Clinical and Forensic

APPLICATIONS MANUAL

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Introduction and Application Hints

CPS analitica for Chemistry

Extraction Hints

Verify sample application pH. Analytes that are not in their proper form (i.e., neutral or charged), will not effectively bind to the sorbent and may result in low or erratic recoveries. The pH of deionized water cannot be correctly determined using pH paper. Use of a calibrated pH meter is necessary.

Always pre-rinse the cartridge with the strongest solution the cartridge will see to ensure the cleanest extraction of your eluate.

Do not allow the sorbent to completely dry out between conditioning steps or before sample application. To ensure properly solvated cartridges, apply each solvent immediately after the previous solvent. Improperly conditioned cartridges may lead to erratic recoveries.

Prior to elution, fully dried cartridges will ensure optimal analyte recovery. To confirm cartridge dryness, touch the sides of the cartridge at the sorbent level at full vacuum. Cartridges should feel about room temperature but not cool. If the cartridge feels cool, water is probably present and still evaporating. Continue drying the cartridge unless otherwise specified in the application note.

Elution rates and soak times specified in the applications are critical for acceptable and consistent recoveries. Hint: When in doubt, slower is always better. 1 mL to 2 mL per minutes is a good general guideline for the sample addition and analyte extraction steps. This recommendation is required for suitable ion exchange extractions.

Always use fresh ammonium hydroxide (NH_4OH) for elutions. NH_4OH rapidly loses its effectiveness when exposed to air. Weak NH_4OH solutions may lead to erratic recoveries.

NH_4OH is more soluble in IPA than CH_2Cl_2. To ensure complete mixing of eluate solvents, add NH_4OH to IPA, then add CH_2Cl_2.

Addition of 1% HCl in MeOH assists in reducing volatility of certain analytes especially sympathomimetic amines.

Certain compounds are slightly volatile. Closely monitor eluate concentration to prevent loss of analyte. Hint: Higher water bath temperatures and lower nitrogen flow rates usually provide optimal results. However, do not exceed 40 °C. Optimal evaporation temp ranges from 35-40 °C. Optimal Nitrogen flow rates are from 5-15 psi.

Solvent quantities for methods are suggested and might be further reduced to meet particular laboratory sample size needs.

Solvents

Acetone; HPLC Grade
Acetonitrile (ACN)(CH_3CN); HPLC Grade
Chloroform ($CHCl_3$); HPLC Grade
Distilled or/ Deionized H_2O
(D.I. H_2O, 5 < pH < 7)
Ethyl Acetate (EtAc); HPLC Grade
Hexane; HPLC Grade
Isopropyl Alcohol (IPA); HPLC Grade
Methanol (CH_3OH): HPLC Grade
Dichloromethane (CH_2Cl_2): HPLC Grade

Solvent mixtures

Acetone / Hexane (1:99)
Acetonitrile / D.I. H_2O (20:80)
Ethyl Acetate / IPA (75:25)
Ethyl Acetate / Hexane (50:50), (75:25)
Methanol / D.I. H_2O (80:20)
Methanol / D.I. H_2O (70:30)
Methanol / D.I. H_2O (10:90)

Use of non-chlorinated elution solvents

In response to environmental concerns over the use of chlorinated compounds in the laboratory, UCT offers these suggested non-chlorinated elution solvents. The recommended parameters have been used successfully on UCT columns by our customers throughout the world and may be routinely used as an alternative to chlorinated elution solvents. You may however see subtle differences on certain compounds due to solubility effects.

Assay	Chlorinated	Non-chlorinated
Opiates	CH_2Cl_2 / IPA/NH_4OH(78:20:2)	EtAc / IPA/NH_4OH (90:6:4)
Propoxyphene	CH_2Cl_2 / IPA/NH_4OH(78:20:2)	EtAc / IPA/NH_4OH (90:6:4)
Cocaine / BE	CH_2Cl_2 / IPA/NH_4OH(78:20:2)	EtAc / CH_3OH/NH_4OH(68:28:4)
Amphetamines	CH_2Cl_2 / IPA/NH_4OH(78:20:2)	EtAc / IPA/NH_4OH (90:6:4)

UCT would like to thank Dr. Leon Glass for his efforts in developing these non chlorinated mixtures.

Reagents

Acetic Acid, Glacial (CH_3COOH):17.4 M
Ammonium Hydroxide (NH_4OH): concentrated (14.8 M)
ß-Glucuronidase: abalone derived
Dimethylformamide (DMF): silylation grade
Hydrochloric Acid (HCl): concentrated (12.1 M)
N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS)
Pentafluoropropionic Acid Anhydride (PFAA or PFPA)
Phosphoric Acid (H_3PO_4): concentrated (14.7 M)
Sodium Acetate Trihydrate ($NaCH_3COO$-3 H_2O): F.W. 136.08
Sodium Borate Decahydrate ($Na_2B_4O_7$-10 H_2O): F.W. 381.37
Sodium Hydroxide, (NaOH): F.W. 40.00
Sodium Phosphate Dibasic, Anhydrous (Na_2HPO_4): F.W. 141.96
Sodium Phosphate Monobasic, Monohydrate ($NaH_2PO_4 \cdot H_2O$): F.W. 137.99

Notes:

Storage of organics in some plastic containers may lead to plasticizer contamination of the solvent or solvent mixture, this may interfere with analyte quantitation. Good laboratory practice dictates all who handle or are potentially exposed to reagents, solvents and solutions used or stored in the laboratory should familiarize themselves with manufacturer's recommendations for chemical storage, use and handling, and should also familiarize themselves with an appropriate Material Safety Data Sheet (MSDS).

How to Prepare Solutions and Buffers

1.0 M Acetic Acid:
To 400 mL D.I. H_2O add 28.6 mL glacial acetic acid. Dilute to 500 mL with D.I. H_2O. Storage: 25 °C in glass or plastic. Stability: 6 months

100 mM Acetic Acid:
Dilute 40 mL 1.0 M acetic acid to 400 mL with D.I. H_2O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months

100 mM Acetate Buffer (pH 4.5):
Dissolve 2.93 g sodium acetate trihydrate in 400 mL D.I. H_2O; add 1.62 mL glacial acetic acid. Dilute to 500 mL with D.I. H_2O. Mix. Adjust pH to 4.5 ± 0.1 with 100 mM sodium acetate or 100 mM acetic acid. Storage: 25 °C in glass or plastic. Stability: 6 months; Inspect daily for contamination.

1.0 M Acetate Buffer (pH 5.0):
Dissolve 42.9 g sodium acetate trihydrate in 400 mL D.I. H_2O; Add 10.4 mL glacial acetic acid. Dilute to 500 mL with D.I. H_2O. Mix. Adjust pH to 5.0 ± 0.1 with 1.0 M sodium acetate or 1.0 M acetic acid. Storage: 25 °C in glass or plastic. Stability: 6 months; Inspect daily for contamination.

100 mM Acetate Buffer (pH 5.0):
Dilute 40 mL 1.0 M acetate buffer to 400 mL with D.I. H_2O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months

7.4 M Ammonium Hydroxide:
To 50 mL D.I. H_2O add 50 mL concentrated NH_4OH. Mix. Storage: 25 °C in glass or fluoropolymer plastic. Stability: Storage condition dependent.

100 mM Hydrochloric Acid:
To 400 mL D.I. H_2O add 4.2 mL concentrated HCI. Dilute to 500 mL with D.I. H_2O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months

Methanol /Ammonium Hydroxide (98:2):
To 98 mL CH_3OH add 2 mL concentrated NH_4OH. Mix. Storage: 25°C in glass or fluoropolymer plastic. Stability: 1 day.

0.35 M Sodium Periodate:
Add 37.5 g sodium periodate to a 500 mL volumetric flask, dilute to volume with D.I. H_2O. Mix. Stability: 2 mos. at room temperature.

CH_2Cl_2 / IPA / NH_4OH (78:20:2):
To 20 mL IPA, add 2 mL concentrated NH_4OH. Mix. Add 78 mL CH_2Cl_2. Mix. Storage: 25 °C in glass or fluoropolymer plastic. Stability: 1 day

100 mM Phosphate Buffer (pH 6.0):
Dissolve 1.70 g Na_2HPO_4 and 12.14 g NaH_2P0_4 H_2O in 800 mL D.I. H_2O. Dilute to 1000 mL using D.I. H_2O. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH). Storage: 5°C in glass. Stability: 1 month; Inspect daily for contamination.

500 mM Phosphoric Acid:
To 400 mL D.I. H_2O add 17.0 mL concentrated phosphoric acid. Dilute to 500 mL with D.I. H_2O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months

1.0 M Sodium Acetate:
Dissolve 13.6 g sodium acetate trihydrate in 90 mL D.I. H_2O. Dilute to 100 mL with D.I. H_2O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months

100 mM Sodium Acetate:
Dilute 10 mL 1.0 M sodium acetate to 100 mL with D.I. H_2O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months

100 mM Sodium Borate:
Dissolve 3.81 g Na2B4O7•10 H_2O in 90 mL D.I. H_2O. Dilute to 100 mL with D.I. H_2O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months.

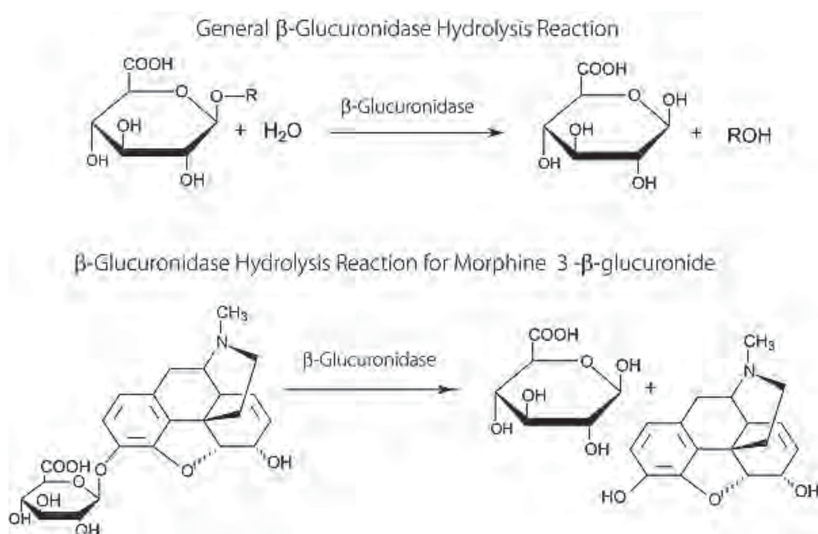
100 mM Sodium Phosphate Dibasic:
Dissolve 2.84 g Na_2HPO_4 in 160 mL D.I. H_2O. Dilute to 200 mL using D.I. H_2O. Mix. Storage: 5 °C in glass. Stability: 1 month; Inspect daily for contamination.

100 mM Sodium Phosphate, Monobasic:
Dissolve 2.76 g NaH_2PO_4-H_2O in 160 mL D.I. H_2O. Dilute to 200 mL with D.I. H_2O. Mix. Storage: 5 °C in glass. Stability: 1 month. Inspect daily for contamination.

100 mM Sulfuric Acid:
To 400 mL D.I. H_2O add 2.7 mL concentrated H_2SO_4. Dilute to 500 mL with D.I. H_2O. Mix.
Storage: 25 °C in glass or plastic. Stability: 6 months

Why is Selectrazyme® β –Glucuronidase derived from red abalone (*Haliotis rufescens*) needed in everyday sample prep?

- In the body's attempt to eliminate xenobiotics, glucuronidation occurs, where the drug is conjugated with glucuronic acid by the human UDP-glucuronosyltransferase family of enzymes. Similar conjugation reactions occur with isoforms of sulfotransferases yielding the sulfate conjugate.
- The glucuronides formed are more polar (water soluble) than the parent compound (original drug) and are generally excreted via the kidney into urine.
- For proper detection of the parent compound and phase 1 metabolites, hydrolysis may be required. This technique is therefore frequently applied to biological fluids, primarily urine.
- Selectrazyme® β -Glucuronidase catalyzes hydrolysis of β-D-glucuronic acid allowing for the determination of total drug concentration versus solely free drug concentration(s).



Benefits to Hydrolyzing with Selectrazyme® β -Glucuronidase

1. Cleaner extracts
2. Stability of analytes due to mild hydrolysis conditions
3. Minimum artifacts present in extracts

Comparing Efficiency of Various Sources of Enzyme

Journal of Analytical Toxicology, Vol. 19, May/June 1995

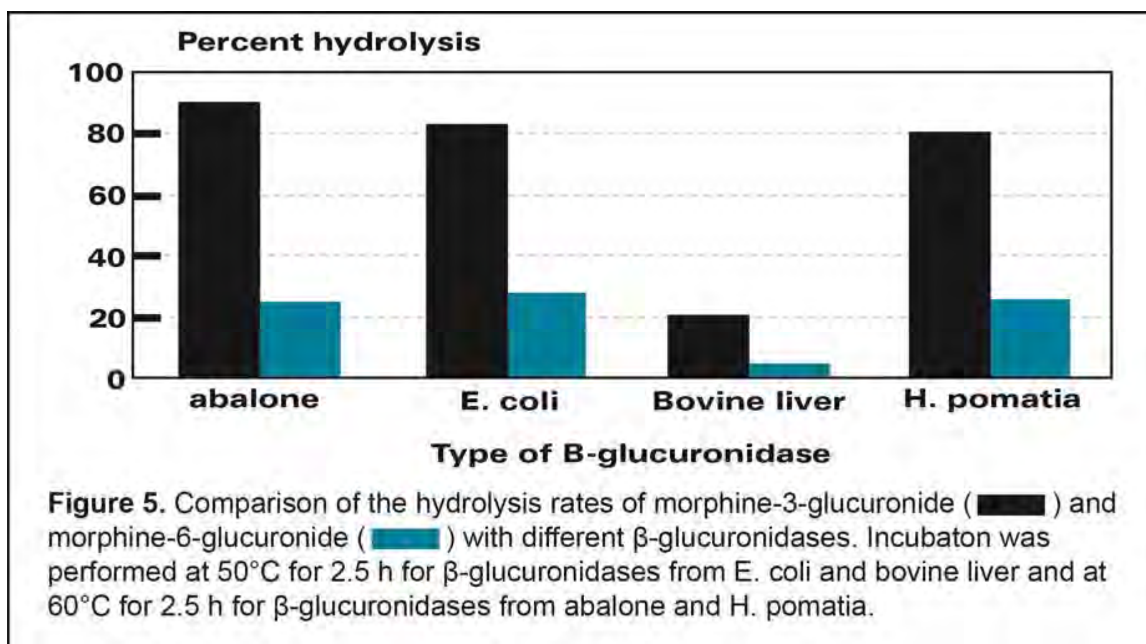


Figure 5. Comparison of the hydrolysis rates of morphine-3-glucuronide ( ) and morphine-6-glucuronide ( ) with different β-glucuronidases. Incubaton was performed at 50°C for 2.5 h for β-glucuronidases from E. coli and bovine liver and at 60°C for 2.5 h for β-glucuronidases from abalone and H. pomatia.

Hydrolysis using Selectrazyme® β -Glucuronidase results in a sample solution containing a significant amount of solubilized enzyme. If not effectively eliminated, this material can accumulate on the head of a guard or analytical column; significantly increasing column backpressure and reducing column lifetime. Strongly consider using SPE prior to analytical injection to not only prolong the life of your HPLC column, but also to produce the cleanest, most accurate results.

Why We Derivatize:

1. To reduce the polarity and enhance the volatility of high molecular weight polar drugs making them more suitable for analysis via GC-MS (Figure 1).
2. To increase the molecular weight of very volatile drugs, thereby resulting in a more complex mass spectrum that improves the selectivity for that particular drug. An important consideration in derivatizing drugs for GC/MS analysis is that the spectrum of the resulting compounds should contain at least three ions that are unique to that analyte and not a result of the matrix.

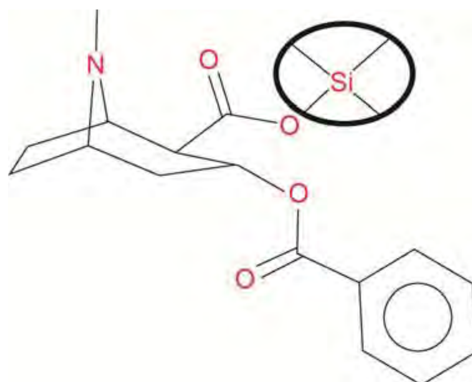


Figure 1. Trimethylsilyl derivative of benzoylecgonine. The underivatized compound has a carboxyl group and is too polar to pass through a GC column.

How to choose a derivatizing agent

Common derivatizing agents for drugs are trifluoroacetic anhydride (TFAA), pentafluoroacetic anhydride (PFAA), and N,O-bis(trimethylsilyl)- trifluoroacetamide (BSTFA, MTBSTFA). TFAA and PFAA react with alcohols, phenols and amines to form floroacyl esters and amides. The main disadvantage of the acid anhydrides is that they are extremely corrosive and can damage the capillary column of the instrument. Excess derivatization reagent must be removed by evaporation prior to injection and the derivatized analytes must be dissolved in a volatile solvent.

For most drugs, conversion to the trimethylsilyl (TMS) ethers, esters and amides, using BSTFA (or MTBSTFA) is found to be very simple and effective. When preparing these derivatives, it is necessary to evaporate the drug extracts to complete dryness prior to derivatization. There is no need to remove any excess BSTFA (MTBSTFA) prior to injection like what is required with agents such as HFAA and PFAA because it does not pose a risk to instrumentation. Another advantage of BSTFA (or MTBSTFA) is that it boils at 145°C at atmospheric pressure; therefore the solvent is not likely to evaporate when stored, increasing its shelf-life.

Derivatizing agents are usually stored at room temperature or in a dessicator. Refrigeration should be avoided due to humid conditions shortening the life and effectiveness of the product.

Common compounds found in a forensic/clinical setting along with their targeted functional groups and derivatizatizing agent of choice for are listed in Table1.

Drug	Derivatized Functional Group	Derivative (BSTFA unless other specified)
amphetamine	-N_2H	Spectrum of BSTFA derivative not definitive; therefore prepare the 4-carbethoxyhexafluorobutyryl amide (4-CB)
methamphetamine	-NH_2	Spectrum of BSTFA derivative not definitive; therefore prepare the 4-carbethoxyhexafluorobutyryl amide (4-CB)
phentermine	-NH_2	Spectrum of BSTFA derivative not definitive; therefore prepare the 4-carbethoxyhexafluorobutyryl amide (4-CB)
cocaine	none	
benzoylecgonine	-CO_2H	TMS ester
morphine	-OH (two)	di-TMS ether
codeine	-OH	mono-TMS ether
6-monoacetylmorphine	-OH	mono-TMS ether
dihydrocodeine	-OH	mono-TMS ether
hydrocodone	enol –OH formed from =O	mono-TMS ether di-TMS ether
oxycodone	-OH enol –OH formed from =O	TMS ether di-TMS ether
norcodeine	-OH -NH_2	One peak: TMS ether and TMS amide
hydromorphone	-OH enol –OH formed from =O	mono-TMS ether di-TMS ether
oxymorphone	-OH (two) enol –OH formed from =O	mono-TMS ether di-TMS ether tri-TMS ether
phencylidine (PCP)	none	
9-carboxy-11-nor-Δ^9-tetrahydrocannabinol	-OH -CO_2H	One peak: mono-TMS ether and mono-TMS ester

Classification of Derivatizing Agents

Silylation

Silylation is the most popular derivatization procedure for GC sample analysis. Silylation reagents are easy to use and readily form derivatives. In silylation, an active hydrogen such as that found in acids, alcohols, thiols, amines, amides, enolizable ketones and aldehydes is replaced by trimethylsilyl (TMS) or t-butyldimethylsilyl (t-BDMS). Compared to their parent compounds, silyl derivatives are more volatile, less polar, and more thermally stable. As a result, GC separation is improved and detection is enhanced.

Acylation

Acylation reagents are typically available as acid anhydrides, acyl derivatives, or acyl halides. Acylation reagents offer similar advantages to silylation reagents. They create less polar, more volatile derivatives. As opposed to silylating reagents, acylating reagents target highly polar, multi-functional compounds, such as carbohydrates and amino acids. Acylating reagents also introduce electron-capturing groups to the derivatized sample; enhancing analytical detection. Acyl halides and acyl derivatives are highly reactive. Typically they are used where steric hindrance may be an issue. Due to the acidic nature of these reagents any excess material or byproducts must be removed prior to sample analysis to prevent GC inlet or column degradation.

Alkylation

Alkylation reagents replace active hydrogens with an alkyl group. These reagents are used to modify compounds having acidic hydrogens, such as carboxylic acids and phenols. Alkylation reagents can be used alone to form esters, ethers, and amides or they can be used in combination with acylation or silylation reagents. Esterification is the most popular method of alkylation. Alkyl esters are stable and form quickly and quantitatively. Alteration of the length of the substituted alkyl group can be used to alter the GC retention times of derivatives.

Good HPLC Practices

Technical Tip #1:
HPLC Column Care: Based upon decades of HPLC Technical Service experience, the number one mistake, made by experienced and novice chromatographers alike, is the improper changing of mobile phase solvents, such as in the two scenarios below. Incorrect washing procedures often lead to precipitated buffer in the column that can cause changes in retention and high back pressure.

Don't Do:
Scenario 1: An analyst stores his column in high percent organic solvent. He places the column on the HPLC and begins to equilibrate the column with a buffered mobile phase.

Do:
Wash the column with > 50 percent D.I. H_2O before beginning the equilibration with buffer.

Don't Do:
Scenario 2: An analyst finishes his method development project that used a buffered mobile phase. He begins pumping a high percent organic through the column to store it in that solvent.

Do:
Wash the column with > 50 percent D.I. H_2O before beginning the high percent organic wash.

Technical Tip #2:
To obtain the highest efficiency and greatest sensitivity, use HPLC flow rates that provide the optimum linear velocity (OLV) for the particular column employed. This is more important when using larger particle size packings (e.g., 5µm and larger), and less of an issue when using smaller particle packings (e.g., 3µm and smaller), or core-shell particles. The OLV is related to the optimum flow rate (OFR), and is dependent on the internal diameter (i.d.) of the HPLC column:

Column i.d. (mm)	Typical OFR (mL/min)
2.1	0.21
3	0.43
4	0.76
4.6	1.0
10	4.7

III. Technical Tip #3:
Running HPLC chromatograms at elevated temperatures can provide several advantages:

- Lowers mobile phase solvent viscosity and column/system backpressure
- Enhances peak efficiency and sensitivity
- Helps to prevent perturbations in baseline due to room temperature fluctuations
- Provides somewhat faster elution times
- Helps to provide more reproducible chromatographic results

However, when performing chiral separations, a lower column temperature is often advantageous in obtaining greater resolution between enantiomers.

IV. Technical Tip #4:
The standard operating pH range for most silica-based HPLC columns is pH 2 to 8. However, some C18 phases (e.g., UCT Selectra® C18) contain high carbon loading (19% C) that enables the analyst to run methods at an elevated pH (pH 10 or even pH 12) for an extended period of time, if necessary. The higher carbon load is thought to partially shield the silica backbone from alkaline hydrolysis.

V. Technical Tip #5:
Particularly in performing LC-MS method development work, try using Hydrophilic Interaction Liquid Chromatography (HILIC) instead of Reversed Phase Mode (RP) chromatography. The HILIC Mode uses higher concentrations of organic in the mobile phase that allows more efficient desolvation in the mass spectrum instrument. This typically results in increased sensitivity. There are a variety of columns available that can perform HILIC separations, such as the Selectra® PFPP and Selectra® Mixed-Mode I phases.

V1. Technical Tip #6:
When a mass spectrometer is used as the LC detector (LC-MS), the mobile phase must be volatile in order for the LC-MS interface to adequately vaporize it. If you utilize a buffer that is non- volatile, such as phosphate or citrate, a large quantity of it could pass into ESI or APCI, initiating a large amount of salt formation in the MS. This build up has been known to quench ionization in electrospray LC-MS, leading to lower sensitivity of analytes.

