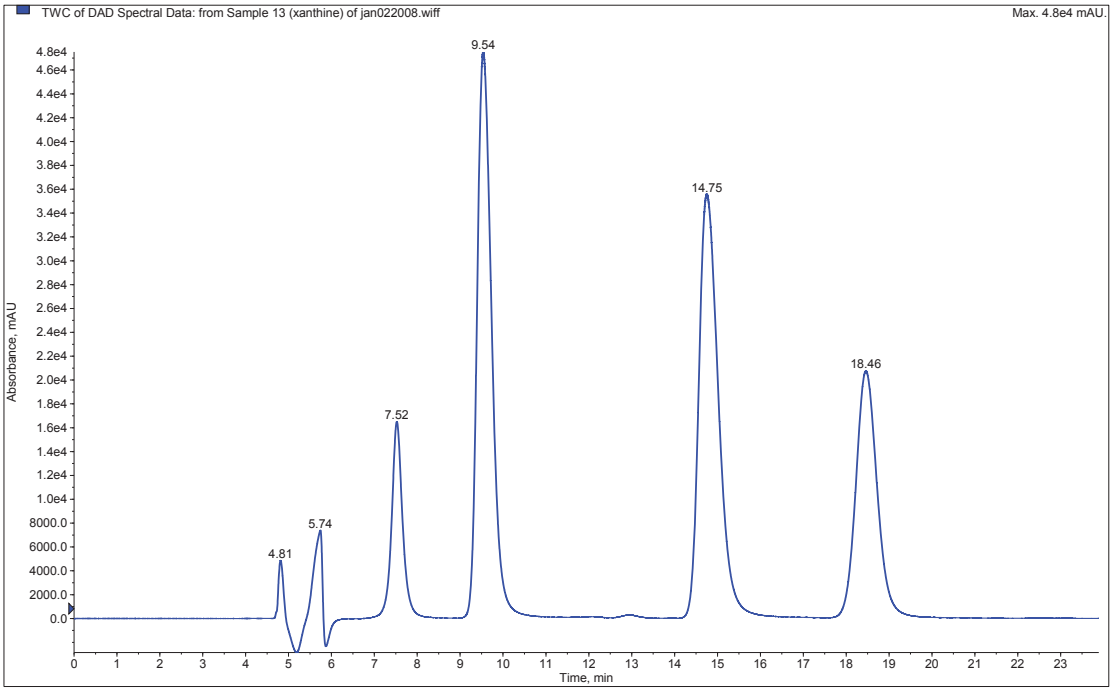
Compound

Theobromine: 7.5 minutes

Theophylline: 9.5 minutes

Caffeine: 14.5 minutes

*8-Chlorotheophylline: 18.0 minutes



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

Part #

CSDAU – CLEAN SCREEN® DAU

SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

or

SLC-18100ID21-3UM – Selectra® C18 HPLC Column, 100 x 2.1 mm, 3 µm

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 ± 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® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   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_2O
   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