Buffers	pH Range	LC-MS Compatible
Phosphate (pK_1)	1.1 – 3.1	X
Phosphate (pK_2)	6.2 – 8.2	X
Phosphate (pK_3)	11.3 – 13.3	X
Acetate*	3.8 – 5.8	YES
Citrate (pK_1)	2.1 – 4.1	X
Citrate (pK_2)	3.7 – 5.7	X
Citrate (pK_3)	4.4 – 6.4	X
Trifluoroacetic acid (0.1%)	2.0	YES
Phosphoric acid (0.1%)	2.0	X
Formic acid (0.1%)	2.7	YES
Ammonium formate	2.7 – 4.7	YES
Ammonium bicarbonate	6.6 – 8.6	YES
Borate	8.3 -10.3	YES
*suitable for LC-MS as ammonium acetate		

Screening Methods

CPS analitica for Chemistry

ACIDIC/NEUTRAL/BASIC ANALYTES IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN XCEL® I EXTRACTION COLUMN

Part #:
CSXCE106 – CLEAN SCREEN XCEL® I 130 mg, 6 mL Tube
BETA-GLUC-10 – SELECTRAZYME® Beta-glucuronidase
SLDA50ID21-5UM – Selectra® DA HPLC Column 50 x 2.1 mm, 5µm

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
   NOTE: See Hydrolysis step if required

   Hydrolysis: To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase.
   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 1-2 hours at 65°C.
   (Hydroxylamine can be added to sample here if oxime derivative is preferred.)
   Allow sample to cool

2. **APPLY SAMPLE:**

   Load sample directly to column without any preconditioning
   Pull sample through at a rate of 1-2 mL/ minute
   Dry column thoroughly under full vacuum or positive pressure for 1 minute

3. **WASH 1 (ACIDIC/ NEUTRAL –FRACTION 1):**
   1 x 1 mL of 0.1M Acetic Acid

   - Apply pressure to column for ~1minute(vacuum (10mm Hg) or positive pressure(~80-100 psi) to make certain the entire sample and any residual is pulled through to waste

   1 x 2 mL Hexane to remove residual aqueous phase

   - Dry column (5 minutes at full vacuum or pressure)

4. **ELUTION 1 (ACIDIC/ NEUTRAL COMPOUNDS – FRACTION 1):**

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

5. **DRY ELUTE:**

   Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C

   Reconstitute with 100 µL of Ethyl Acetate or Mobile Phase

6. **WASH 2 (BASIC COMPOUNDS-FRACTION 2):**
   1 x 1 mL of 2% Acetic Acid/98% Methanol

   Dry column 5 minutes at full vacuum (10mm Hg) or positive pressure (~80-100 psi)

7. **ELUTION 2 (BASIC COMPOUNDS-FRACTION 2):**

   1 x 1 mL of CH_2Cl_2/ IPA/ Ammonium Hydroxide (78/20/2).

8. **DRY ELUTE:**

   Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C. Take care not to overheat or over evaporate. Certain compounds are heat labile, such as the amphetamines and phencyclidine. Reconstitute with 100 µL of Ethyl Acetate or Mobile Phase

Notes:
(1) Fraction 1 (Acid Neutrals) and Fraction 2 (Bases) can be combined together if need be. The Acid/ Neutral fraction tends to be dirtier than the Basic one, so for more effective results, keep fractions separate.

(2) A keeper solvent such as DMF can be used to prevent the volatilization of amphetamines and phencyclidine.Use 30-50 µL of high purity DMF in the sample (Fraction 2) before evaporation.

(3) A 1% HCl in CH_3OH solution has been used to prevent volatization by the formation of the hydrochloric salt of the drugs. Add 1 drop of the solution prior to evaporating than continue to dryness.

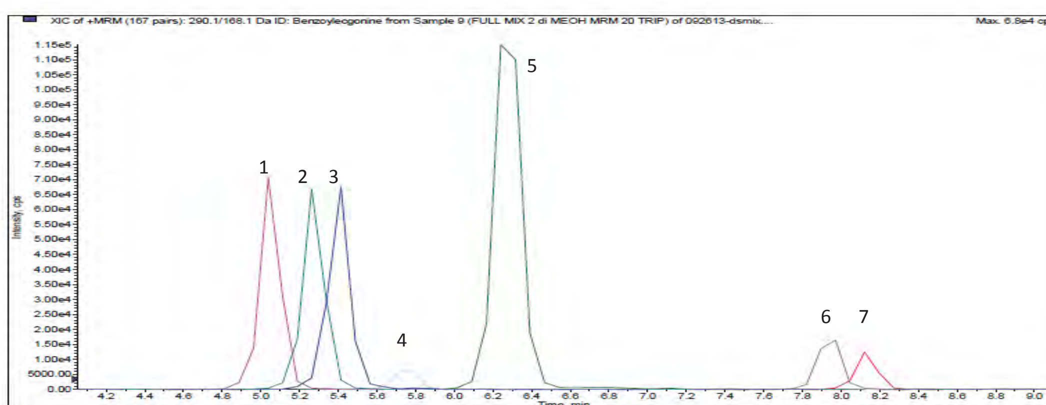
(4) The hexane wash step can be removed if user is looking to analyze for Parent THC

(5) To extract the benzodiazepine group at higher recovery, following the elution of the Acidic/Neutral drugs, a second elution can be done prior to moving on to the second wash phase. The second elution solvent would consist of 98% Ethyl Acetate/ 2% Ammonium Hydroxide.

INSTRUMENT CONDITIONS (LC-MS/MS):

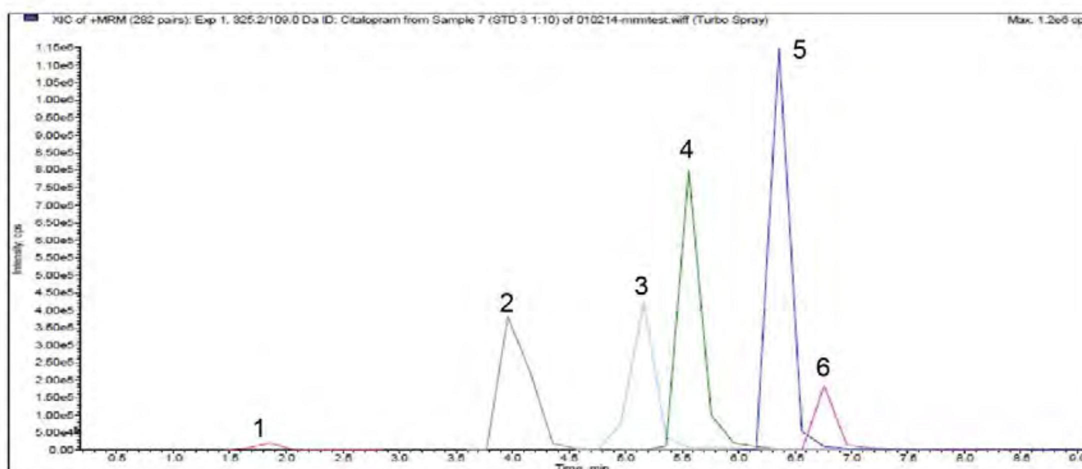
<u>CHROMATOGRAMS</u>

Basic Panel 1



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Tapentadol	**222.2**	**107.2**	**5.10**
2. Tramadol	**264.2**	**58.0**	**5.25**
3. Benzoylecgonine	**290.1**	**168.1**	**5.40**
4. Meperidine	**248.2**	**220.0**	**5.75**
5. Cocaine	**304.1**	**182.1**	**6.30**
6. Fentanyl	**337.2**	**188.2**	**7.90**
7. Buprenorphine	**468.3**	**396.3**	**8.15**

Basic Panel 2



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Clonidine	**230.0**	**213.0**	**1.80**
2. Ketamine	**238.1**	**125.0**	**4.00**
3. Mirtazepine	**266.2**	**195.1**	**5.10**
4. Clozapine	**327.1**	**270.1**	**5.60**
5. Citalopram	**325.2**	**109.0**	**6.40**
6. Norfluoxetine	**296.2**	**134.2**	**6.80**

PARAMETERS

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

Flow Rate: 0.5 mL/minute **Polarity**: Positive

Injection Volume: 20 µL

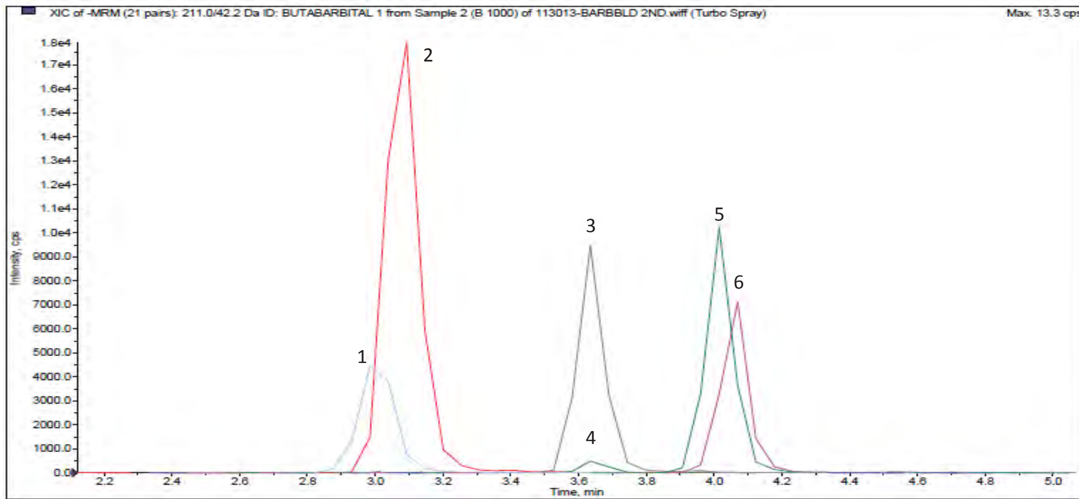
LC Column: Selectra® DA HPLC Column 50 x 2.1mm 5 µm

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

Gradient:

Time	%A	%B
0.00	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

Barbiturates



Analyte	MRM Transitions		Relative Retention Time (minutes)
	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	

REPRESENTATIVE ANALYTES EXTRACTED

AMPH/METHAMP
MDMA/MDA/MDEA
OPIATES(12)
METHADONE/EDDP
SYMPATHOMIMETICS

MEPERIDINE/NORMEPERIDINE
COCAINE/BE/EME
TCA'S(7)
BARBITURATES
FENTANYL/NORFENTANYL

CARBEMAZEPINE
IBUPROFEN
CARISOPRODAL/MEPROBAMATE

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

Part #:
CSDAU – CLEAN SCREEN® DAU
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
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
   Note: See Hydrolysis step if required

   Hydrolysis: To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase.
   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 1-2 hours at 65°C.
   (Hydroxylamine can be added to sample here if oxime derivative is preferred.)
   Allow sample to cool

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 (10 minutes at full vacuum or pressure)
   1 x 2 mL hexane to remove residual aqueous phase

5. **ELUTE ACIDIC AND NEUTRAL DRUGS (FRACTION 1):**
   1 x 3 mL Hexane: Ethyl Acetate (50:50)
   Collect eluate at 1 to 2 mL/minute

6. **DRY ELUATE:**
   Evaporate to dryness at < 40°C
   Reconstitute with 100 µL of Ethyl Acetate or Mobile Phase

7. **WASH COLUMN:**
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

8. **ELUTE BASIC ANALYTES:**
   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute
   NOTE: Prepare elution solvent daily
   Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

9. **DRY ELUATE:**
   Evaporate to dryness at < 40°C. Take care not to overheat or over evaporate. Certain compounds are heat labile, such as the amphetamines and phencyclidine.
   Reconstitute with 100 µL Ethyl Acetate or Mobile Phase

Notes:

(1) Fraction 1 (Acid Neutrals) and Fraction 2 (Bases) can be combined together if need be. The Acid/ Neutral fraction tends to be dirtier than the Basic one, so for more effective results, keep fractions separate.

(2) A keeper solvent such as DMF can be used to prevent the volatilization of amphetamines and phencyclidine.Use 30-50 µL of high purity DMF in the sample (Fraction 2) before evaporation.

(3) A 1% HCl in CH_3OH solution has been used to prevent volatization by the formation of the hydrochloric salt of the drugs. Add 1 drop of the solution prior to evaporating than continue to dryness.

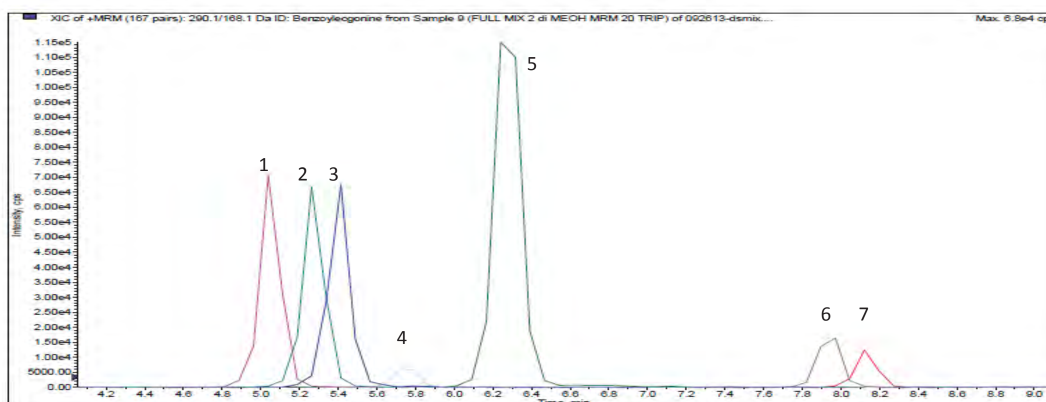
(4) The hexane wash step can be removed if user is looking to analyze for Parent THC

(5) To extract the benzodiazepine group at higher recovery, following the elution of the acidic/neutral drugs, a second elution can be done prior to moving on to the second wash phase. The second elution solvent would consist of 98% Ethyl Acetate/ 2% Ammonium Hydroxide.

INSTRUMENT CONDITIONS (LC-MS/MS):

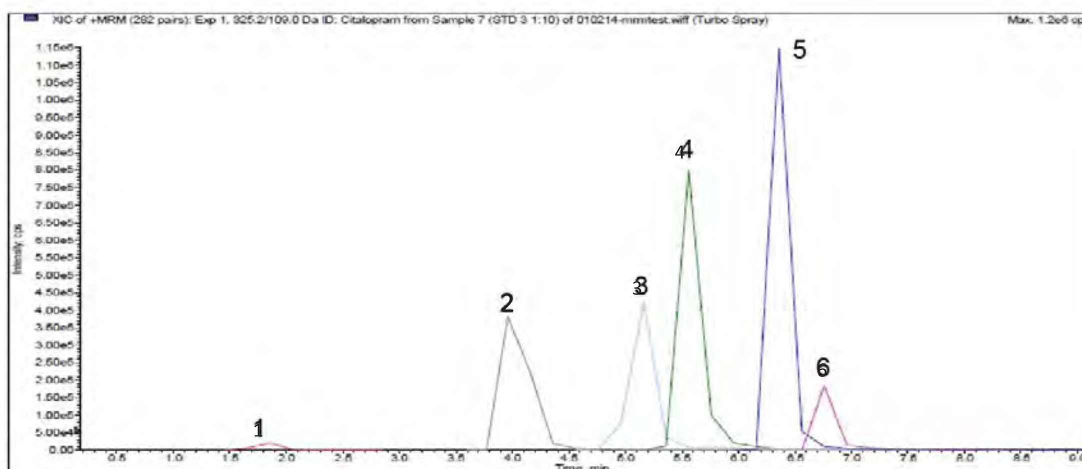
CHROMATOGRAMS

Basic Panel 1



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylecgonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Clonidine	**230.0**	**213.0**	**1.80**
2. Ketamine	**238.1**	**125.0**	**4.00**
3. Mirtazepine	**266.2**	**195.1**	**5.10**
4. Clozapine	**327.1**	**270.1**	**5.60**
5. Citalopram	**325.2**	**109.0**	**6.40**
6. Norfluoxetine	**296.2**	**134.2**	**6.80**

PARAMETERS

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

Flow Rate: 0.5 mL/minute **Polarity**: Positive

Injection Volume: 20 µL

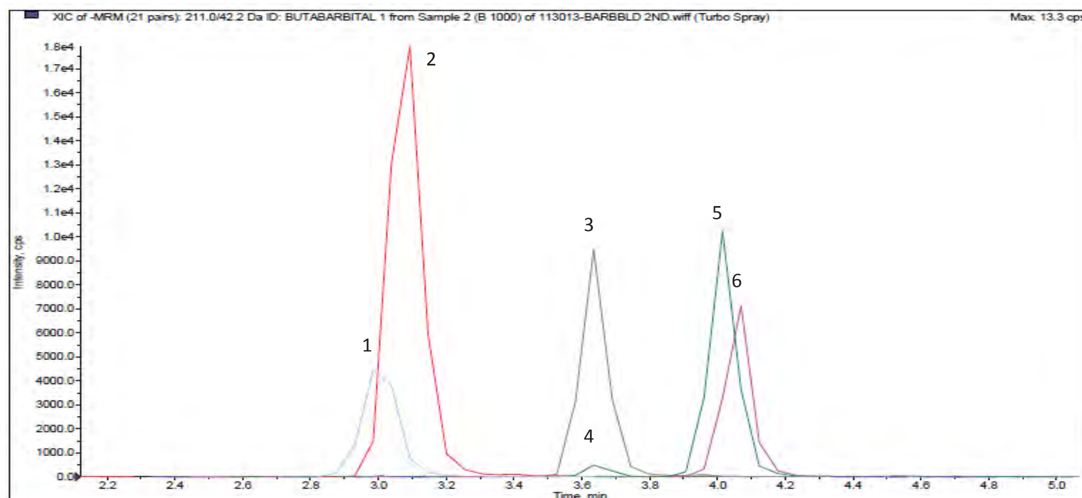
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Gradient:

Time	%A	%B
0.00	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

Barbiturates



Analyte	MRM Transitions		Relative Retention Time (minutes)
	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	

CLEAN SCREEN® DAU
Forensic Applications

Data Provided By:
City of Philadelphia,
Department of Public Health Office of the Medical Examiner
321 University Avenue Philadelphia, Pennsylvania 19104

The following are some of the many compounds that have been extracted from forensic samples with the CLEAN SCREEN® DAU bonded silica extraction cartridge (Part #: CSDAU303):

I. ACIDIC / NEUTRAL DRUG FRACTION (A)

Acetaminophen
Barbiturates
Benzoic acid
Caffeine
Carbamazepine
Carisoprodol
Chlorpropamide
Clonazepam
Cotinine
Diazepam
Glutethimide and metabolite
Ibuprofen
Meprobamate
Methyl salicylate
Nordiazepam
Phenytoin
Primidone
Salicylic acid
Theophylline
Thiopental

II. BASIC DRUG FRACTION (B)

Amantadine
Amitriptyline and metabolite
Amphetamine
Benzocaine
Benzoylecgonine
Benztropine
Bromodiphenhydramine
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Cocaine and metabolite
Codeine
Cresol
Dextromethorphan
Dextrorphan
Thioridazine
Dihydrocodeine
Dihehydramine
Doxepin and metabolite
Ephedrine
Fluoxetine
Imipramine and metabolite
Ketamine
Lidapine
Loxapine
Meperidine
Methadone and metabolite
Methamphetamine
Methyl ρ-aminobenzoate
Methyl benzoate
Methyl ecgonine
Methylparaben
Trazodone
Methylphenidate
Methyprylon and metabolites
Morphine
Nicotine
Oxycodone
Pentazocine
Phencyclidine
Phenethylamine
Phentermine
Phenylpropanolamine
Procaine
Propoxyphene and metabolite
Propylparaben
Tranylcypromine
Trifluoperazine
Trimipramine

Acid & Neutral Drugs

CPS analitica for Chemistry

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

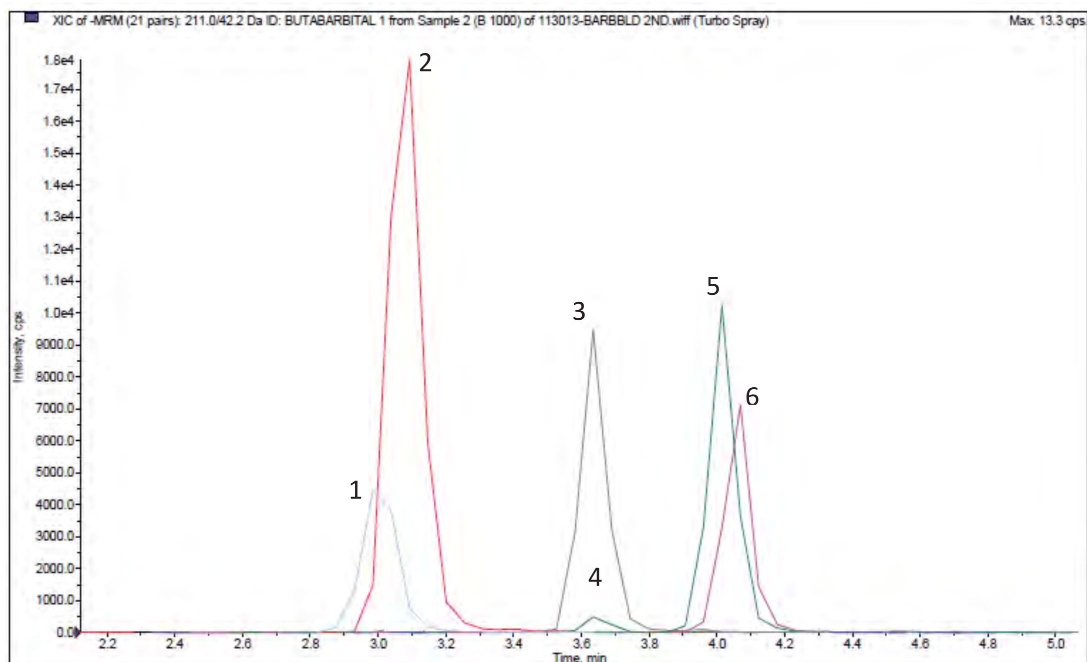
7. **RECONSTITUTE / DERIVATIZE:**

   - **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
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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

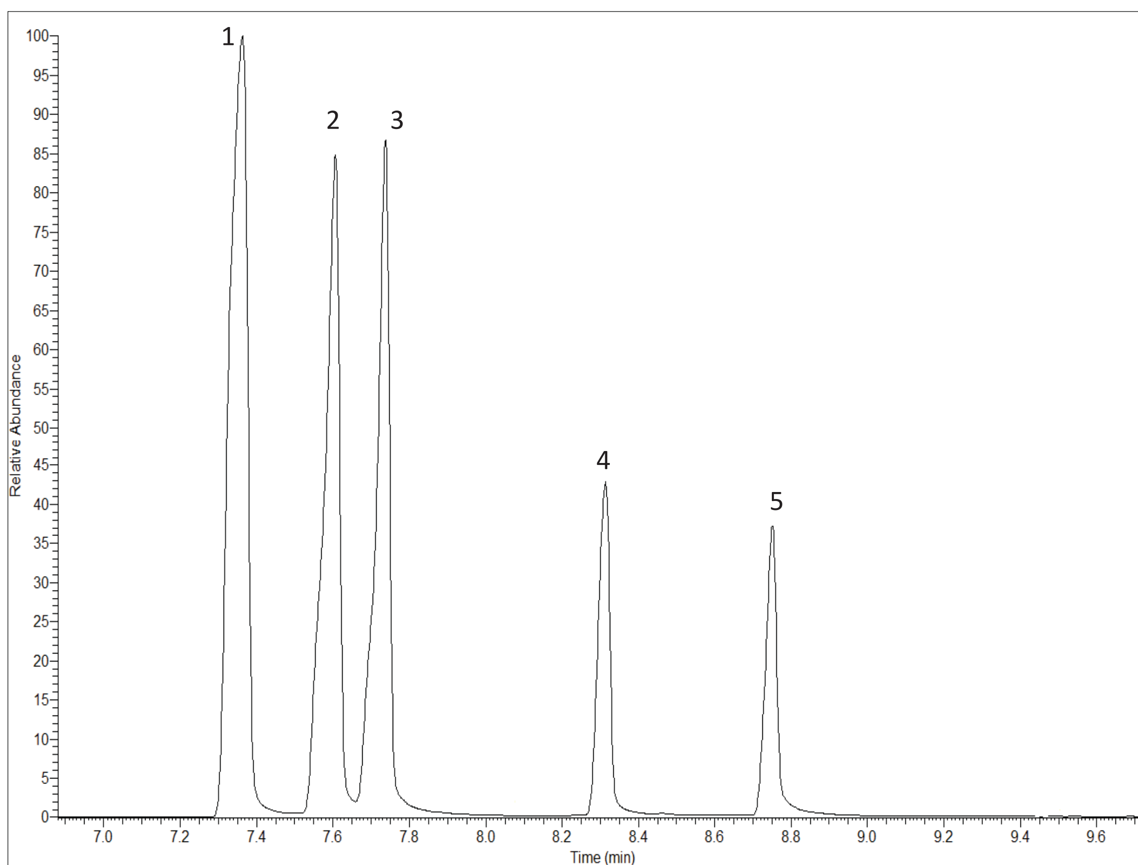
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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):