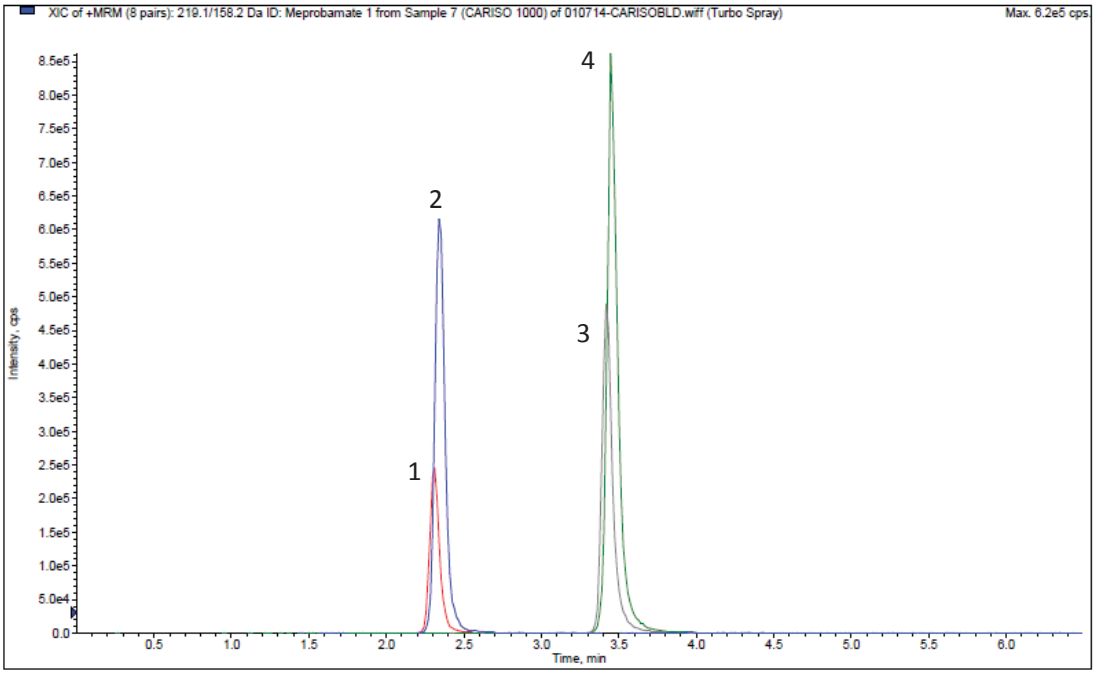
6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

7. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10-15 µL.
   - **GC-MS**: Dissolve residue in 100 µL 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_7	226.2	165.1	2.32
2.MEPROBAMATE	219.1	158.2	2.34
3.CARISOPRODOL D_7	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_2O

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

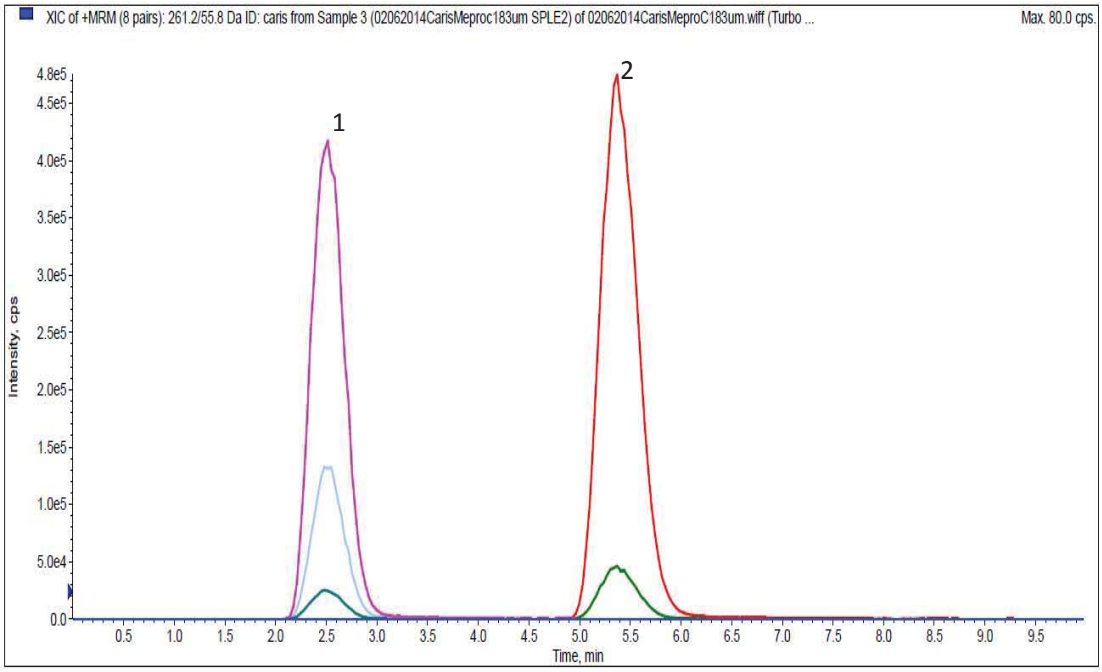
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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_7	226.2	165.4	
2.CARISOPRODOL	261.1	176.1	5.36
CARISOPRODOL D_7	268.2	183.2	

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

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

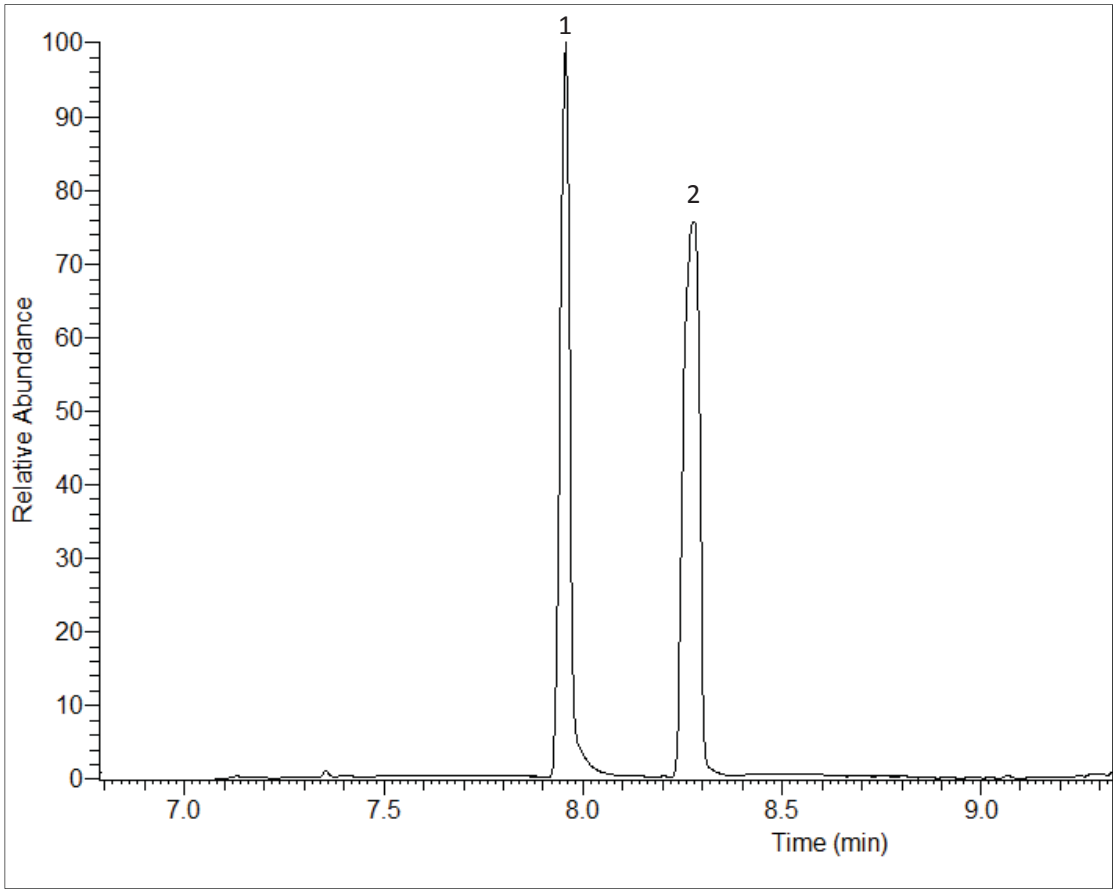
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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_7	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 ºC, MS Source: 350 ºC, MS Quad: 150 ºC

EtG/EtS IN URINE BY LC-MS/MS USING 500 MG CLEAN-UP® QAX EXTRACTION COLUMN

Part #

CUQAX156 – CLEAN-UP QAX 500 mg, 6 mL Tube

1. **PREPARE SAMPLE:**
   To 0.5 mL of urine containing deuterated analogues of EtG/EtS
   Add 4 mL of D.I. H_2O
   Mix/vortex