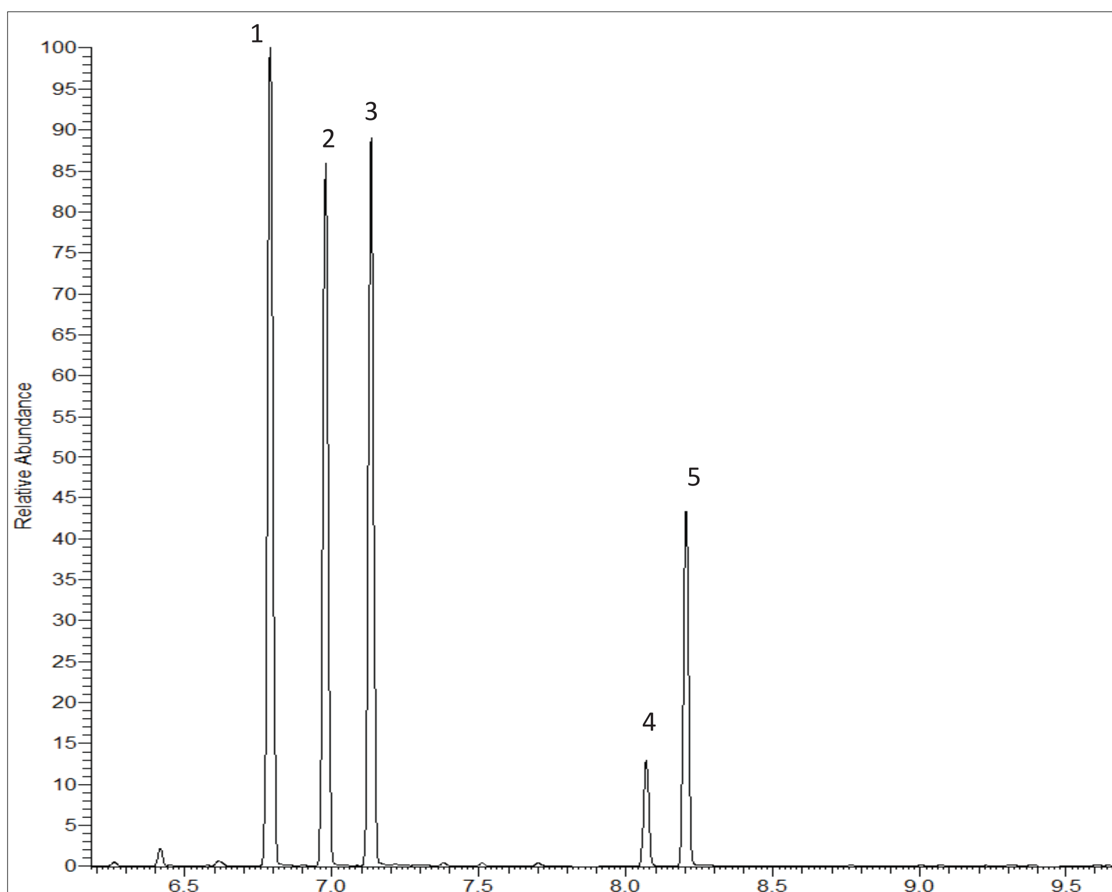
CHROMATOGRAM 1 (UNDERIVATIZED)



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

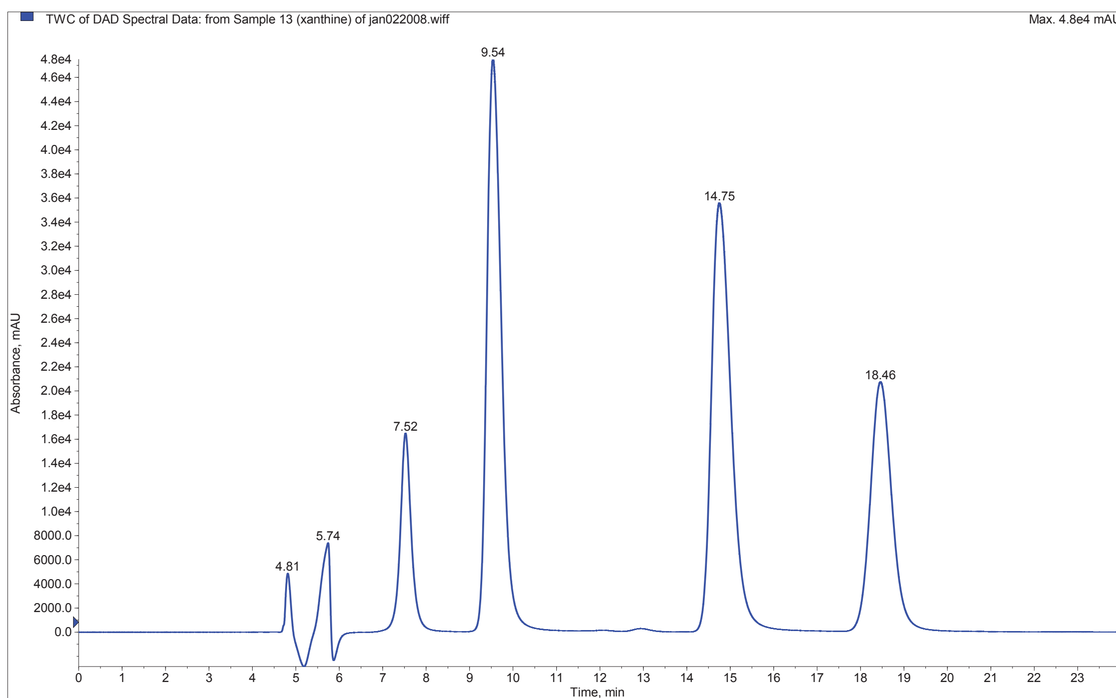
Column Temperature: ambient

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

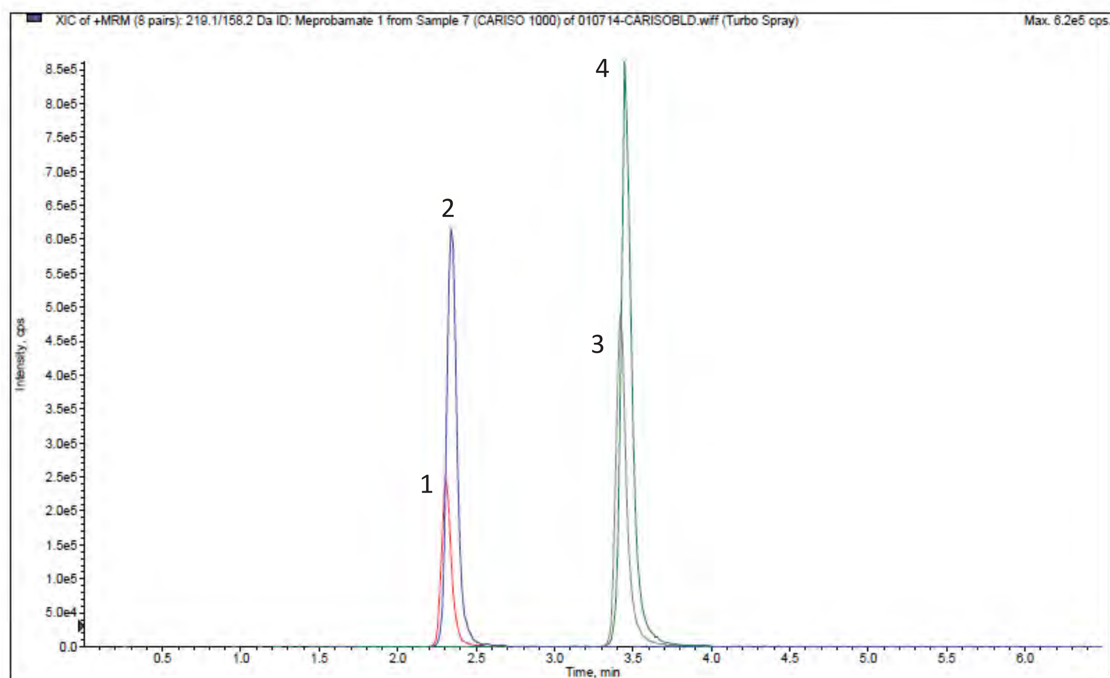
6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

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

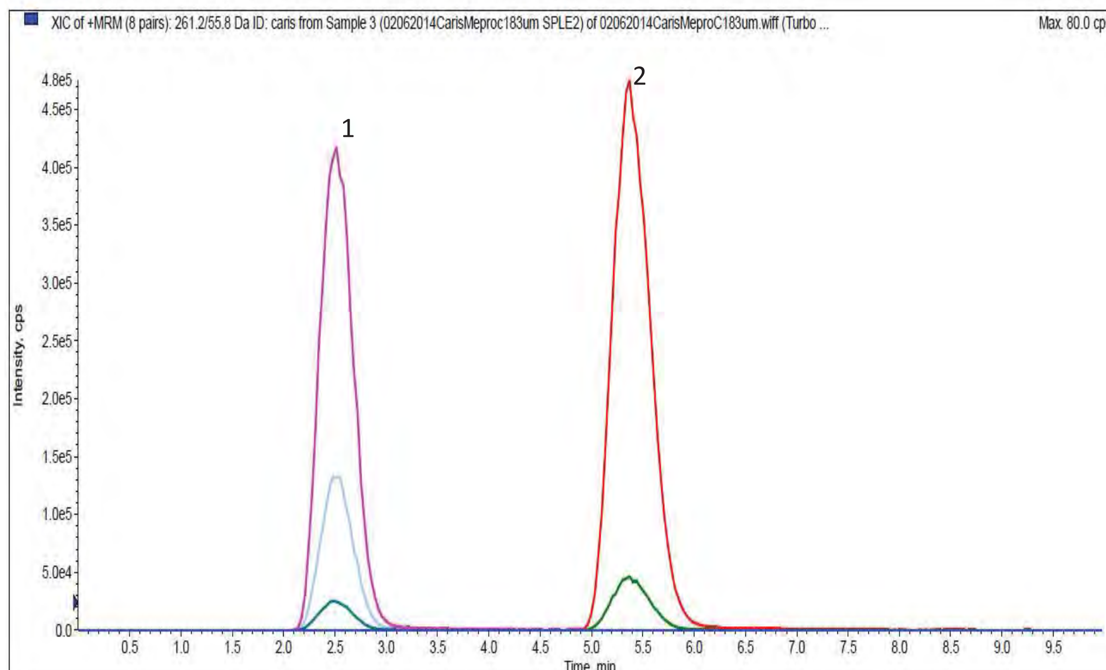
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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

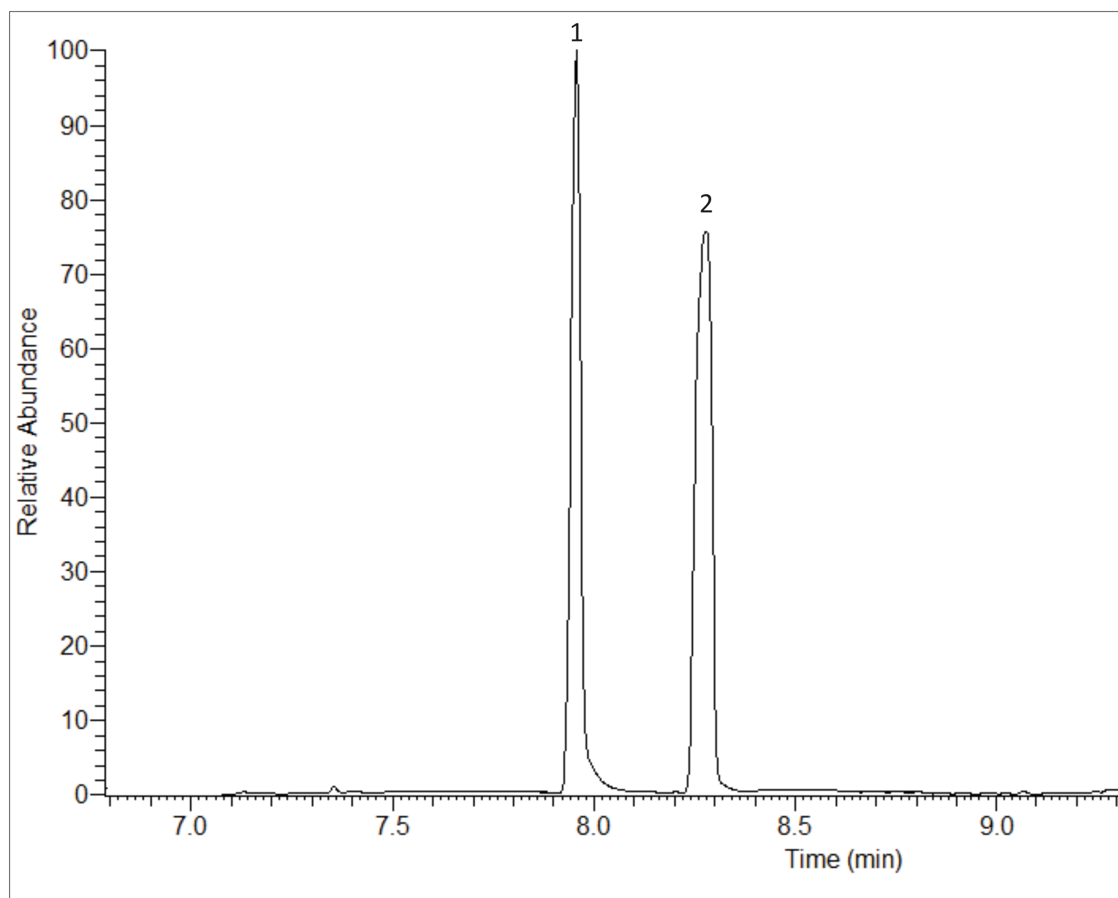
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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

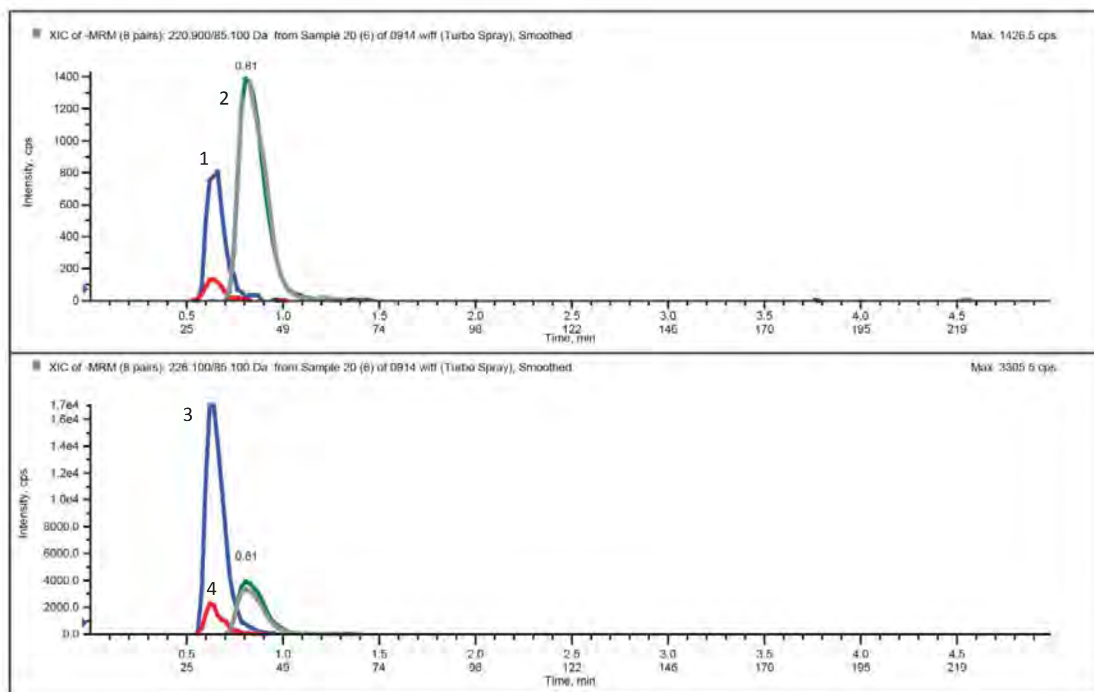
   Collect eluate at 1 to 2 mL/minute

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).

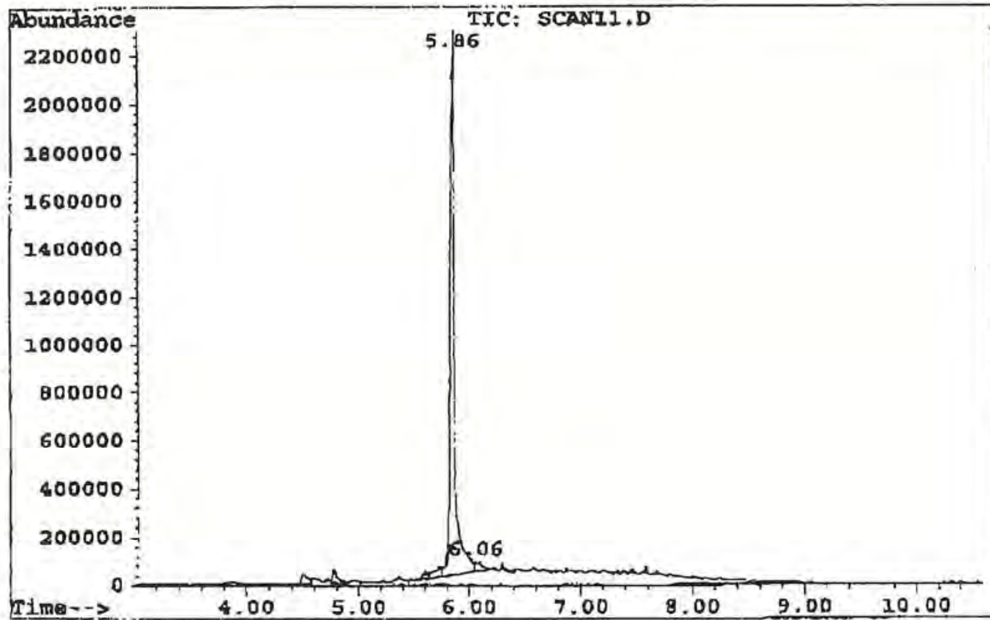
NOTES:

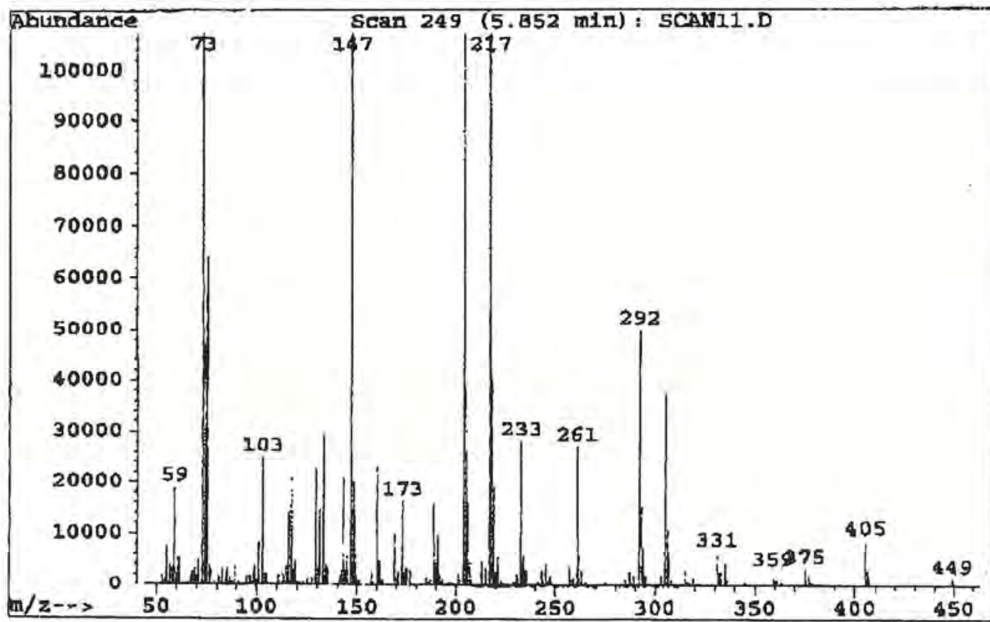
The prepared buffer should be filtered 30-45 minutes (equilibrated) before analysis for constant results.

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.

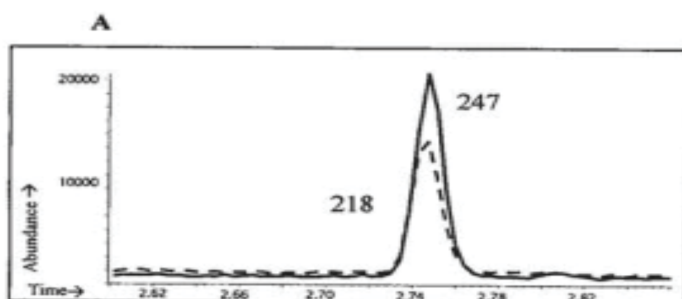
6. **DRY ELUATE:**
   Dry under a stream of nitrogen at < 35 °C.

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



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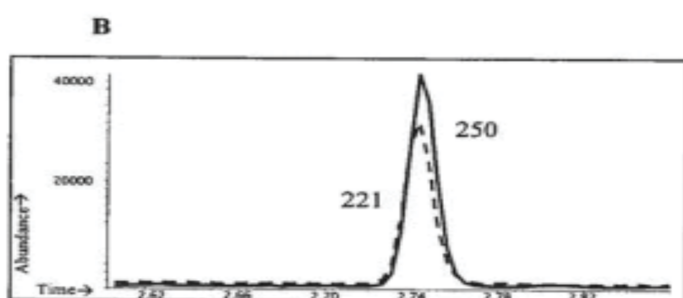


Analyte	Quantify Ion	Qualifier Ion	Relative Retention Time (minutes)
1. Methylmalonic Acid	247	218	2.76
2. Methylmalonic Acid-D_3	250	221	2.74

PARAMETERS

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

GC capillary column: RtxR-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.