2. **CONDITION CLEAN-UP® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   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_2O
   1 x 3 mL Methanol
   Dry column (**10 minutes** at full vacuum or pressure)

5. **ELUTE EtG/EtS ANALYTES:**
   2 x 3 mL 6% Acetic Acid/94% Methanol

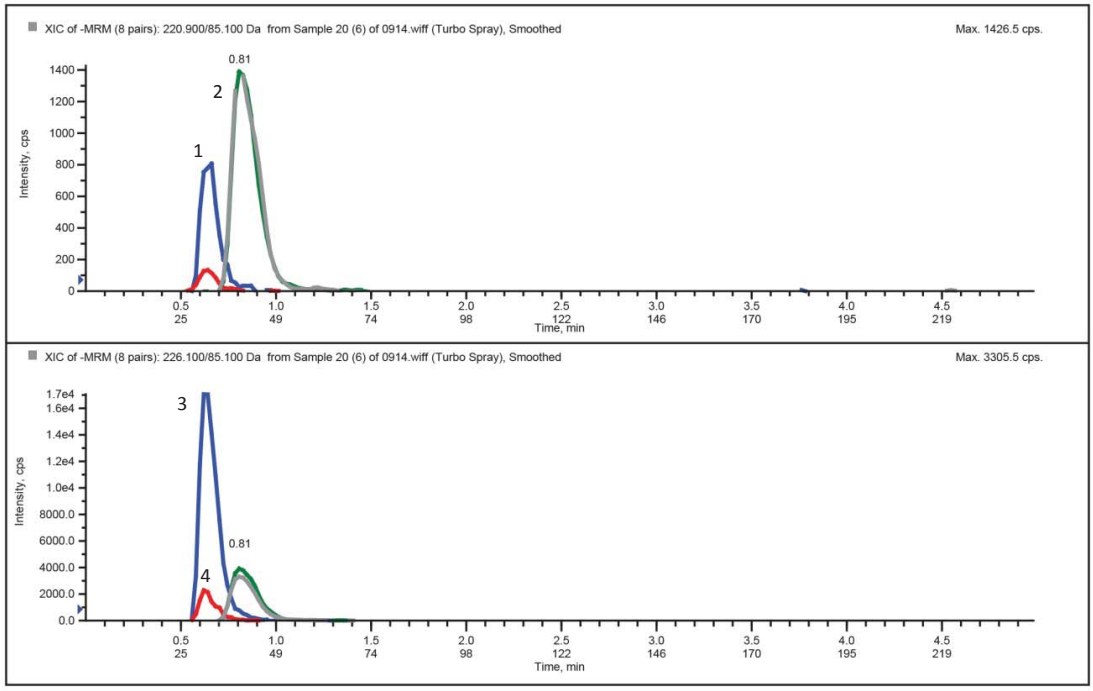
   Collect eluate at 1 to 2 mL/minute

6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

7. **RECONSTITUTE:**
   with 50-100 µL of Mobile Phase

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAMS



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. EtS	125.1	95.8	0.65
2. EtG	220.9	75.1	0.83
3. EtS D_5	130.1	97.8	0.63
4. EtG D_5	226.1	74.9	0.81

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

Mobile Phase B: 0.1% Formic Acid in ACN

Flow Rate: 0.35 mL/minute

Polarity: Negative

Injection Volume: 20 µL

LC Column: Diamond Hydride LC Column 100 x 2.1 mm (4 µm)

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

Isocratic:

Time	%A	%B
0.00	50	50
5.00	50	50

LC/MS METHOD FOR EXTRACTING ETHYL GLUCURONIDES FROM URINE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

Part #:

CSETG203 – CLEAN SCREEN® ETG 200 mg, 3 mL Tube

1. **PREPARE SAMPLE:**

   Add 50 µL of Formic Acid to 1 mL of urine. (Internal standard EtG –d5 at 200 ng/mL.)
   Centrifuge for 10 minutes at 3000 rpm.
   Decant solution onto SPE cartridge previously conditioned with 2 mL of 1% Formic Acid.
   Wash sample column with 2 mL D.I. H2O and dry at 10 mm Hg for 10 minutes.
   Elute the EtG with 2 mL of 1% Formic Acid/ Methanol solution.
   Evaporate to dryness under stream of nitrogen.
   Reconstitute with 1 mL of Methanol. The solution
   should be filtered through a 0.2 µm filter for LC/MS analysis.

2. **SUGGESTED LC/MS PROCEDURE:**

   Prepare 1.0 M ammonium acetate buffer by weighing 3.8 g ammonium acetate and dilute to 5L.
   (Option: 0.77 g diluted to 1L D.I. H2O). This solution should be filtered through 0.2 µm filer for LC use.
   LC Mobile Phase –Ammonium Acetate: Acetonitrile (10: 90) at a flow rate of 0.2 mL/minute.
   Injection Volume – 10 mL.
   Detection Limit – 10 ng/mL

3. **SUGGESTED LC/MS/MS PARAMETERS:**

   Tuning the MS:

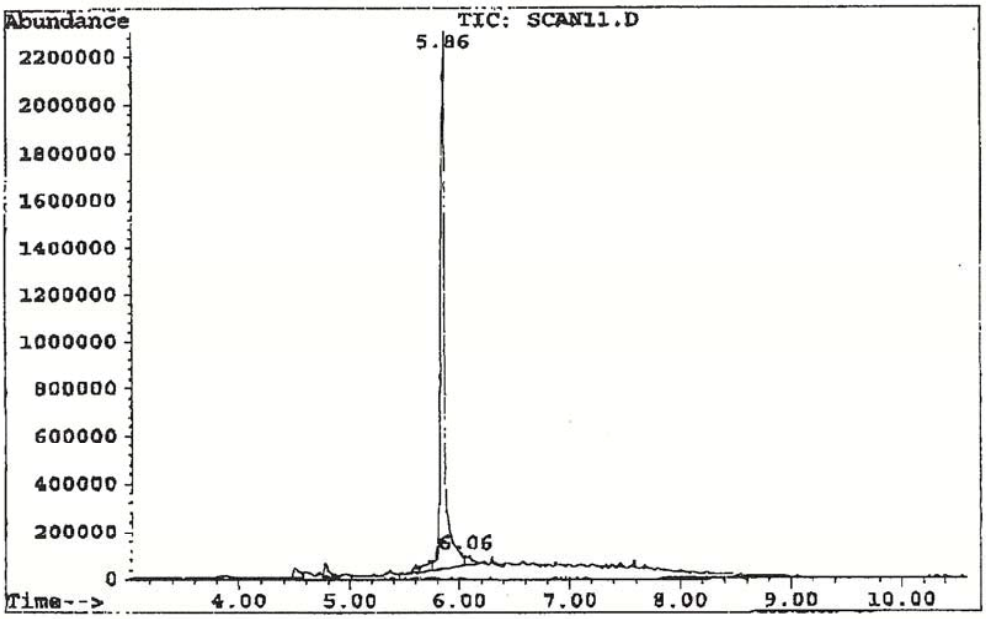
   Tune MS using PPG
   Tune MS using 500 ng/mL EtG, mobile phase 0.2 mL/min, EtG solution 1 µL/min. Optimize ion source and mass analyzer to signal 221 m/z. Determine the collision voltage for ion 75 m/z and reference ions 85 and 113 m/z. Tune file uses scan rate of 0.3 s; acquisition time 6 minutes.
   Quantifier ion is 75 and qualifier ions are 85 and 113. Collision voltage 75(16), 85(16) / and 113 (14.5).