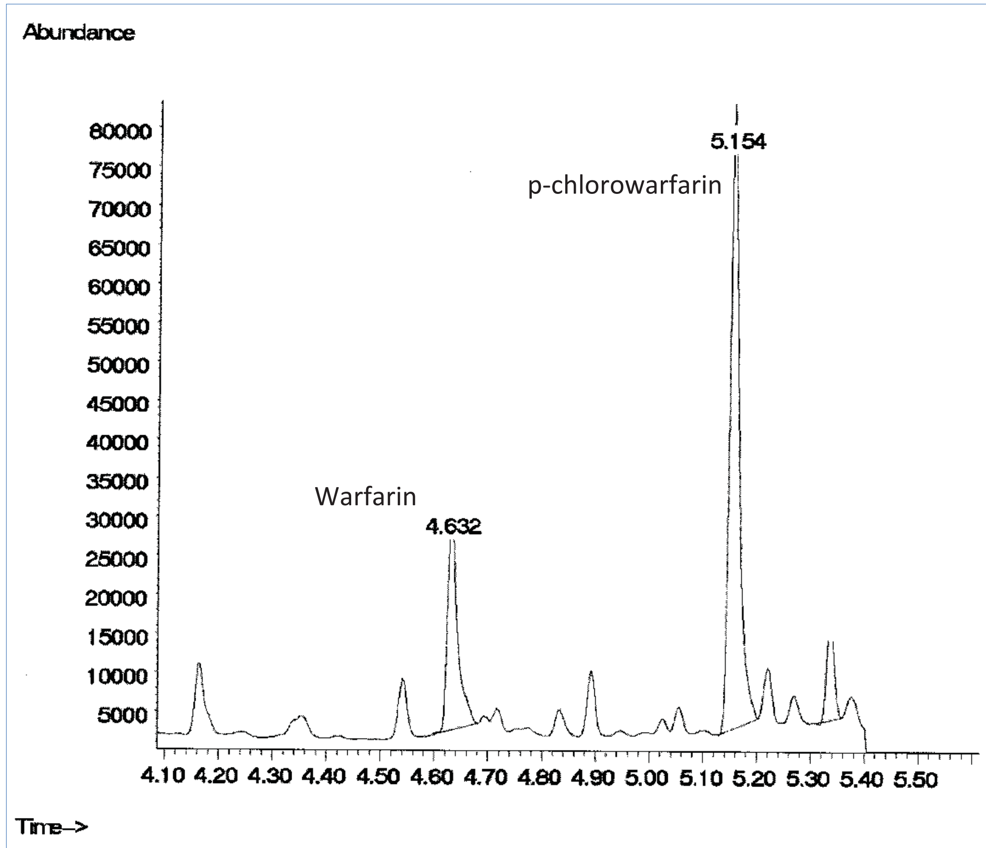
6. **Collect eluates at approx 1-2 mL/minute**

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)



Basic Drugs

CPS analitica for Chemistry

ANTIDEPRESSANTS 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

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 acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE ANTIDEPRESSANTS:**
   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2 v/v)
   Collect eluate at 1 to 2 mL/minute
   or
   1 x 3 mL Ethyl Acetate/ IPA/ NH_4OH (78:20:2 v/v)

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add Ethyl Acetate (pH 11-12)

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

7. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.
   - **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

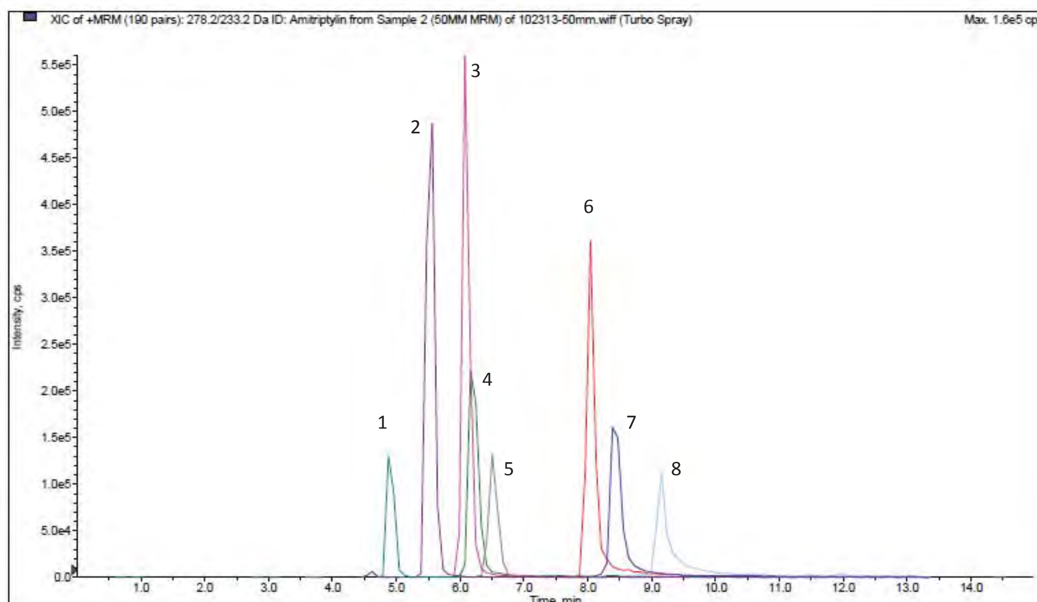
   Alternate Derivatization

   Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and react at 70 °C for 30 minutes; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.Venlafaxaine	278.2	260.2	4.90
2.Zolpidem	308.2	235.2	5.50
3.Trazadone	372.2	176.1	6.05
4.PCP	244.2	86.1	6.20
5.Quintiapine	384.2	253.1	6.50
6.Imipiramine	281.2	86.1	8.40
7.Amitriptyline	278.2	233.2	8.42
8.Sertraline	306.1	159	9.25

PARAMETERS

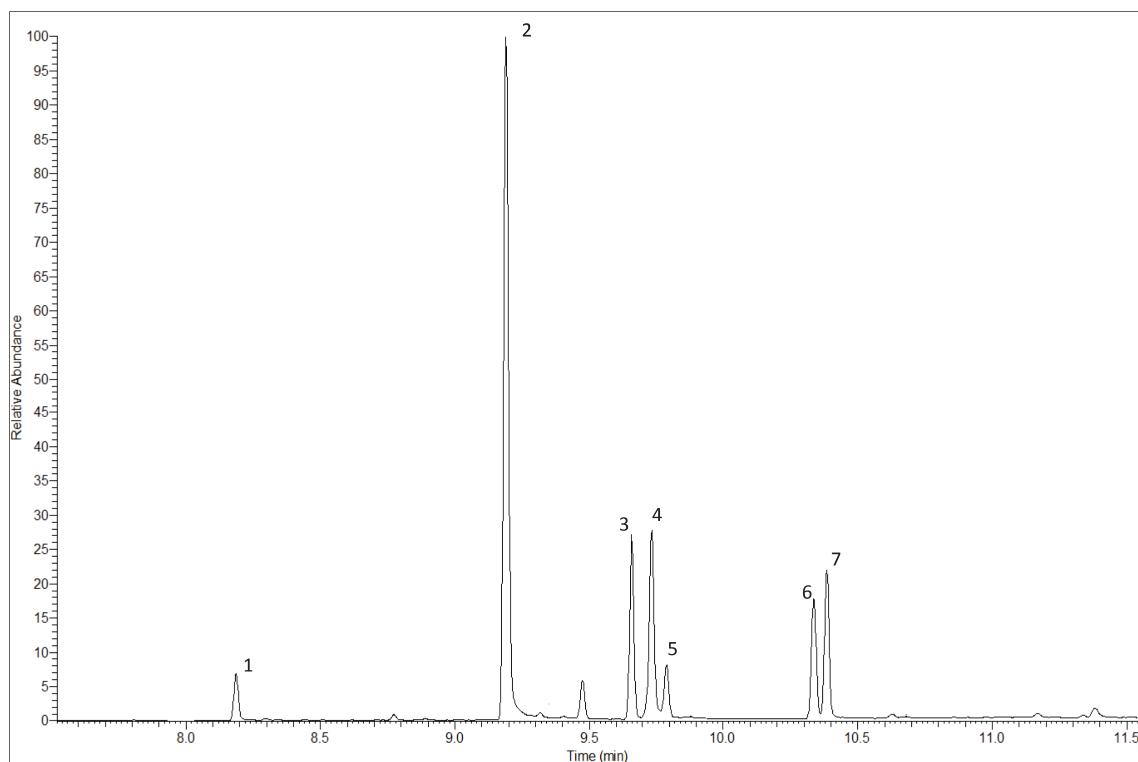
Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.5 mL/minute
Polarity: Positive
Injection Volume: 20 µL
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time minutes
1. Fluoxetine	309	91	104	8.19
2. Venlafaxine	134	179	202	9.19
3. Amitriptyline	115	203	202	9.66
4. Nortryptyline	189	202	115	9.72
5. Imipramine	193	280	234	9.77
6. Sertraline	274	262	159	10.34
7. Citalopram	324	208	238	10.39

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 (1.2 mL/minute)

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

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

Part #
CSDAU - CLEAN SCREEN® DAU SPE cartridge
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50x2.1 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
   NOTE: See Hydrolysis step if required

Hydrolysis: To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase. Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C. (Hydroxylamine can be added to sample here if oxime derivative is preferred.) Allow sample to cool

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 acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE BASIC ANALYTES:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute

   NOTE: Prepare elution solvent daily
   Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

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

7. **RECONSTITUTE / DERIVATIZE:**

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 20 µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

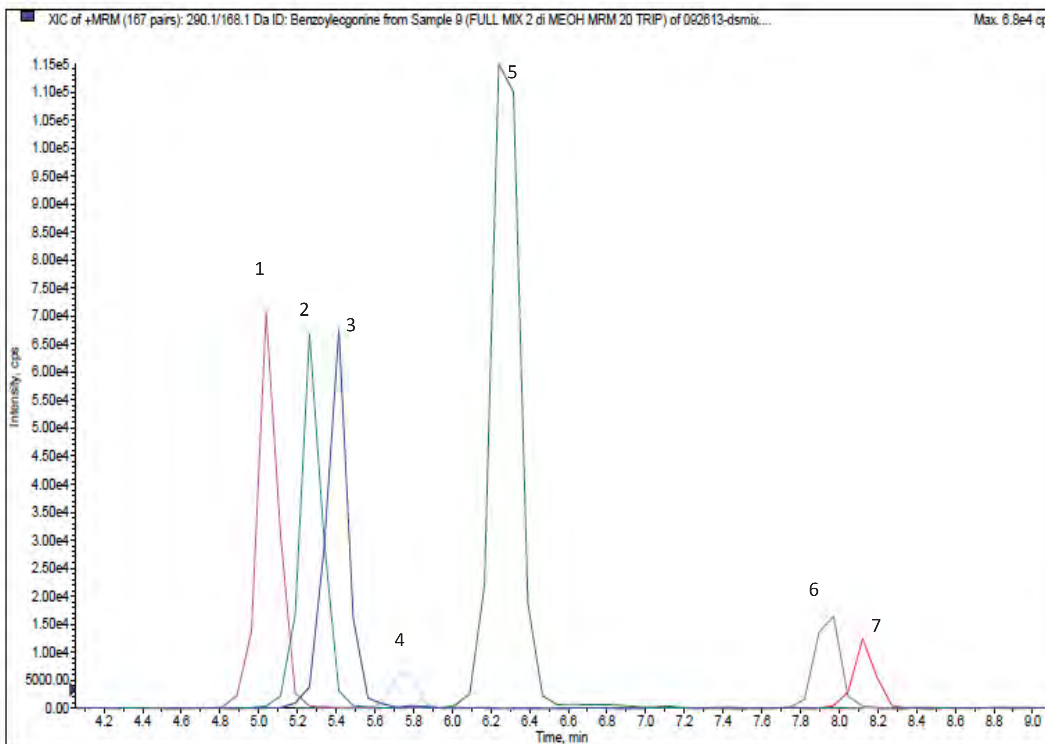
Alternate Derivatization

Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and react at 70 °C for 30 minutes; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

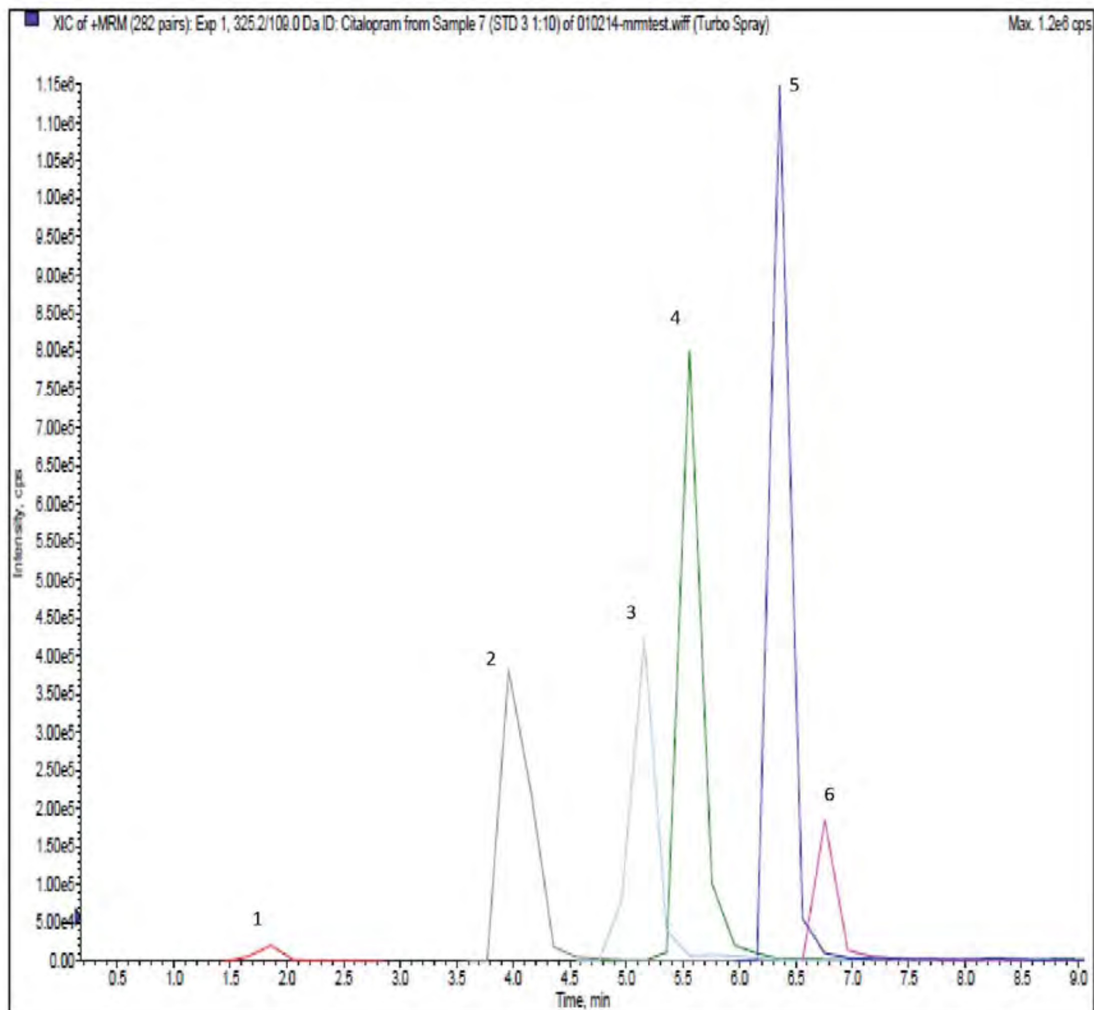
CHROMATOGRAMS

Basic Panel 1



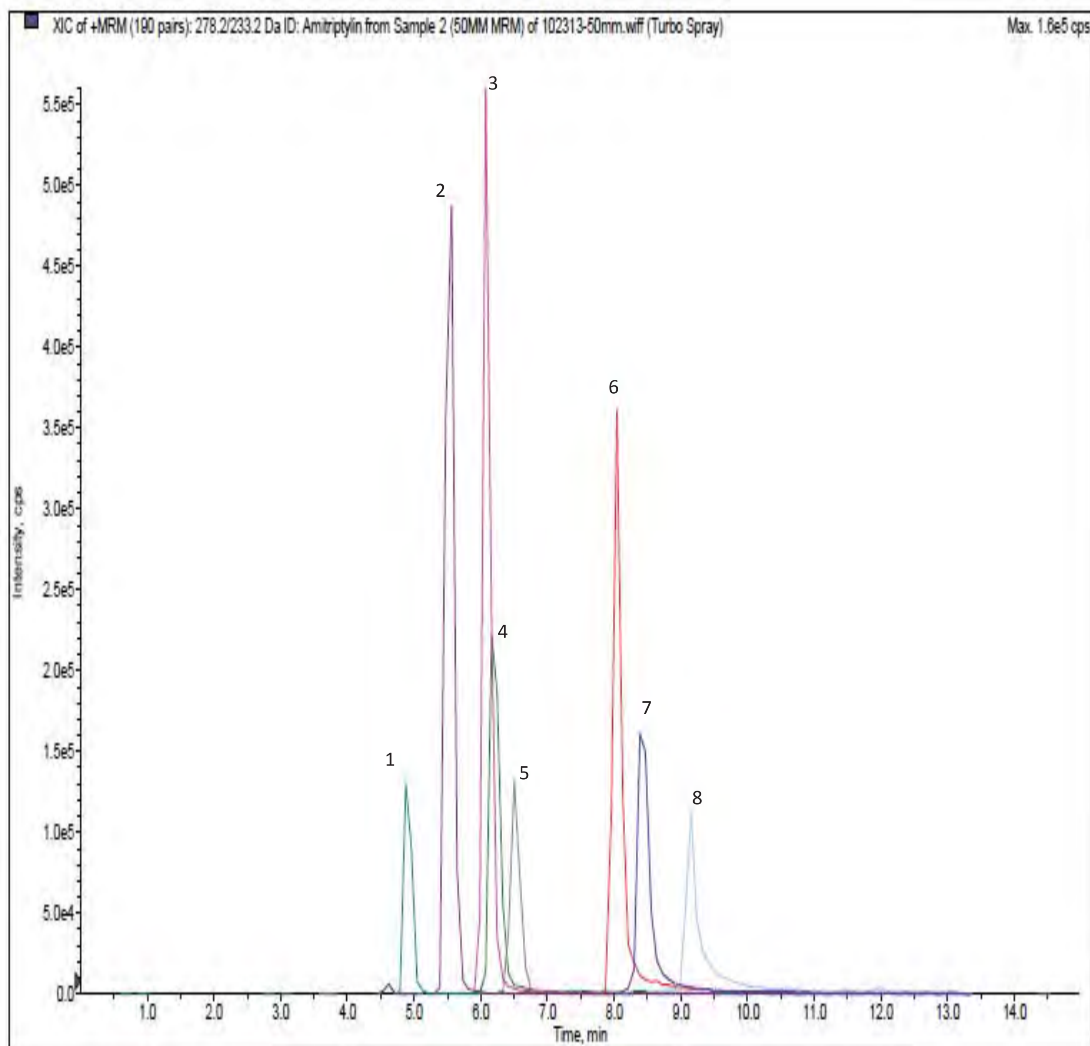
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.Tapentadol	222.2	107.2	5.10
2.Tramadol	264.2	58.0	5.25
3.Benzoylecgonine	290.1	168.1	5.40
4.Meperidine	248.2	220.0	5.75
5.Cocaine	304.1	182.1	6.30
6.Fentanyl	337.2	188.2	7.90
7.Buprenorphine	468.3	396.3	8.15

Basic Panel 2



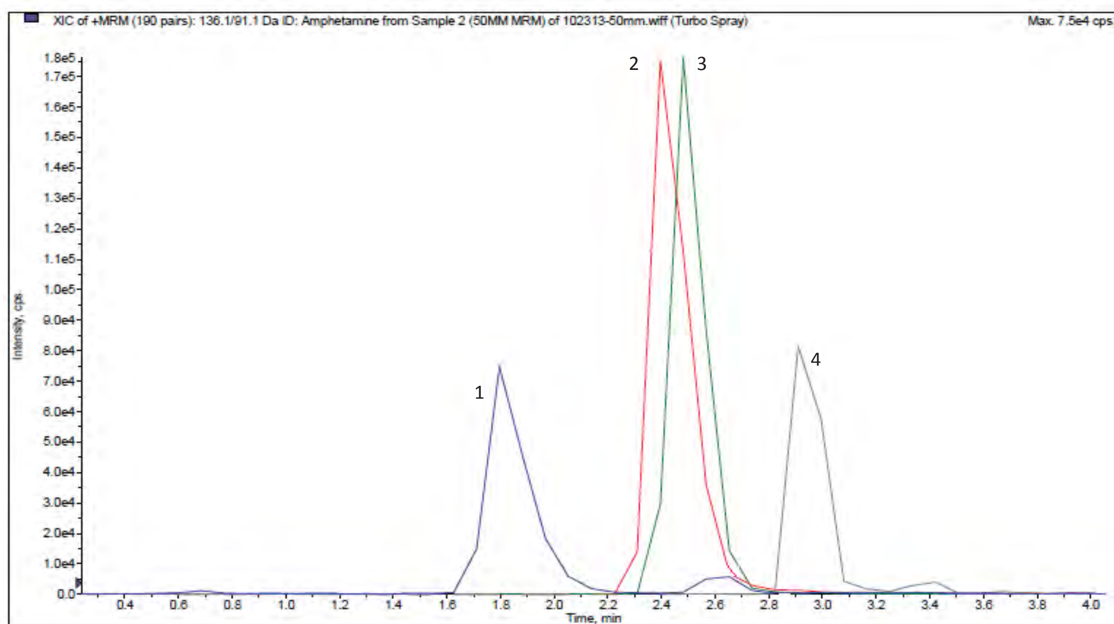
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxaine	278.2	260.2	4.90
2. Zolpidem	308.2	235.2	5.50
3. Trazadone	372.2	176.1	6.05
4. PCP	244.2	86.1	6.20
5. Quintiapine	384.2	253.1	6.50
6. Imipiramine	281.2	86.1	8.40
7. Amitriptyline	278.2	233.2	8.42
8. Sertraline	306.1	159	9.25

Amphetamine Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	136.1	91.1	1.18
2. Methamphetamine	150.1	91.1	2.40
3. MDA	180.1	105.0	2.45
4. MDMA	194.1	105.1	2.95

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 20 µL

L 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	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

REPRESENTATIVE ANALYTES EXTRACTED

AMPH/METHAMP
SYMPATHOMIMETICS
TCA'S(7)

MDMA/MDA/MDEA
MEPERIDINE/NORMEPERIDINE
CYCLOBENZAPRINE
DIPHENHYDRAMINE

OPIATES(7)
PCP
FENTANYL/NORFENTANYL
CITALOPRAM

METHADONE/EDDP
COCAINE/BZE
SERTRALINE TRAMADOL/NORTRAM
CLONIDINE

BASIC ANALYTES IN BLOOD/URINE/SERUM BY LC-MS/MS OR GC-MS CLEAN SCREEN® XCEL I 96 WELLPLATE

Part #
WSH96EXE11 – CLEAN SCREEN XCEL® I 130 mg, 96 well plate
BETA-GLUC-10 – Selectrazyme® Beta-Glucuronidase
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**

   To 1-2 mL whole blood, plasma/ serum or urine add 500 µL 100mM phosphate buffer (pH 6.0)

   Add appropriate volume and concentration of internal standard.

   Note: See Hydrolysis step if required

   Hydrolysis: To 1-2 mL of urine sample, add 500 µL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase. Optionally, add 500 µL of acetate buffer and 25 µL of concentrated β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C. (Hydroxylamine can be added to sample here if oxime derivative is preferred.)
   Allow sample to cool
   Do not adjust pH~ sample is ready to be added to the extraction plate.

2. **APPLY SAMPLE**

   Load sample directly to column without any preconditioning.
   Pull sample through at a rate of 1-2 mL/ minute.
   Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. **WASH**

   1 x 1 mL 98% Methanol: 2% Acetic Acid
   Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. **ELUTION**

   1 x 1 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. **DRY ELUTE**

   Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. **RECONSTITUTE / DERIVATIZE**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.
   - **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

   Alternate Derivatization

   Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and
   react at 70 °C for 30 minutes; Cool and inject 1-2 µL

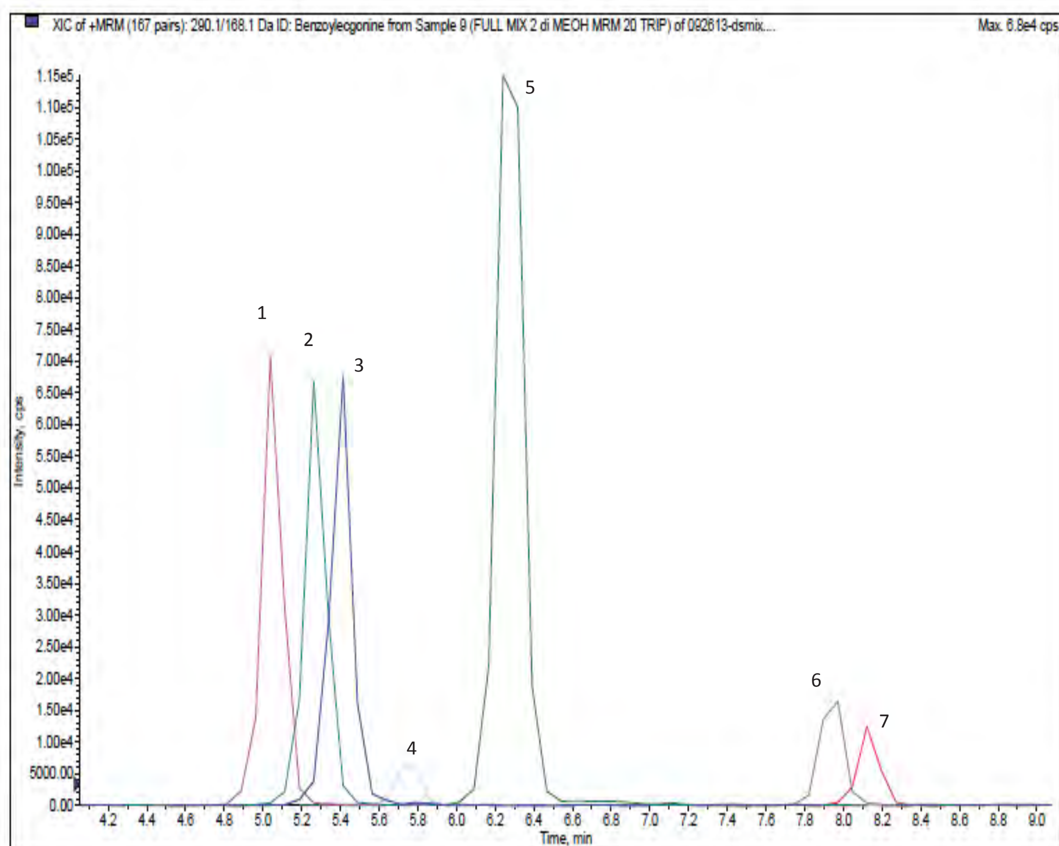
NOTES

(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis, if being used. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.

INSTRUMENT CONDITIONS (LC-MS/MS):

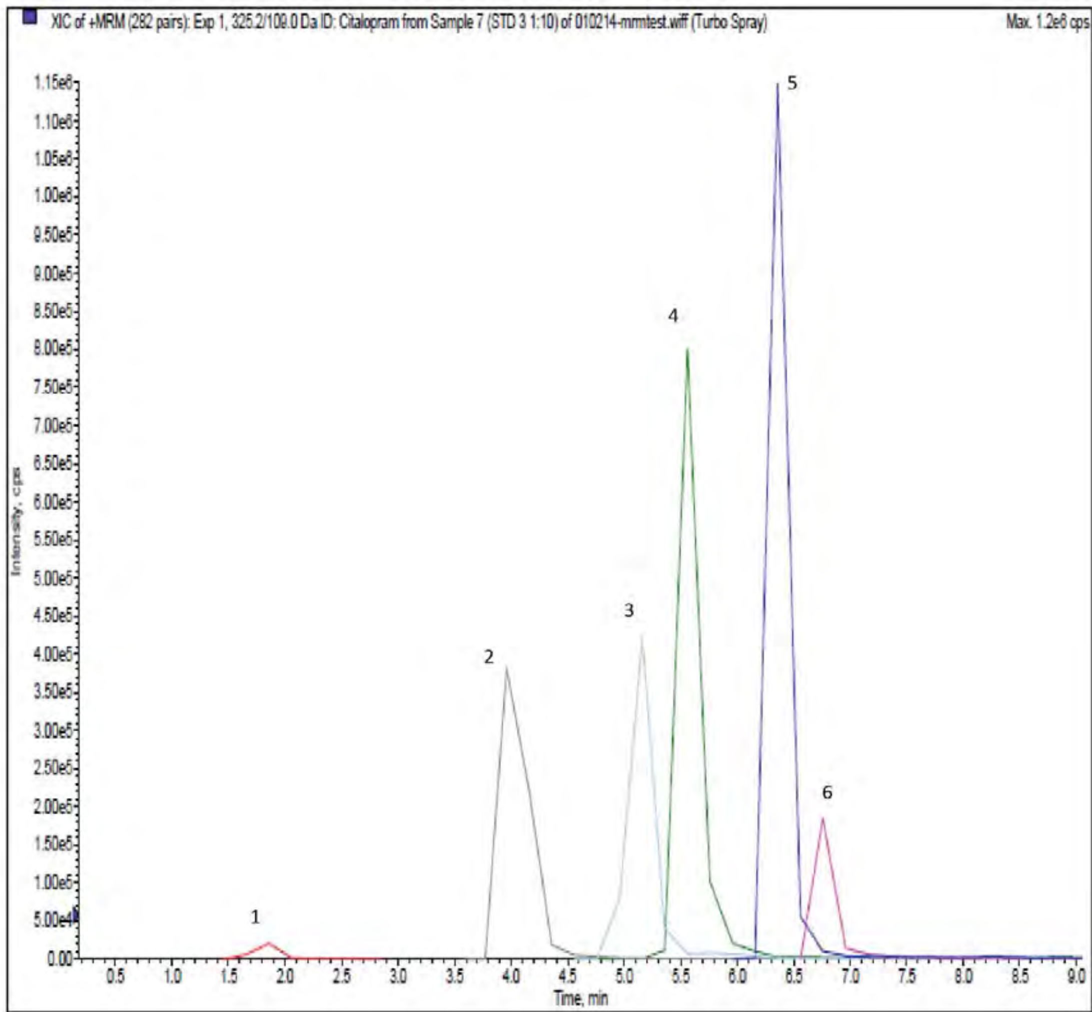
CHROMATOGRAMS

Basic Panel 1



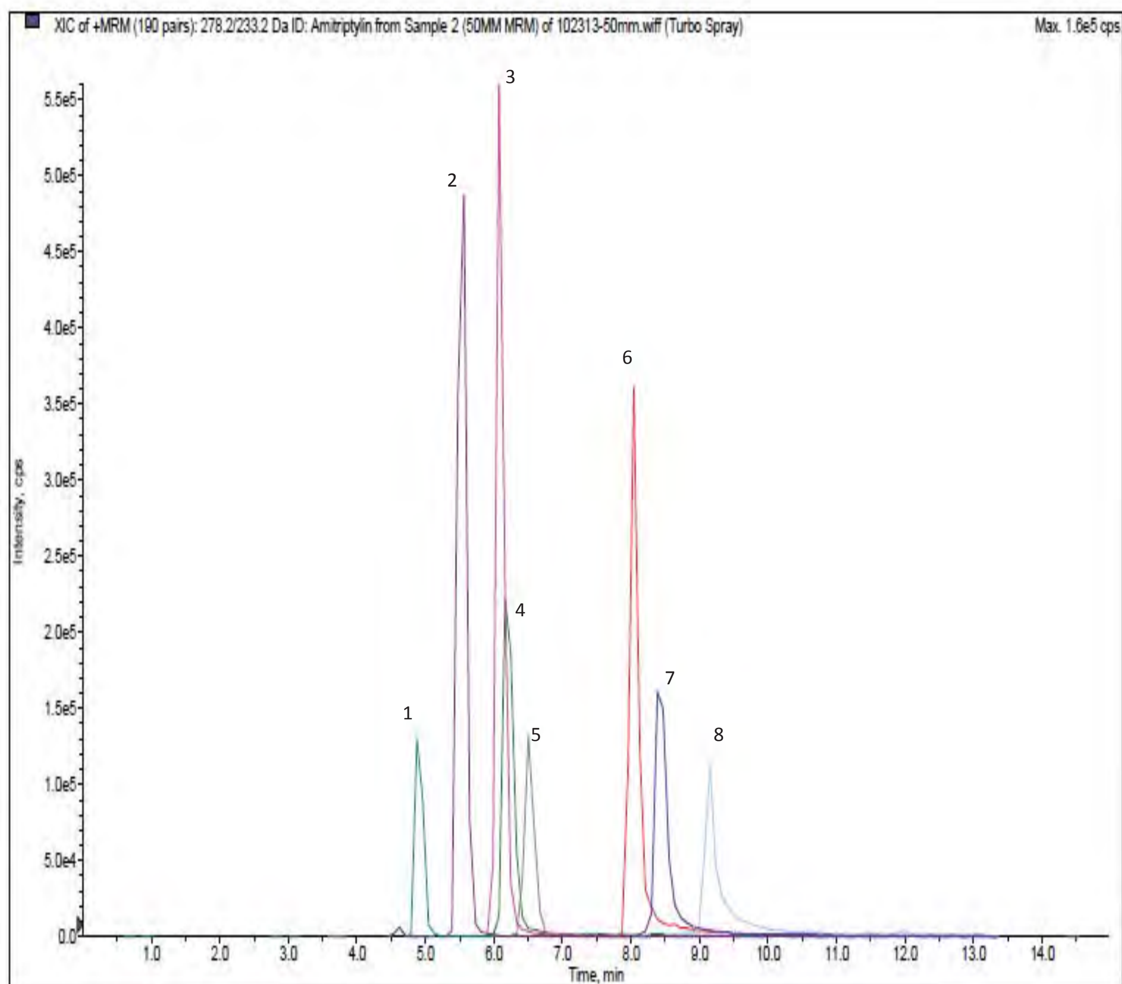
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylecgonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



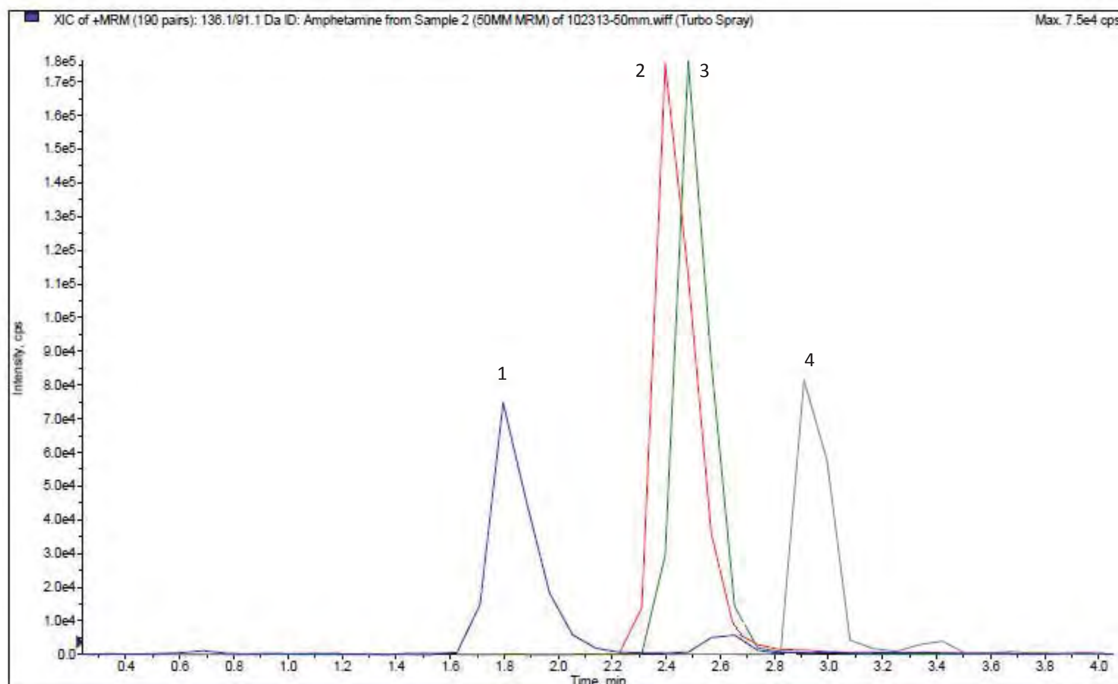
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxaine	278.2	260.2	4.90
2. Zolpidem	308.2	235.2	5.50
3. Trazadone	372.2	176.1	6.05
4. PCP	244.2	86.1	6.20
5. Quintiapine	384.2	253.1	6.50
6. Imipiramine	281.2	86.1	8.40
7. Amitriptyline	278.2	233.2	8.42
8. Sertraline	306.1	159	9.25

Amphetamine Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	136.1	91.1	1.18
2. Methamphetamine	150.1	91.1	2.40
3. MDA	180.1	105.0	2.45
4. MDMA	194.1	105.1	2.95

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 20 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	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

REPRESENTATIVE ANALYTES EXTRACTED

AMPH/METHAMP
SYMPATHOMIMETICS
TCA'S(7)
MDMA/MDA/MDEA
MEPERIDINE/NORMEPERIDINE
CYCLOBENZAPRINE
DIPHENHYDRAMINE
OPIATES(7)
PCP
FENTANYL/NORFENTANYL
CITALOPRAM
METHADONE/EDDP
COCAINE/BZE
SERTRALINE TRAMADOL/NORTRAM
CLONIDINE

BASIC ANALYTES IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN XCEL® I EXTRACTION COLUMN

Part #
CSXCE111 – CLEAN SCREEN XCEL® I 130 mg, 1 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
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
Note: See Hydrolysis step if required

Hydrolysis: To 1-2mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase. Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C.
(Hydroxylamine can be added to sample here if oxime derivative is preferred.)
Allow sample to cool

2. **APPLY SAMPLE**

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. **WASH**

1 x 3 mL 98% Methanol: 2% Acetic Acid
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. **ELUTION**

1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. **DRY ELUTE**

Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. **RECONSTITUTE / DERIVATIZE**

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 20 µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

Alternate Derivatization

Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and react at 70 °C for 30 minutes; Cool and inject 1-2 µL

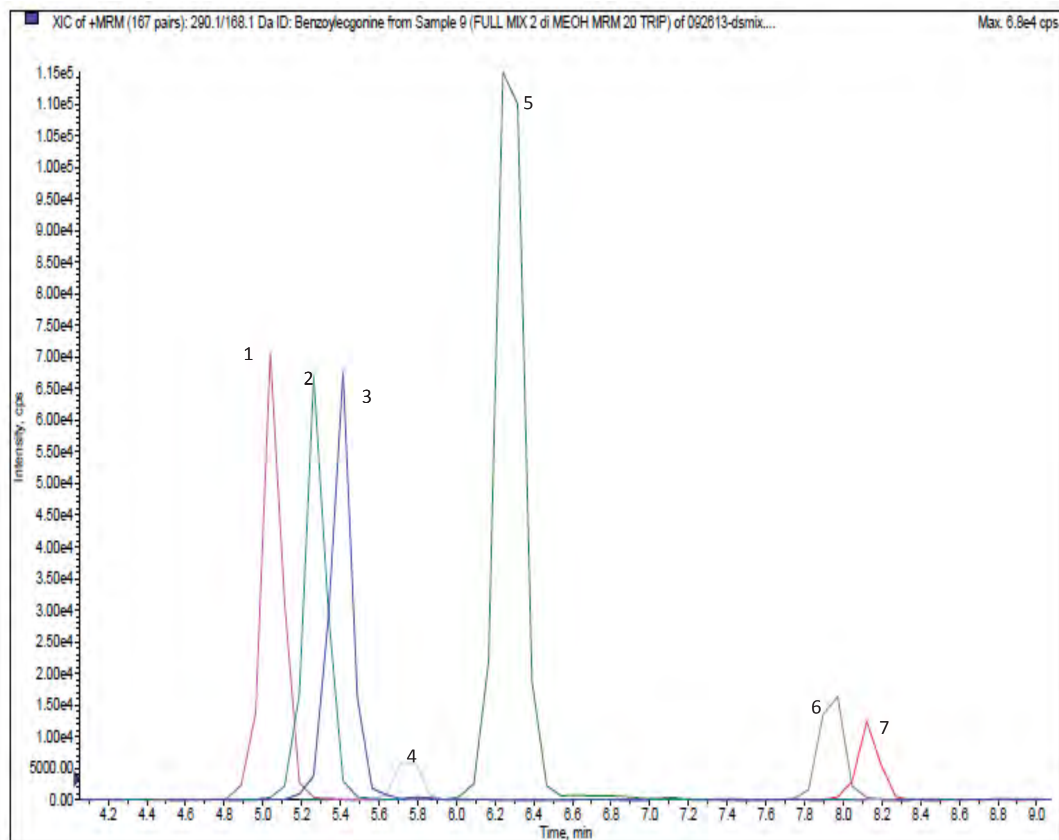
NOTES

(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis, if being used. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.

INSTRUMENT CONDITIONS (LC-MS/MS):

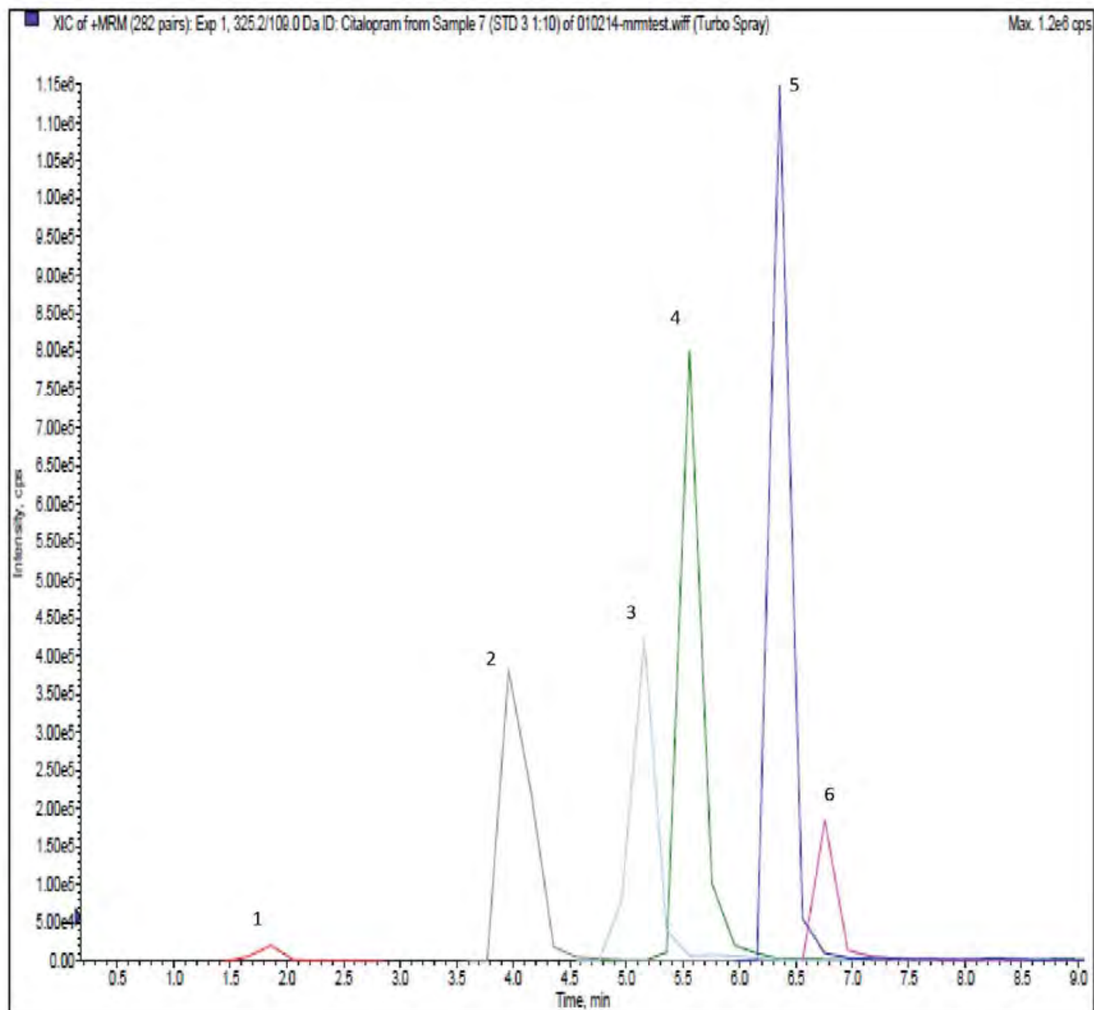
CHROMATOGRAMS

Basic Panel 1



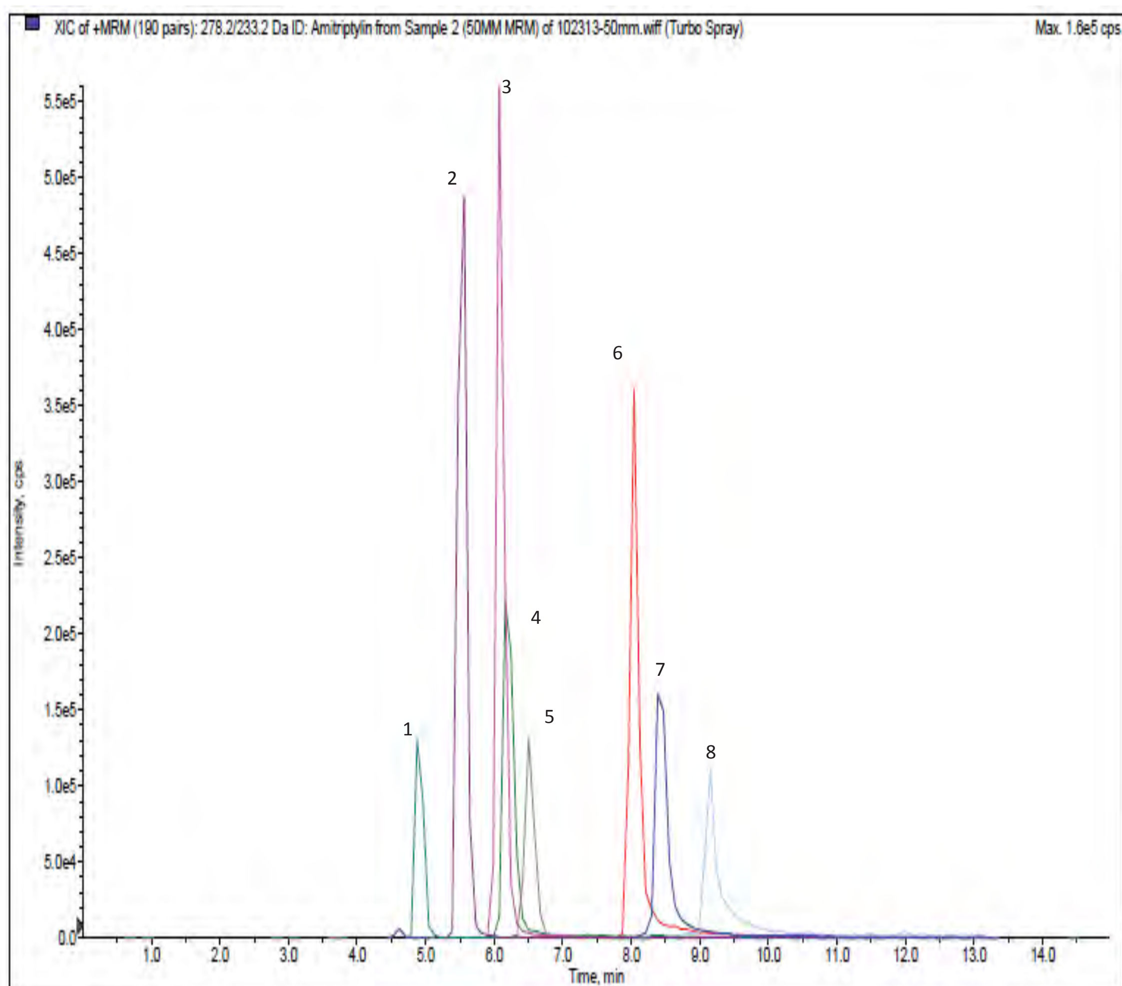
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylecgonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



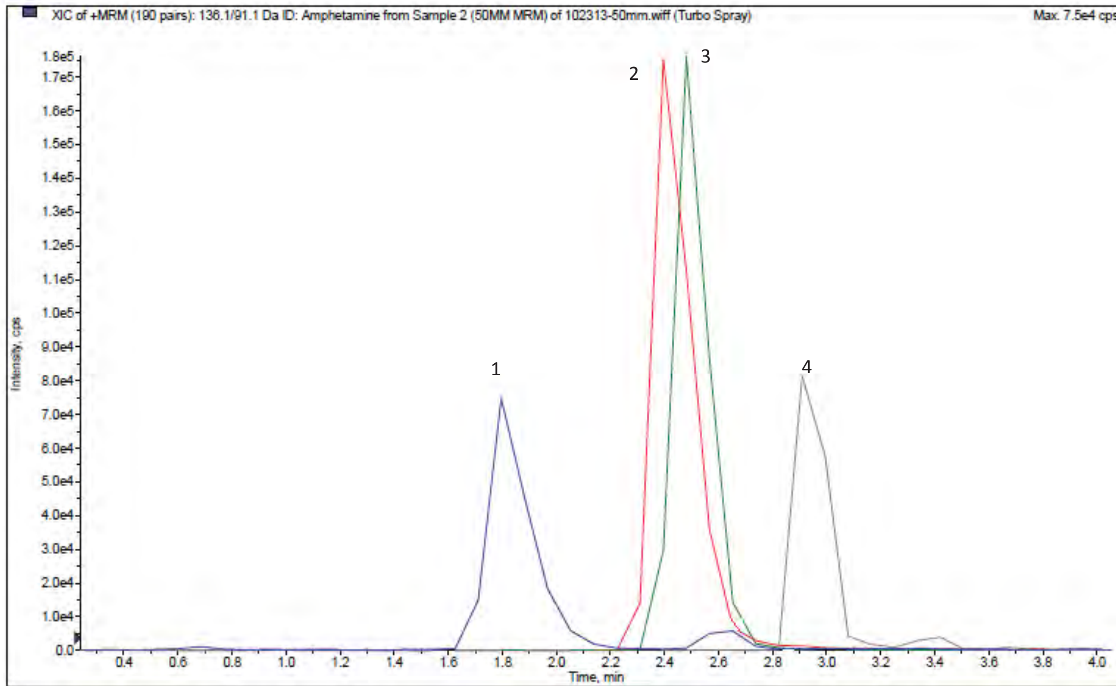
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxaine	278.2	260.2	4.90
2. Zolpidem	308.2	235.2	5.50
3. Trazadone	372.2	176.1	6.05
4. PCP	244.2	86.1	6.20
5. Quintiapine	384.2	253.1	6.50
6. Imipiramine	281.2	86.1	8.40
7. Amitriptyline	278.2	233.2	8.42
8. Sertraline	306.1	159	9.25

Amphetamine Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.Amphetamine	136.1	91.1	1.18
2.Methamphetamine	150.1	91.1	2.40
3.MDA	180.1	105.0	2.45
4.MDMA	194.1	105.1	2.95

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.5 mL/minute
Polarity: Positive
Injection Volume: 20 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	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

REPRESENTATIVE ANALYTES EXTRACTED

AMPH/METHAMP
SYMPATHOMIMETICS
TCA'S(7)
MDMA/MDA/MDEA
MEPERIDINE/NORMEPERIDINE
CYCLOBENZAPRINE
DIPHENHYDRAMINE
OPIATES(7)
PCP
FENTANYL/NORFENTANYL
CITALOPRAM
METHADONE/EDDP
COCAINE/BZE
SERTRALINE TRAMADOL/NORTRAM
CLONIDINE

BETA BLOCKERS IN BLOOD OR URINE FOR GC/MS CONFIRMATIONS USING 200 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #:
ZSDAU020 without Tips
or
ZCDAU020 with CLEAN-THRU® Tips

1. **PREPARE SAMPLE:**
   To 1 mL of Acetate buffer (pH 4.5) add 1 mL of blood or urine. Add 2 mL of Acetate buffer (pH 4.5).
   Mix/vortex
   Centrifuge as appropriate.