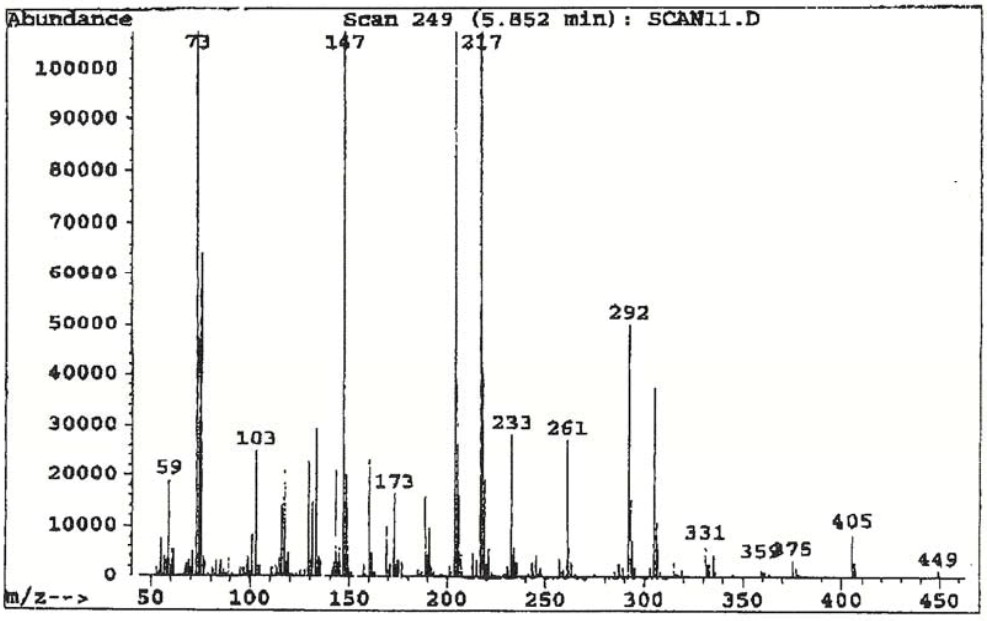
NOTES:

The prepared buffer should be filtered 30-45 minutes (equilibrated) before analysis for constant results.
After sample elution from the column, the LC must be programmed to flush the column using an Acetonitrile / DI water gradient (50/50 to 90/10) to avoid carryover from previous specimen.

ETHYL GLUCURONIDES

Chromatogram





METHYLMALONIC ACID FROM SERUM OR PLASMA FOR GC/MS ANALYSIS USING: 500 mg CLEAN-UP® QAX EXTRACTION COLUMN

Part #:
CUQAX15Z – CLEAN-UP® QAX 500 mg, 10mL Tube
SMSTFA-1-1 – SELETRA-SIL® MSTFA w/ 1% TMCS

1. **PREPARE SAMPLE:**
   Add 100 µL of internal standard D_3-MMA and 1 mL of acetonitrile to 1 mL of plasma or serum.
   Vortex for 20 sec.
   Centrifuge for 5 min at 2000 rpm.

2. **CONDITION CLEAN-UP® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.

3. **APPLY SAMPLE:**
   Decant supernatant onto SPE column.

4. **WASH COLUMN:**
   1 x 10 mL of D.I. H_2O.
   Dry with vacuum for 3 min.
   1 x 5 mL of CH_3OH.
   Dry with vacuum for 3 min.
   1 x 2 mL of methyl-tert-butyl ether (MTBE).
   Dry with vacuum for 3 min.

5. **ELUTE METHYLMALONIC ACID:**
   1 x 5 mL of 3% Formic Acid in MTBE; collect at 1 to 2 mL/min.