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 Acetate Buffer (pH 4.5).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

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

4. **WASH COLUMN:**
   2 x 1 mL Acetone/ Methanol (1:1) aspirate.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE BETA BLOCKERS:**
   1 x 1 mL CH_2Cl_2/ IPA/NH4OH (78:20:2).
   Collect the eluate by gravity.
   NOTE: Prepare elution solvent fresh daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

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

7. **DERIVATIZE:**
   Derivatization Solution: Methaneboronic acid at 5 mg/mL
   prepared in dry Ethyl Acetate (use molecular sieve).
   Store this solution at –20 °C (freezer conditions) until use.

   Reaction Mixture
   Add 100 µL of the Methaneboronic acid solution (see above).
   Mix/vortex.
   React 15 minutes at 70 °C. Remove from heat source to cool.

   NOTE: Do not evaporate this solution.

8. **ANALYSIS:**
   Inject 1 to 2 µL sample.

Reference:
Branum G, Sweeney S, Palmeri A, Haines L and Huber C
The Feasibility of the Detection and Quantitation of ß Adrenergic Blockers By Solid Phase Extraction and Subsequent Derivatization with Methaneboronic Acid. Journal of Analytical Toxicology 22: 135-141 (1998)

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM

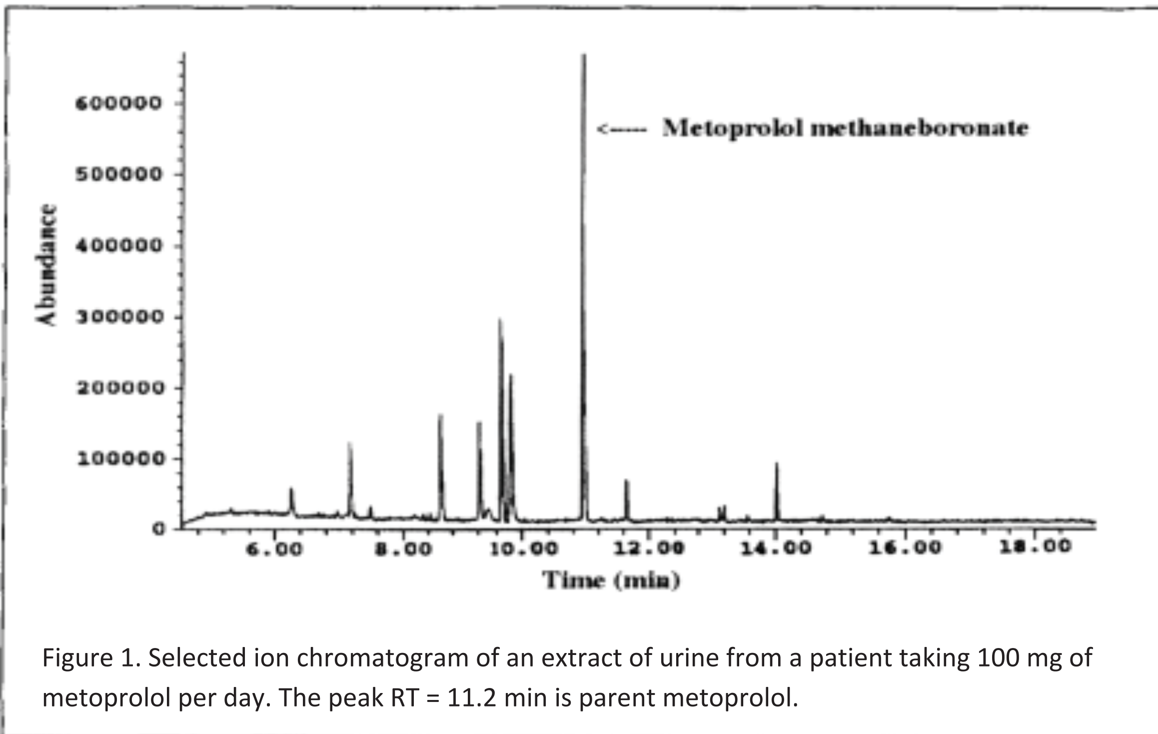


Figure 1. Selected ion chromatogram of an extract of urine from a patient taking 100 mg of metoprolol per day. The peak RT = 11.2 min is parent metoprolol.

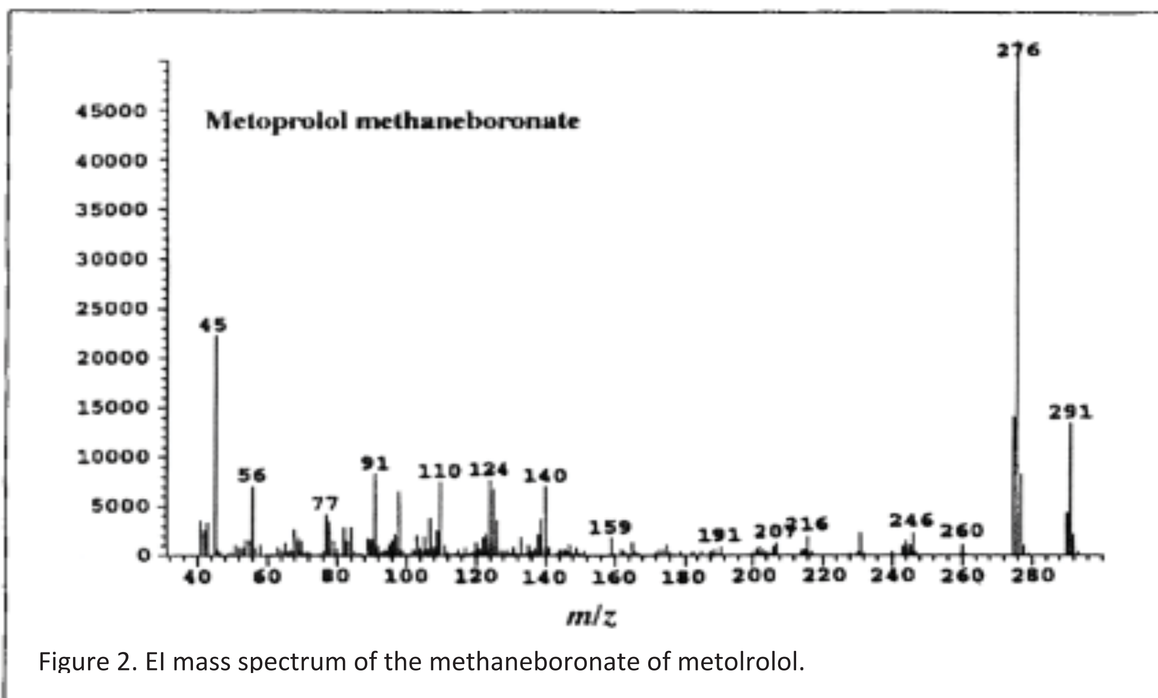


Figure 2. EI mass spectrum of the methaneboronate of metolrolol.

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Acebutolol	246	299	360
Alprenolol	258	273	138
Atenolol	275	290	164
Betaxolol	316	331	246
Bisoprolol	230	334	349
Carteolol	301	316	218
Isoproteronol (IS)	202	244	259
Metoprolol	276	291	140
Propranolol	283	268	128
Soltalol	281	296	239

PARAMETERS

GC/MS: Agilent - 5971/ 5890 GC/MS System with 7683B ALS System
GC capillary column: Rtx-5sil MS 30m X 0.25mm, 0.25µm
Injector: 2µL Splitless, 250°C
Oven temperature program: 110°C for 1 min; 20°C/min to 170°C; 7°C/min to 225°C; 24°C/min to 290°C for 10 min
Carrier gas: Helium

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

Part #
CSDAU206 – CLEAN SCREEN® DAU 200 mg, 6 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

1. **PREPARE SAMPLE:**

Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
Add 1 mL of blood, plasma/ serum, 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

Urine: PREPARE ENZYME HYDROLYSIS OF GLUCURONIDES:
To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0)
containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated
β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool.
Do not adjust pH~ sample is ready to be added to the extraction column.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
1 x 3 mL CH_3OH
1 x 3 mL D.I. H_2O
1 x 1 mL 100 mM Acetate buffer (pH 5.0)
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 acetate buffer (pH 5.0)
1 x 3 mL CH_3OH
Dry column (5-10 minutes at full vacuum or pressure)

5. **ELUTE BUPRENORPHINE/NORBUPRENORPHINE:**
1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2 v/v)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

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

7. **RECONSTITUTE / DERIVATIZE:**

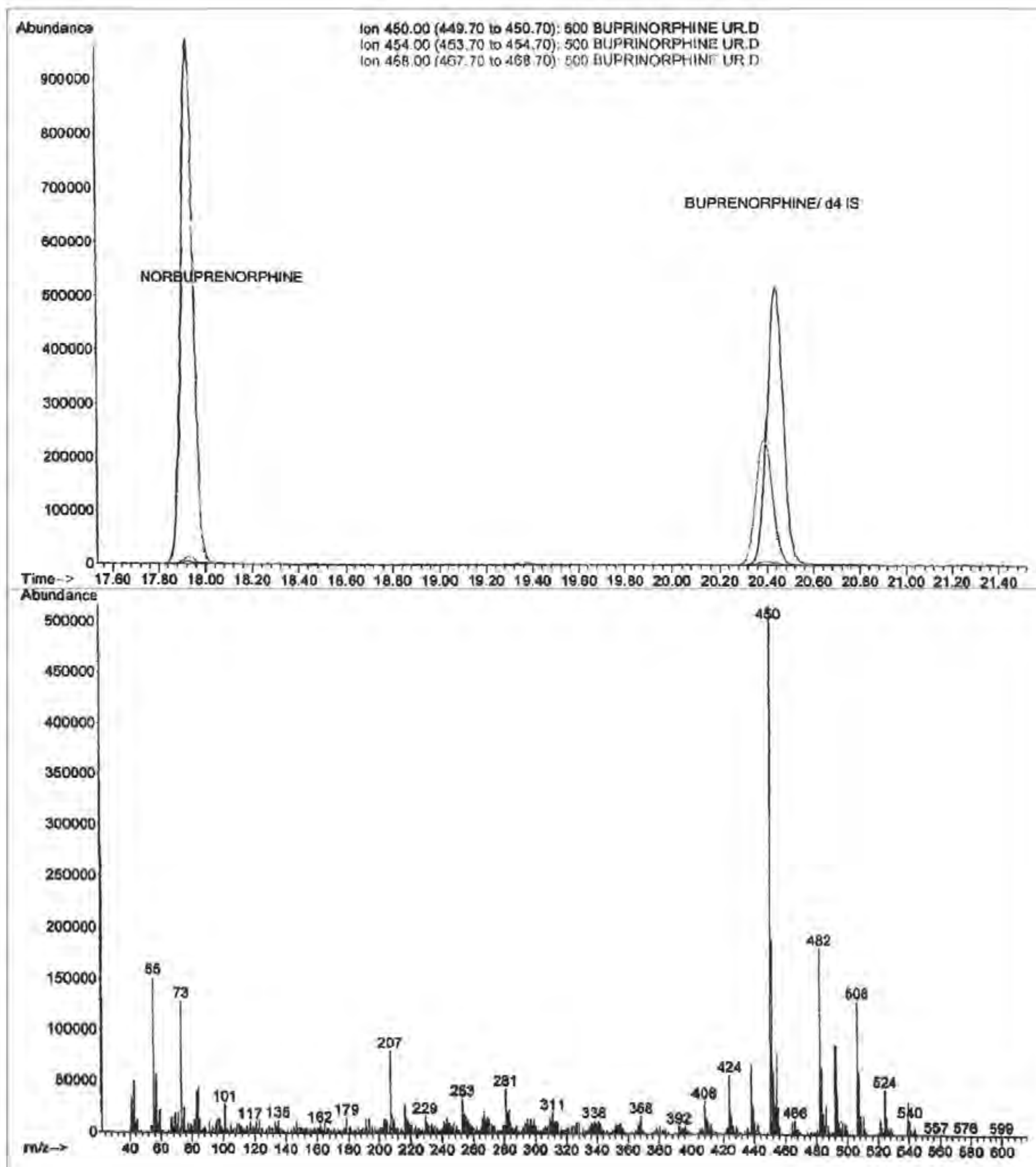
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10 µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

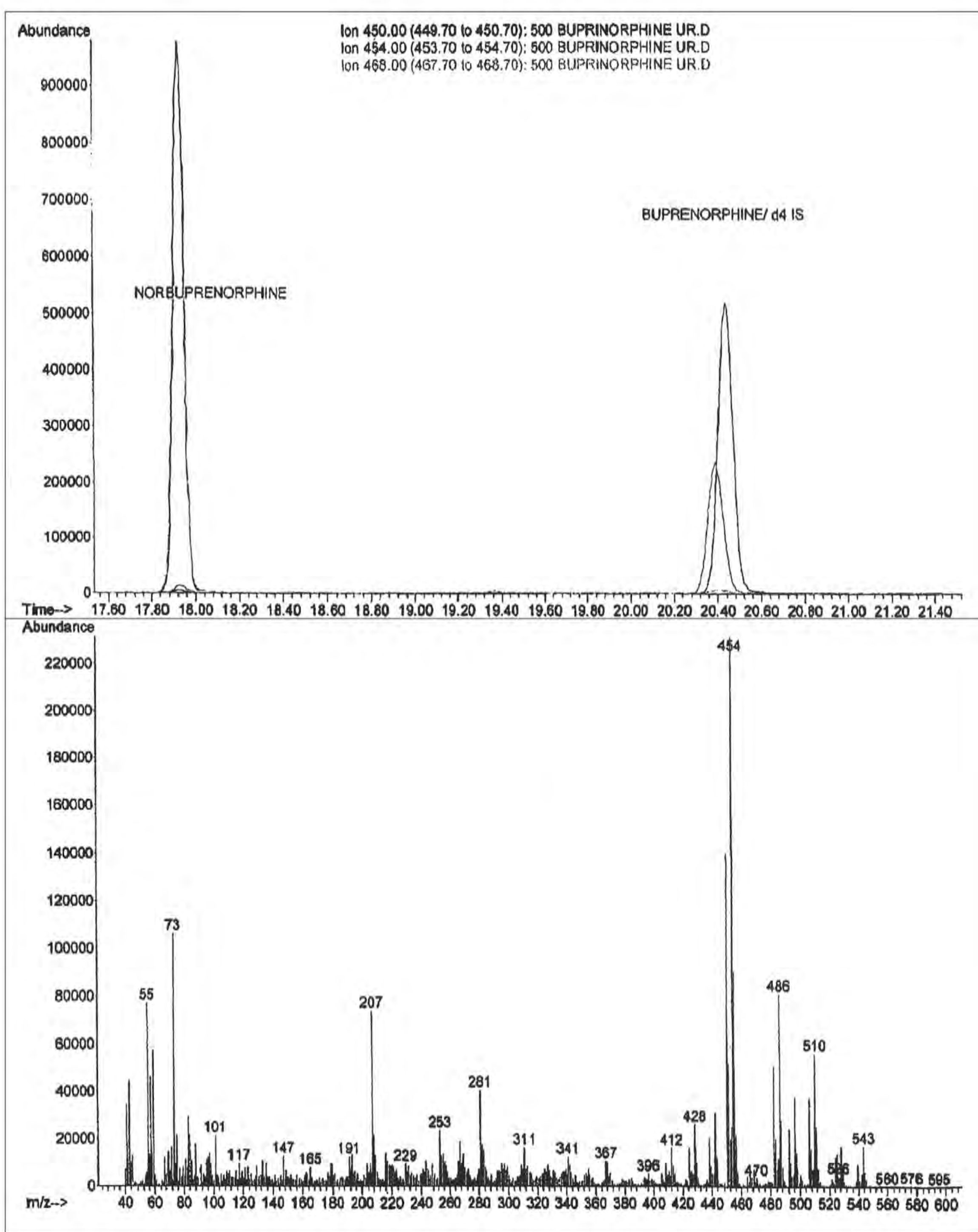
Alternate Derivatization

- Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of 50 µL BSTFA w/ 1% TMCS react at 70 °C for 30 minutes; Cool and inject 1-2 µL

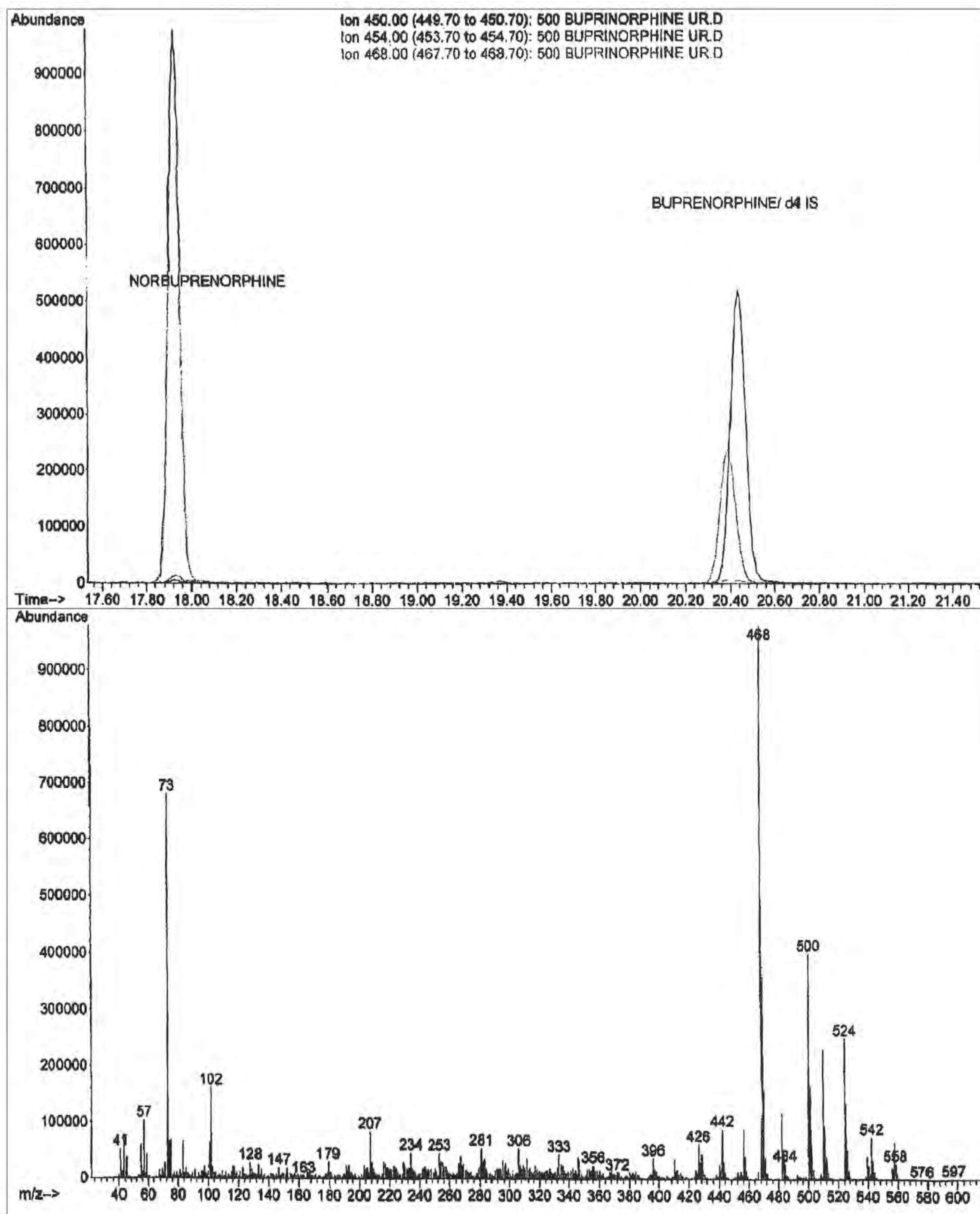
INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAMS




Abundance
Ion 450.00 (449.70 to 450.70): 500 BUPRINORPHINE UR.D
Ion 454.00 (453.70 to 454.70): 500 BUPRINORPHINE UR.D
Ion 468.00 (467.70 to 468.70): 500 BUPRINORPHINE UR.D
NORBUPRENORPHINE
BUPRENORPHINE/ d4 IS
Time-->
Abundance
m/z-->

-



Analyte	Primary Ion	Secondary Ion	Tertiary Ion
Buprenorphine-D_4-TMS	454	486	510
Buprenorphine-TMS	450	482	506
Norbuprenorphine-TMS	468	500	524
Norbuprenorphine-D_3-TMS	471	503	527

CLOZAPINE AND METABOLITES IN WHOLE BLOOD, SERUM/PLASMA AND URINE USING 200 mg CLEAN-UP® EXTRACTION COLUMN AND LC-MS/MS or HPLC-UV ANALYSIS

PART #:

CECNP123 – CLEAN-UP® CYANOPROPYL 200 mg, 3 mL Tube

1. **PREPARE SAMPLE:**

   To 1 mL of 100 mM prosphate buffer (pH 6.0) add internal standard.*

   Add 1 mL of blood, serum/plasma or urine. Add 2 mL of 100 mM phosphate buffer (pH 6.0).

   Mix/vortex Centrifuge as appropriate.

2. **CONDITION CLEAN-UP® CECNP123 EXTRACTION COLUMN:**

   1 x 1 mL Methanol.

   1 x 1 mL H_2O.

   1 x 0.5 mL 100 mM phosphate buffer (pH 6.0).

   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 1 mL D.I. H_2O

   1 x 0.5 mL 1% NH_4OH in D.I. H_2O.

   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE CLOZAPINE:**

   1 x 0.2 mL 1% NH_4OH in Methanol.

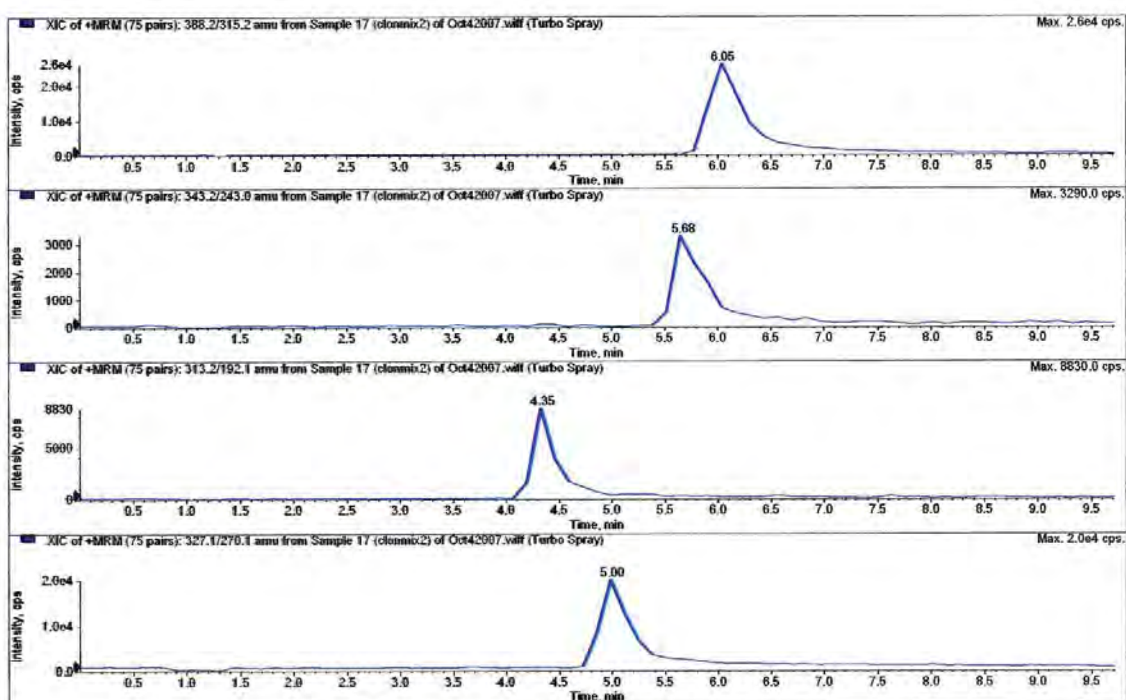
   Collect eluate at 1-2 mL /minute.

   Inject 5 µL (LC-MS/MS).

   Inject 20 µL (HPLC-UV).

INSTRUMENT CONDITIONS:
Column: 150 x 2.1 mm (3 µm) Zorbax: Agilent Technologies.
Mobile phase: Acetonitrile: 0.1% Formic Acid (33:67).
Flowrate: 0.35 mL/min.
Column Temperature: ambient.
Detector: API 2000 MS/MS.
HP1100 Diode-Array (230 nm).

Compound	MRM Transistion
Clozapine	327.1/270.1
Desmethylclozapine	313.1/192.1
Clozapine-N-oxide	343.1/243.1
*Flurazepam	388.1/315.2



GABAPENTIN/PREGABALIN/BACLOFEN IN BLOOD, PLASMA/SERUM BY LC-MS/MS OR GC-MS 200 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #
ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
Or
SMTBSTFA-1-1 – SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS
SLDA100ID21-5UM – Selectra® DA HPLC Column, 100 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**
   To 0.2-0.5 mL of sample add 1 mL of acetone dropwise while vortexing
   Add internal standards
   Mix/vortex and let stand for 5 minutes
   Transfer organic phase to clean tube
   Evaporate to dryness.
   Add 3 mL of 100 mM HCl
   Vortex mix and centrifuge as appropriate

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM HCl
   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 Ethyl Acetate
   1 x 3 mL Hexane
   Dry column (10 minutes at full vacuum or pressure)

5. **ELUTE GABAPENTIN/PREGABALIN/BACLOFEN:**
   1 x 3 mL CH_3OH containing 2% NH_4OH
   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 50 µL of Ethyl Acetate and 50 µL of BSTFA w/1% TMCS;
     Cap and heat at 70 °C for 30 minutes;
     Remove and allow to cool.
     Inject 1-2 µL

Alternate Derivatization

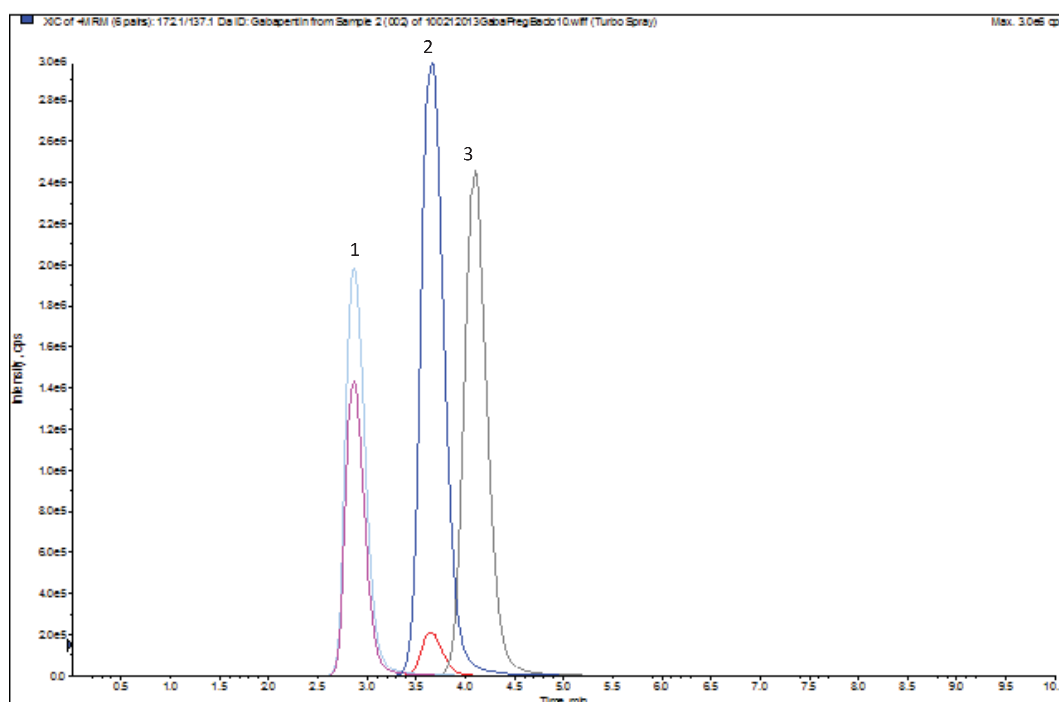
- 50 µL of Ethyl Acetate and 50 µL of MTBSTFA w/1% TBDMCS

GC-MS IONS

Compound	Primary	Secondary	Tertiary
Gabapentin-TMS	210	225	182
Gabapentin D_{10}-TMS	220	235	192

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Pregabalin	160.1	97.1	2.96
2. Gabapentin	172.1	67.1	3.66
3. Baclofen	214.0	150.8	4.32

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 10 µL

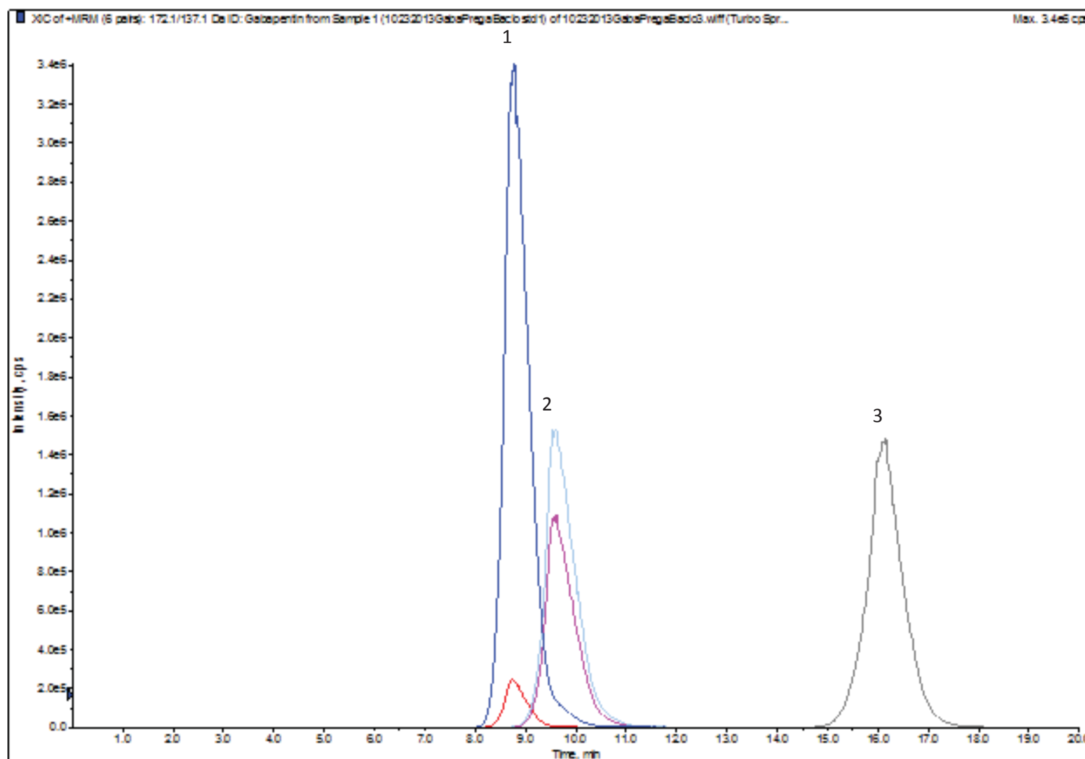
LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

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

Isocratic:

Time	%A	%B
0.00	85	15
11.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Gabapentin	172.1	67.1	8.78
2. Pregabalin	160.1	97.1	9.56
3. Baclofen	214.0	150.8	16.10

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

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

Isocratic:

Time	%A	%B
0.00	85	15
20.00	STOP	

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

Part #
ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube
SLPFPP100ID21-3UM – Selectra® PFPP 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 2 mL 200 mM HCl
   Dry column (5 minutes at full vacuum or pressure).
   1 x 3 mL Methanol
   Dry column (5 minutes at full vacuum or pressure).

5. **ELUTE NICOTINE, COTININE, ANABASINE:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

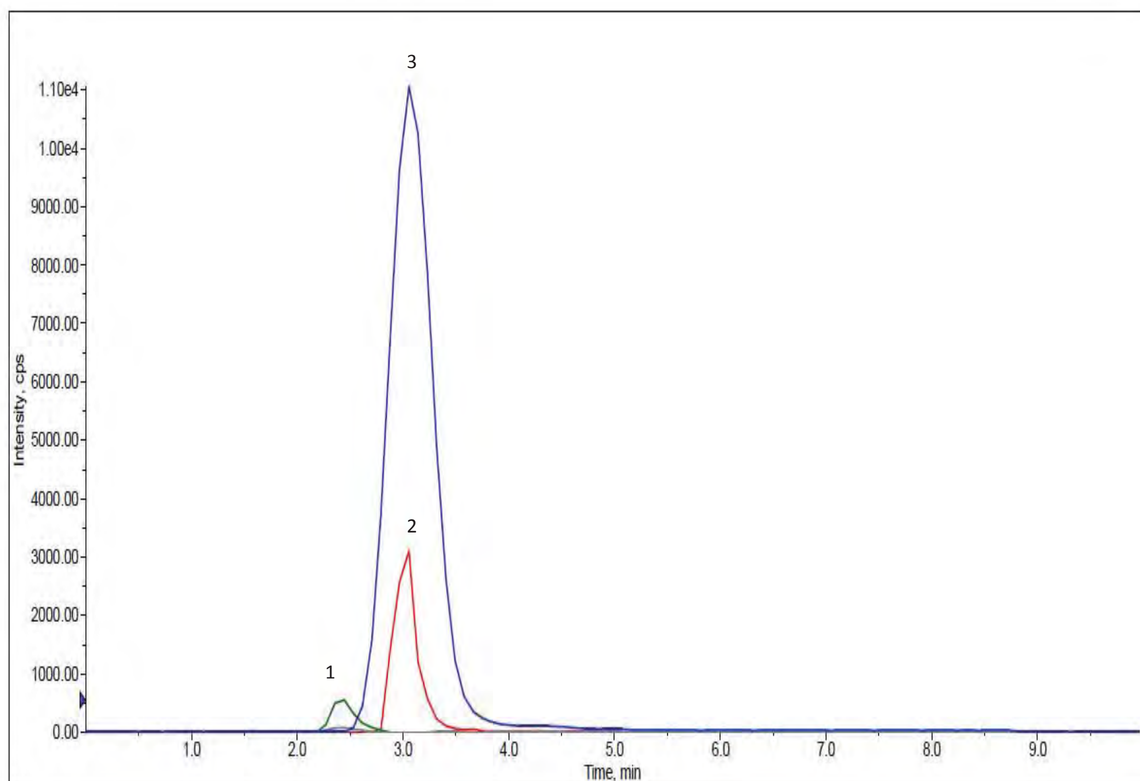
6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

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

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Cotinine	117.2	80.1	2.42
Cotinine D_3	180.2	101.2	-
2. Nicotine D_4	167.2	136.1	3.03
3. Nicotine	163.2	132.2	3.06

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® PFPP HPLC Column 100 x 2.1 mm 3 µm

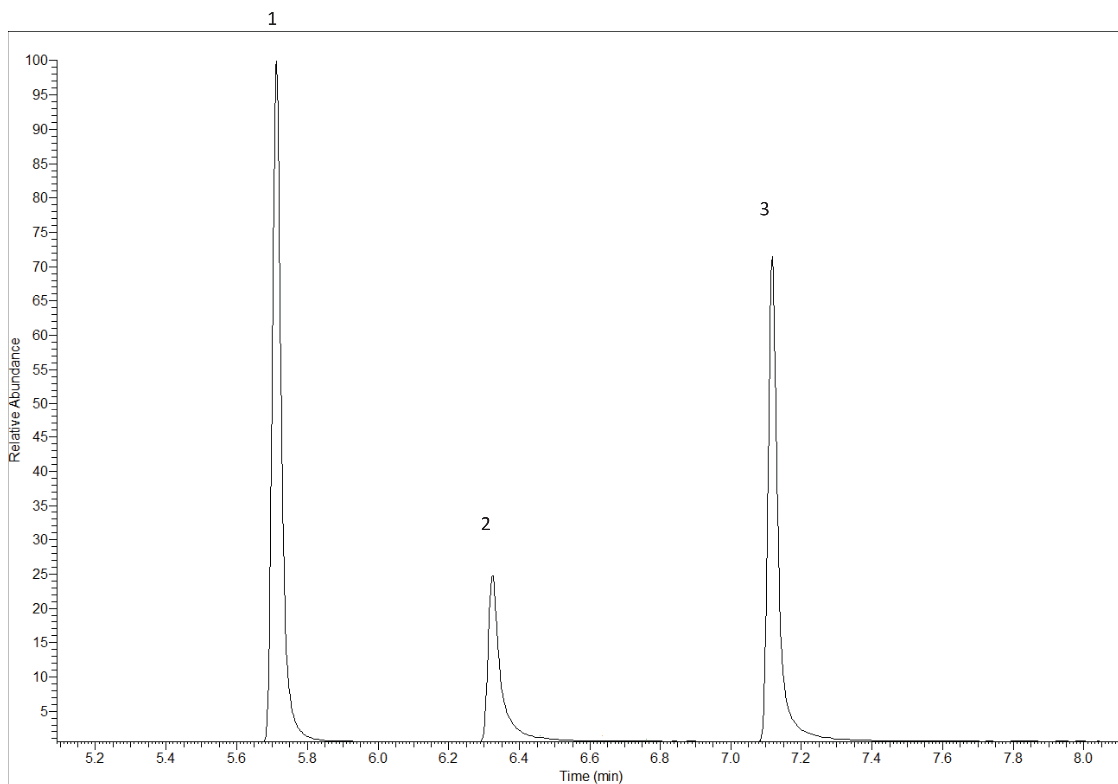
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	80	20
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Nicotine	84	133	162	5.71
Nicotine D_4	88	137	166	-
2. Anabasine	84	105	133	6.32
3. Cotinine	98	119	176	7.12
Cotinine D_4	101	122	179	-

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: 50 °C (0.5) to 320 °C (30 °C/ minute): hold (5 minutes)

Carrier gas: Helium (1.2 mL/ minute)

MSD condition: Aux temperature: 280 ºC, MS Source: 300 ºC, MS Quad: 150 ºC

PAROXETINE IN BLOOD, PLASMA/ SERUM AND URINE.
LC-MSMS CONFIRMATIONS USING 200 mg CLEAN SCREEN® DAU EXTRACTION COLUMN
Part #:
ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards*.
   Add 1 mL whole blood, Serum/Plasma or Urine. Add 2 mL of 100 mM phosphate buffer (pH 6.0).
   Vortex and centrifuge as appropriate.

2. **CONDITION 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 < 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
   1 x 3 mL CH_3OH
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE PAROXETINE:**
   1 x 3 mL Ethyl Acetate: Acetonitrile: NH_4OH (78:20:2)
   Collect eluate at 1-2 mL / minute.

6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40 °C
   Dissolve residue in 100 µL Methanol.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, LLC)

Mobile phase:

Time	Acetonitrile	0.1% Formic Acid aq
0	10	90
15	50	50
16	10	90
20	10	10

Flow rate: 0.35 mL/ minute

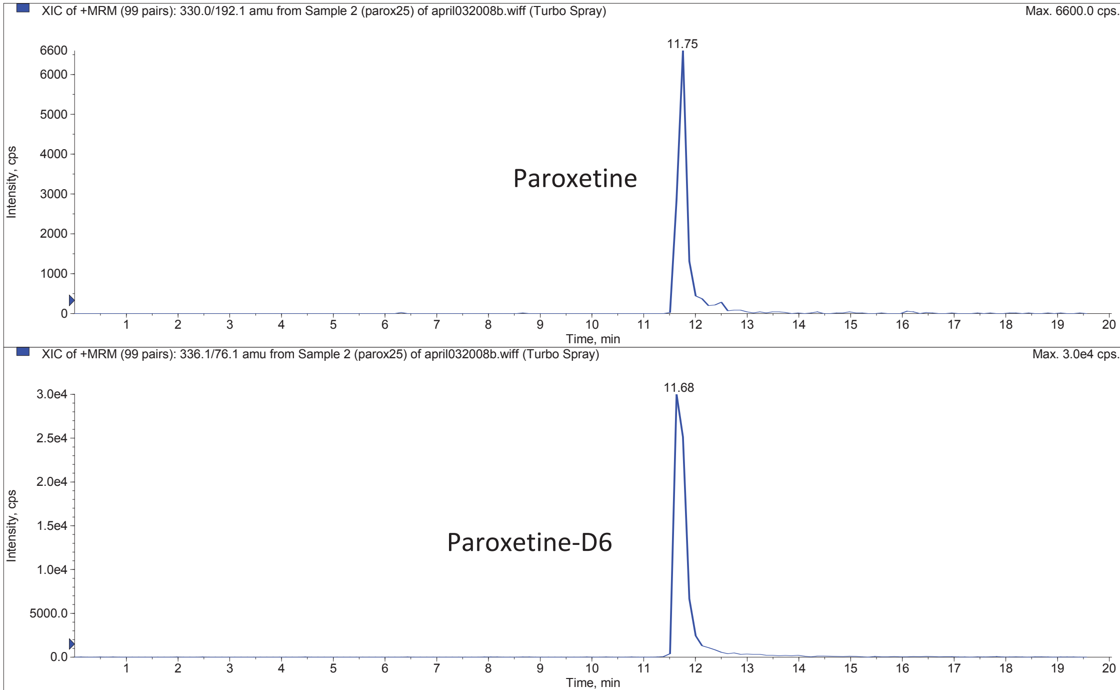
Injection Volume: 5 µL

Column Temperature: ambient

Detector: API 2000 MS/MS.

Compound	MRM Transition
Paroxetine	330.0 / 190.1
Paroxetine-D6	336.0 / 76.1

CHROMATOGRAM :



QUETIAPINE IN BLOOD, PLASMA/SERUM, URINE AND TISSUE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

PART #:
ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10mL Tube

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard.* Add 1 mL blood, plasma/serum, urine or 1 g (1:4) tissue homogenate
   Add 2 mL of 100 mM phosphate buffer (pH 6.0).
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge as appropriate.

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 < 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 100 mM phosphate buffer (pH 6).
   1 x 3 mL 1.0 M acetic acid.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 3 mL of Hexane.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE QUETIAPINE:**
   1 x 3 mL Ethyl Acetate/ Acetonitrile/ NH_4OH (78:20: 2 v/v).
   Collect eluate at 1-2 mL /minute.
   NOTE: Prepare elution solvent daily

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

7. **Reconsititue sample in 100 µL 0.1% trifluoroacetic acid (aq).**
   Inject 50 µL.

INSTRUMENT CONDITIONS:

Column: C_{18} 150 x 4.6 mm (3 µm) Zorbax (Agilent Technologies).

Mobile phase: Acetonitrile: 0.1% Trifluoroacetic acid (25: 75).

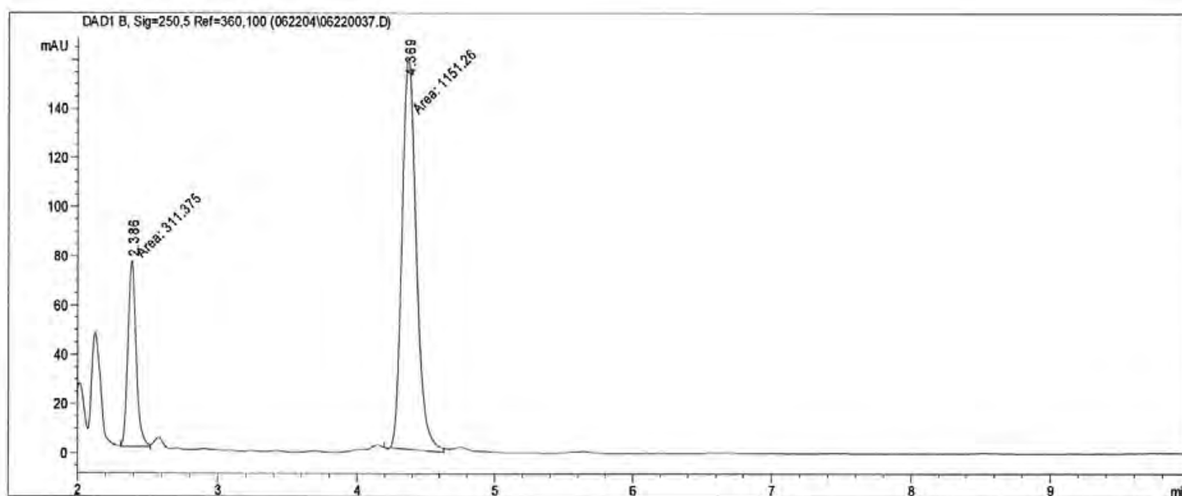
Flowrate: 1 mL / min.

Column Temperature: 35 ˚C.

Detector: Diode Array (250 nm).

Chromatogram:

Quetiapine
Quinidine (internal standard)



Drugs of Abuse

CPS analitica for Chemistry

COCAINE AND BENZOYLECGONINE 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
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA50ID21-5UM - SELECTRA® DA HPLC Column 50 x 2.1 mm, 5µm

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 HCl
   1 x 3 mL CH_3OH.
   Dry column (10 minutes at full vacuum or pressure).

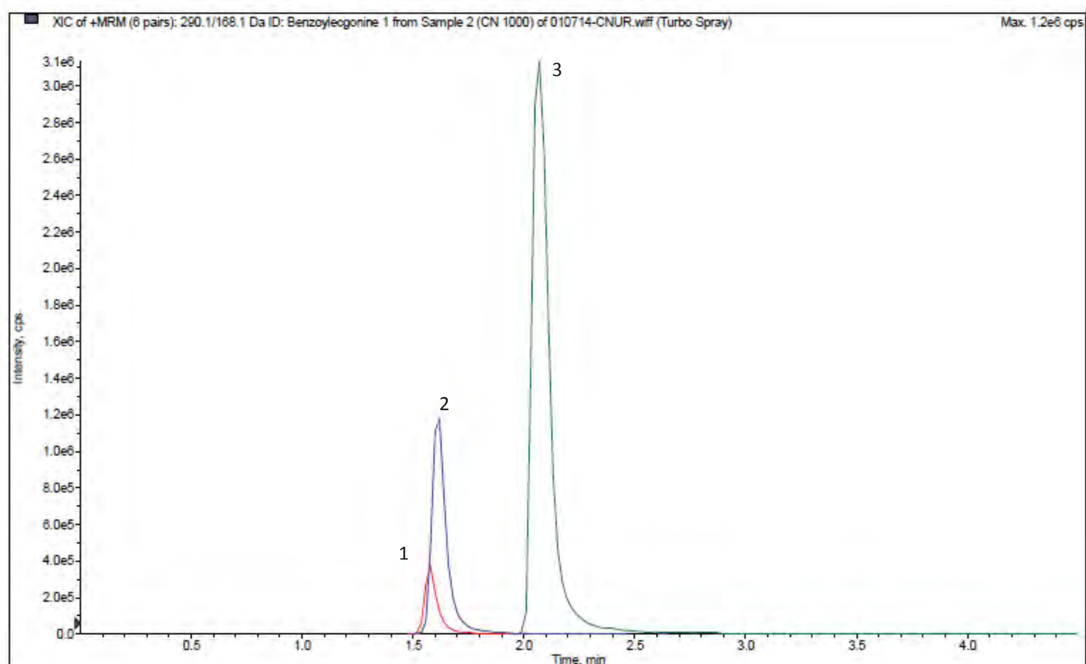
5. **ELUTE COCAINE/METABOLITES:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

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 50 µL of Ethyl Acetate and 50 µL BSTFA w/ 1%TMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1 µL

INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Benzolyecgonine D_8	**298.1**	**171.1**	**1.58**
2. Benzolyecgonine	**290.1**	**168.1**	**1.60**
3. Cocaine	**304.1**	**182.1**	**2.10**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

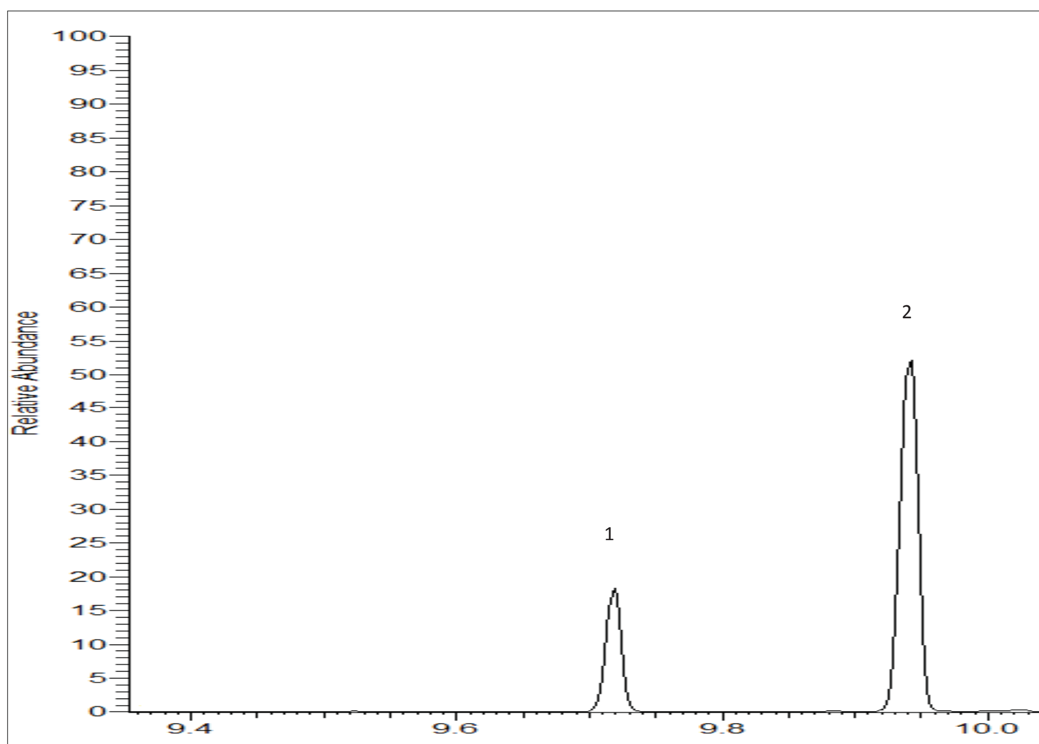
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Gradient:

Time	%A	%B
0.00	75	25
3.00	50	50
3.01	10	90
4.00	75	25
5.50	STOP	

INSTRUMENT CONDITIONS (GC-MS):



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Cocaine	**182**	**198**	**303**	**9.72**
Cocaine D_3	**185**	**201**	**306**	**-**
2. Benzolyecgonine TMS	**240**	**256**	**361**	**9.94**
Benzolyecgonine TMS D_3	**243**	**259**	**369**	**-**

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

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

Injector: 1µL 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 ºC

COCAINE AND BENZOYLECGONINE FROM MECONIUM BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #:
ZSDAU020- CLEAN SCREEN® DAU 200mg, 10 mL Tube
SBSTFA-1-1 –SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA50ID21-5UM-SELECTRA® DA HPLC Column 50 x 2.1MM, 5µm

1. **PREPARE SAMPLE**
   Vortex 0.5 -1 g meconium with 2 mL of Methanol.
   Centrifuge and transfer the supernatant to a clean tube.
   To each tube add 3 mL 100 mM phosphate buffer (pH 6.0), internal standard and vortex.
   Matrix must be more aqueous than organic for good extraction to occur.
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 HCl
   1 x 3 mL CH_3OH.
   Dry column (10 minutes at full vacuum or pressure).