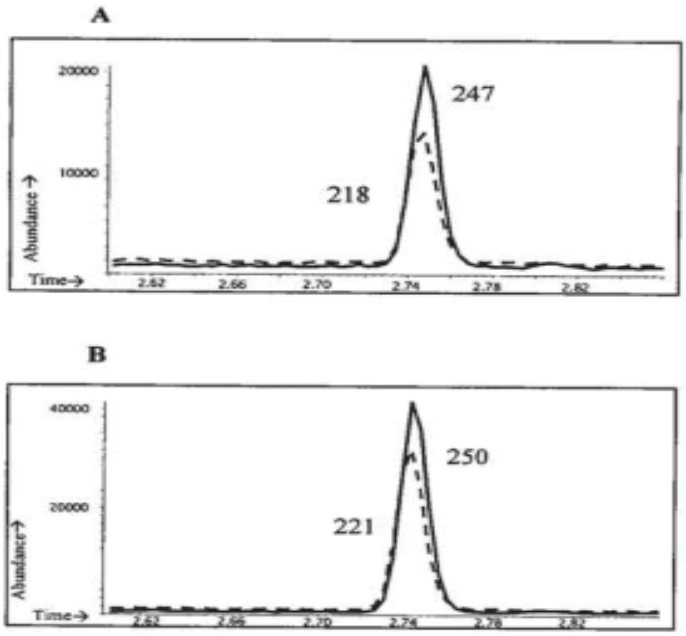
6. **DRY ELUATE:**
   Dry under a stream of nitrogen at < 35 °C.

7. **DERIVATIZE:**
   Reconstitute with 25 µL of MSTFA + 1% TMCS and 25 µL Ethyl Acetate.
   Heat for 20 min at 60 °C.

8. **QUANTITATE:**
   Inject 1 to 2 µL onto gas chromatograph.

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion	Relative Retention Time (minutes)
1. Methylmalonic Acid	247	218	2.76
2. Methylmalonic Acid-D_3	250	221	2.74

PARAMETERS

GC/MS: HP 5890 w/ 5970 MS Detector with 7673 ALS System

GC capillary column: Rtx$_R$-200 MS 20m x 0.18mm, 0.4µm

Injector: 1µL Split 1:20 270°C

Oven temperature program: 100°C for 0.5min; 18°C/min to 160°C; 50°C/min to 300°C for 2.50minutes

Carrier gas: Helium

MSD condition: Aux temperature: 280 ºC, MS Source: 250 ºC, MS Quad: 150 ºC

Compliments of
Mark M. Kusmin and Gabor Kormaromy-Hiller ARUP LABORATORIES

WARFARIN IN WHOLE BLOOD:
MANUAL METHOD FOR GC-MS OR LC CONFIRMATIONS
USING: 200 mg CLEAN-UP® C-30 EXTRACTION COLUMN

Part #:
CEC30203 – CLEAN-UP® C30 200 mg, 3 mL Tube
STMPAH-0-1 – SELECTRA-SIL® TMPAH

1. **PREPARE SAMPLE:**
   To 9 mL of 100 mM phosphate buffer (pH 6.0.0) add internal standard.
   Add 1mL of whole blood) and Mix/vortex.
   Sample pH should be 6.0 + 0.5.
   Adjust pH accordingly with 0.1 M monobasic or dibasic sodium phosphate.
   Centrifuge as appropriate

2. **CONDITION CLEAN-UP® COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM phosphate buffer, (pH 6.0) aspirate.
   NOTE: Aspirate at < 3 inches. Hg to prevent sorbent drying.

3. **APPLY SAMPLE:**
   Load at 1-2 mL/min.

4. **WASH COLUMN:**
   Add 1 x 3 mL of phosphate buffer (0.1 M pH 6)
   Dry under full vacuum for 10 mins
   Add 1 x 3 mL of Hexane
   Dry under full vacuum for 10 mins

5. **ELUTE WARFARIN:**
   Add 2 x 3 mL of Methanol: Ethyl Acetate (12:88)
   Note: Prepare elution solvent daily.

6. **Collect eluates at approx 1-2 mL/minute**

7. **Dry samples:**
   Evaporate to dryness at <40 °C
   Add 50 µL of Ethyl Acetate.
   Add 50 µL of TMAH, and vortex.
   React at for 1 hour at 70 ˚C.
   Cool and inject 1-2 µL onto GC-MS
   Monitor the following ions:

Compound	Primary	Secondary	Tertiary
Warfarin	279	322	280
p-chlorowarfarin (internal standard)	313	315	356

WARFARIN CHROMATOGRAM

GC-MS (methylation)