5. **ELUTE COCAINE/METABOLITES**
   1 x 3 mL CH_2Cl_2/ IPA /NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

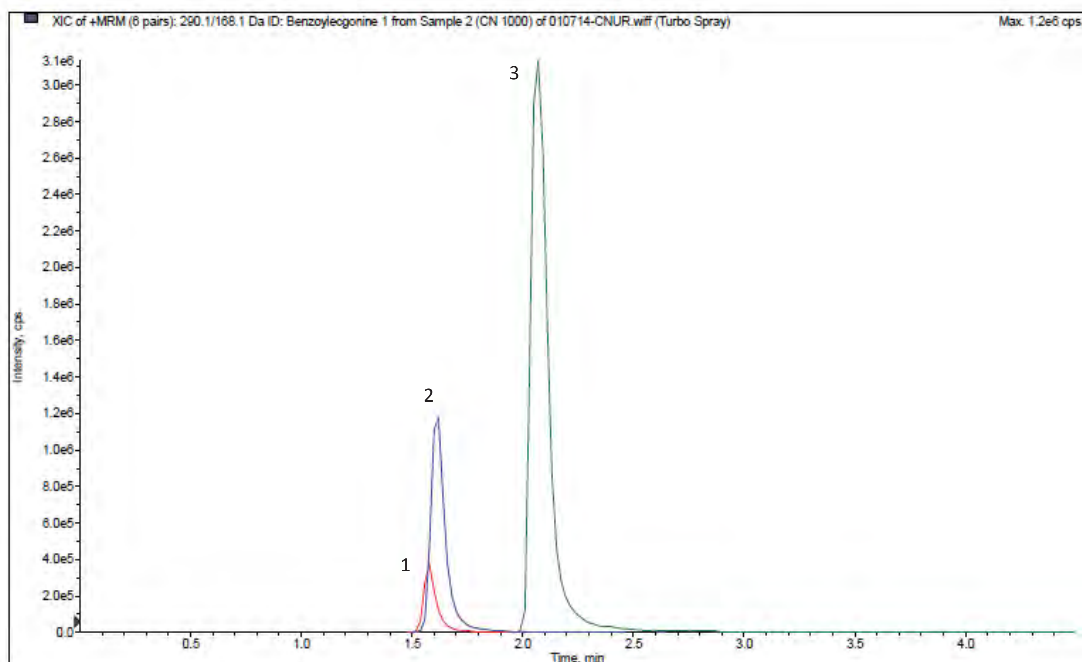
6. **DRY ELUATE**
   Evaporate to dryness at < 40°C.

7. **RECONSTITUTE / DERIVATIZE**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL BSTFA (with 1%TMCS)
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70°C; Cool and inject 1 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q2	
1. Benzolyecgonine D_8	**298.1**	**171.1**	**1.58**
2. Benzolyecgonine	**290.1**	**168.1**	**1.60**
3. Cocaine	**304.1**	**182.1**	2.10

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

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

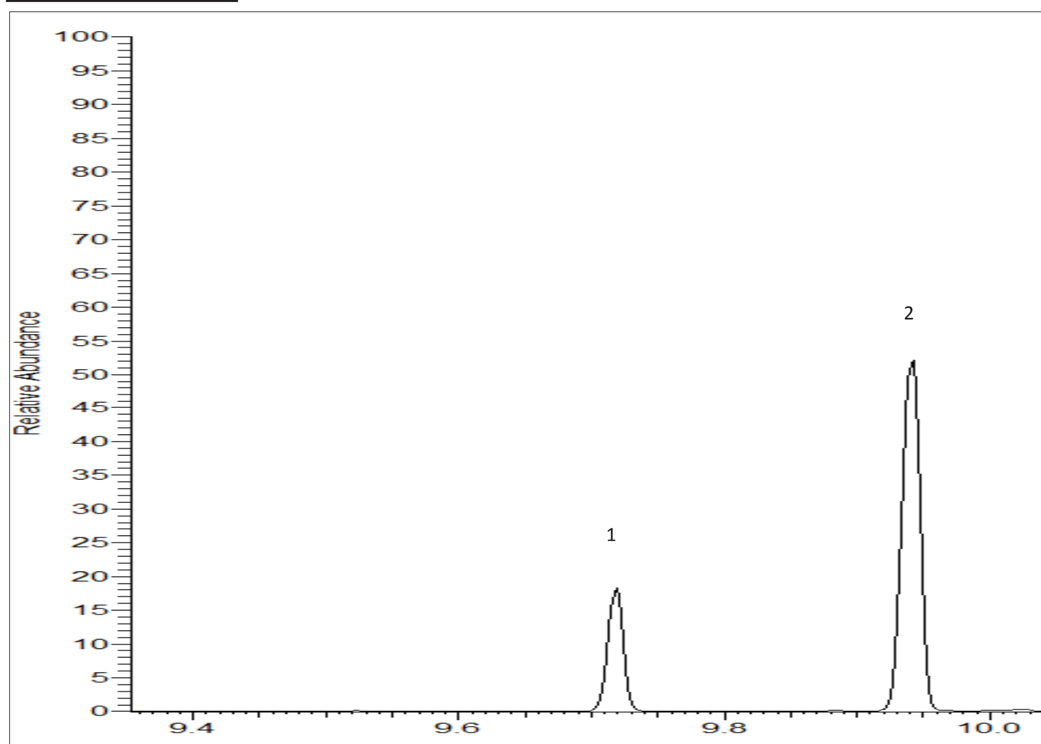
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	75	25
3.00	50	50
3.01	10	90
4.00	75	25
5.50	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
4. Cocaine	182	198	303	9.72
Cocaine D3	185	201	306	-
5. Benzolyecgonine TMS	240	256	361	9.94
Benzolyecgonine TMS D_3	243	259	369	-

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

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

Injector: 1μL 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 ºC

COCAINE AND BENZOYLECGONINE IN URINE BY LC-MS/MS OR GC-MS STRYE SCREEN® DBX EXTRACTION COLUMN

Part #
SSDBX033 – SRYRE SCREEN® DBX 30 mg, 3 mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA50ID21-5UM – SELECTRA® DA HPLC Column 50 x 2.1 mm, 5µm

1. **PREPARE SAMPLE:**
   To 1 mL of urine add internal standard(s) and 300 µL 100mM HCl.
   Mix/Vortex.

2. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute.

3. **WASH COLUMN:**
   1 x 1 mL D.I. H_2O.
   1 x 1 mL 100 mM HCl
   1 x 1 mL CH_3OH.
   Dry column (10 minutes at full vacuum or pressure).

4. **ELUTE COCAINE/METABOLITE:**
   2 x 0.5 mL CH_2Cl_2/ IPA /NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA /NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

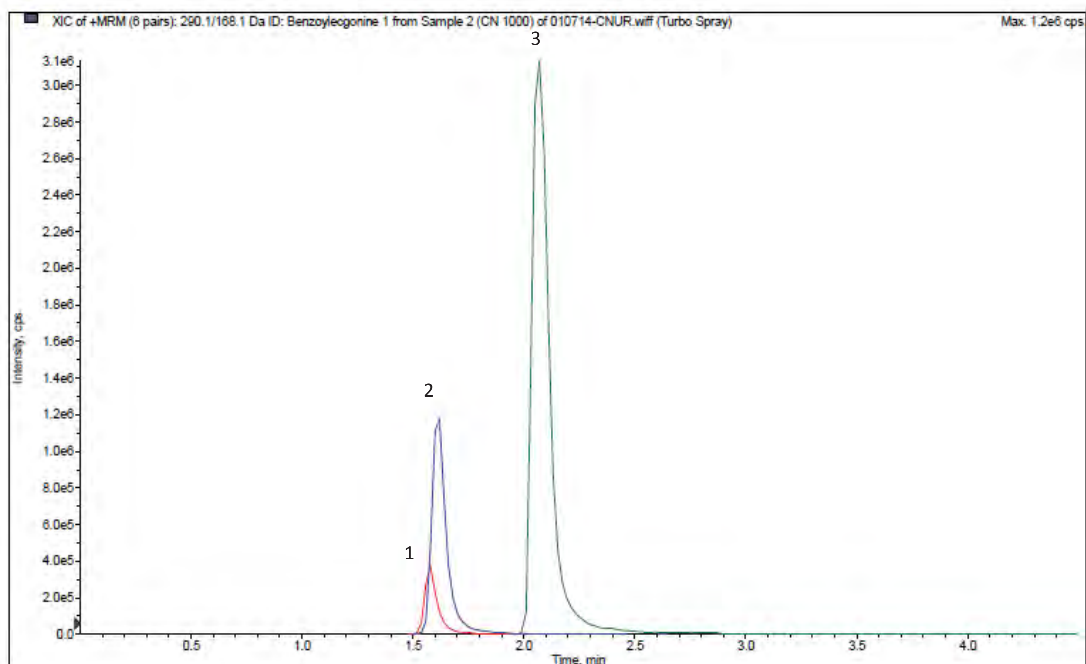
5. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

6. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70°C; Cool and inject 1 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Benzolyecgonine D_8	**298.1**	**171.1**	**1.58**
2. Benzolyecgonine	**290.1**	**168.1**	**1.60**
3. Cocaine	**304.1**	**182.1**	**2.10**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

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

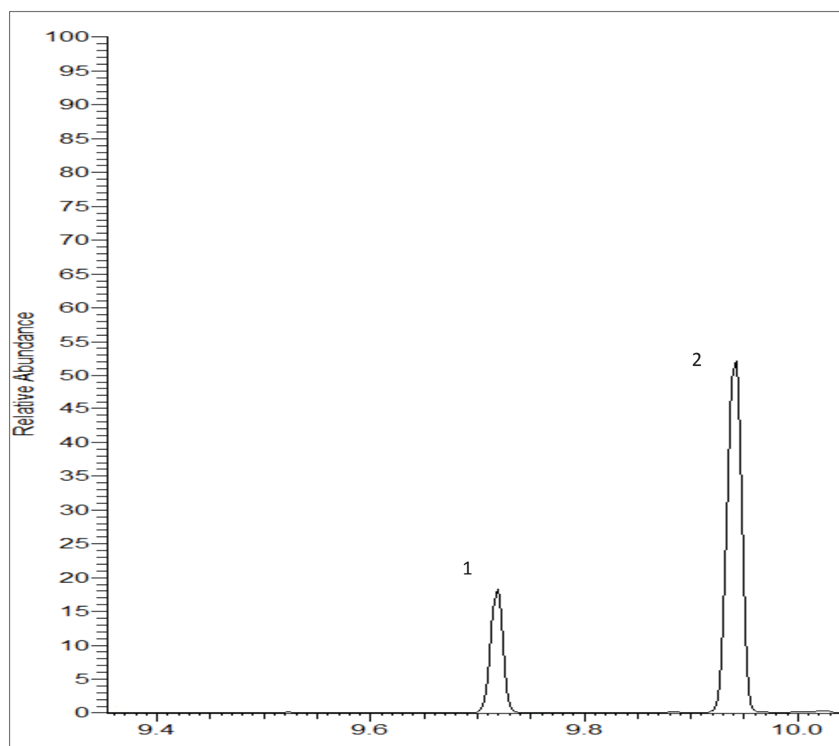
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	75	25
3.00	50	50
3.01	10	90
4.00	75	25
5.50	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
6. Cocaine	182	198	303	9.72
Cocaine D3	185	201	306	-
7. Benzolyecgonine TMS	240	256	361	9.94
Benzolyecgonine TMS D_3	243	259	369	-

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: 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 ºC

COCAINE AND BENZOYLECGONINE IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN XCEL® I EXTRACTION COLUMN

Part #

CSXCE111 – CLEAN SCREEN XCEL® I 130 mg, 1 mL Tube

SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

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

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. APPLY SAMPLE

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. WASH

1 x 2 mL 50:50 Methanol: 100mM HCl
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. ELUTION

1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. DRY ELUTE

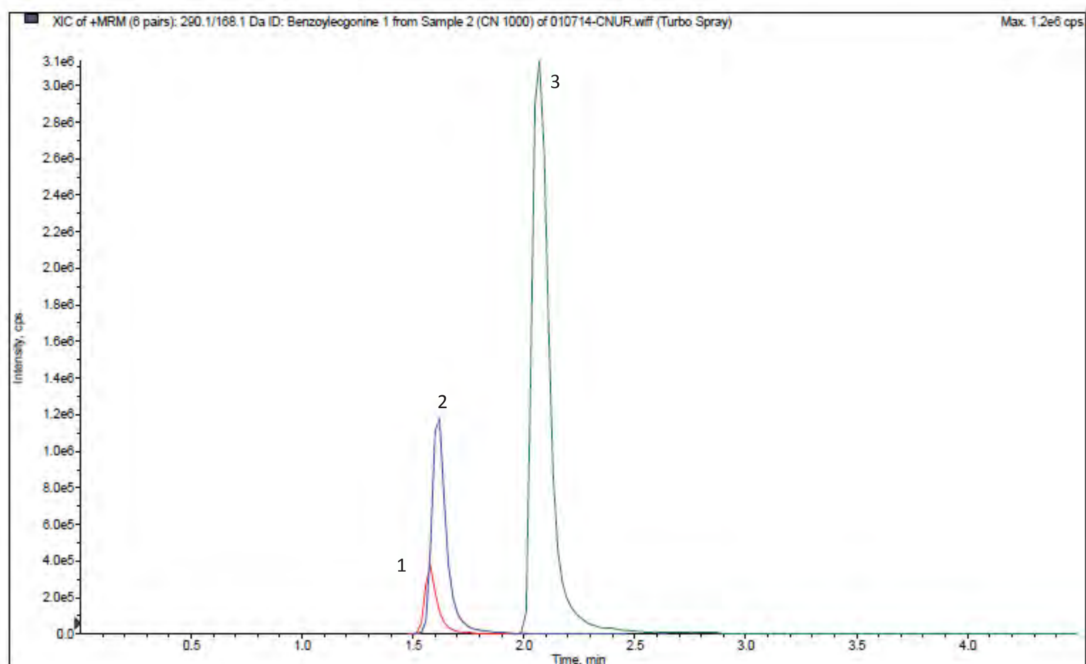
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10 µL.
- **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL BSTFA w/ 1%TMCS
  Overlay with N_2 and cap. Mix/vortex
  React 30 minutes at 70 °C; Cool and inject 1 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Benzolyecgonine D_8	**298.1**	**171.1**	**1.58**
2. Benzolyecgonine	**290.1**	**168.1**	**1.60**
3. Cocaine	**304.1**	**182.1**	**2.10**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

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

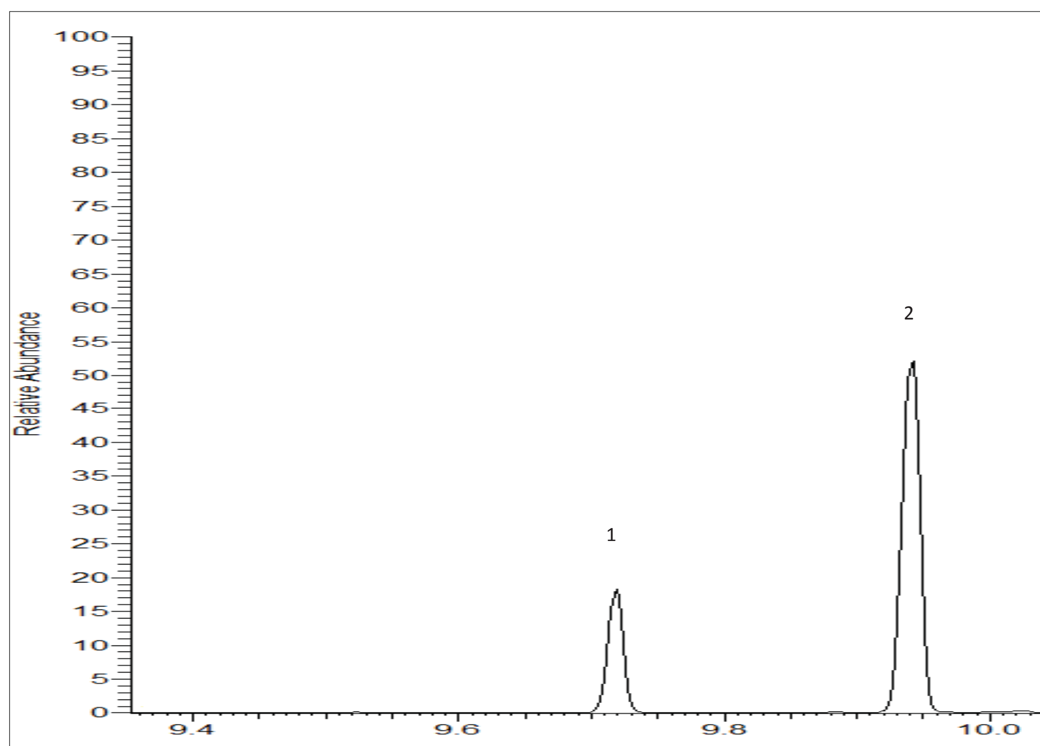
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	75	25
3.00	50	50
3.01	10	90
4.00	75	25
5.50	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Cocaine	**182**	**198**	**303**	**9.72**
Cocaine D3	**185**	**201**	**306**	**-**
2. Benzolyecgonine TMS	**240**	**256**	**361**	**9.94**
Benzolyecgonine TMS D_3	**243**	**259**	**369**	**-**

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: 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 ºC

COCAINE AND METABOLITES IN BLOOD, PLASMA/ SERUM, URINE AND TISSUE FOR GC/MS CONFIRMATIONS

Part #

XRDAH206 - XtrackT® DAU with CLEAN-THRU® Tips 200 mg, 6 mL Tube

XCDAH206 - XtrackT® DAU without Tips

CLTTP050 - CLEAN-THRU® Tips

SPHPH06001-10 - Select pH Buffer Pouches 100mM Phosphate pH 6.0

SBSTFA-1-1 - SELECTRA-SIL® BSTFA w/ 1% TMCS

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0.0) add internal standards
   Add 2 mL of blood, plasma/ serum, urine or 1 g (1:4) tissue homogenate.
   Mix/vortex. 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 as appropriate

2. **CONDITION XTRACKT® DAU EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 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 2 mL 100 mM HCI
   1 x 3 mL CH_30H
   Dry column (5 minutes at full vacuum or pressure)

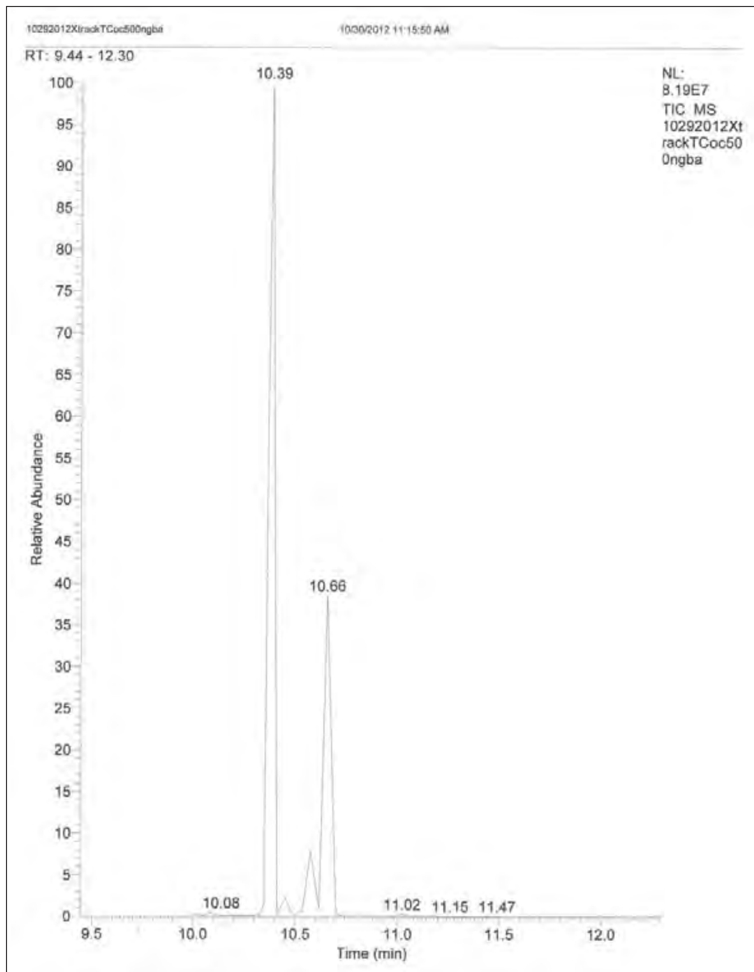
5. **ELUTE COCAINE AND BENZOYLECGONINE:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/ minute

   NOTE: Prepare elution solvent daily. Add IPA/ NH_40H, mix, then add CH_2Cl_2 (pH 11-12)

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

7. **DERIVATIZE:**
   Add 50 L ethyl acetate and 50 L BSTFA w/1% TMCS Overlay with Nitrogen and cap. Mix/vortex
   React 20 minutes at 70 °C
   Remove from heat source to cool
   NOTE: Do not evaporate BSTFA solution

8. **ANALYZE:**
   Inject 1 to 2 µL



Compound	Quantify Ion	Secondary Ion	Tertiary Ion
Cocaine	182	198	303
Cocaine-D_3†	185	201	306
Cocaethylene	196	317	82
Cocaethylene-D_8†	204	325	196
Benzoylecgonine-TMS	240	256	361
Benzoylecgonine-D_3-TMS†	243	259	364

†Suggested internal standard for GC/MS

DULOXETINE IN BLOOD AND URINE BY LC-MS/MS*

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM prosphate buffer (pH 6.0) add internal standard.*
   Add 1 mL of blood or urine. Add 2 mL of 100 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. Mix/vortex.

   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH 6.0).
   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.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE DULOXETINE:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20: 2 v/v). Collect eluate at 1-2 mL /minute.

6. **EVAPORATION:**
   Evaporate eluate under a gentle stream of nitrogen < 40 ºC.

7. **RECONSTITUTE sample in 200 µL of 0.1% Formic Acid.**
   Inject 5 µL.

INSTRUMENT CONDITIONS:

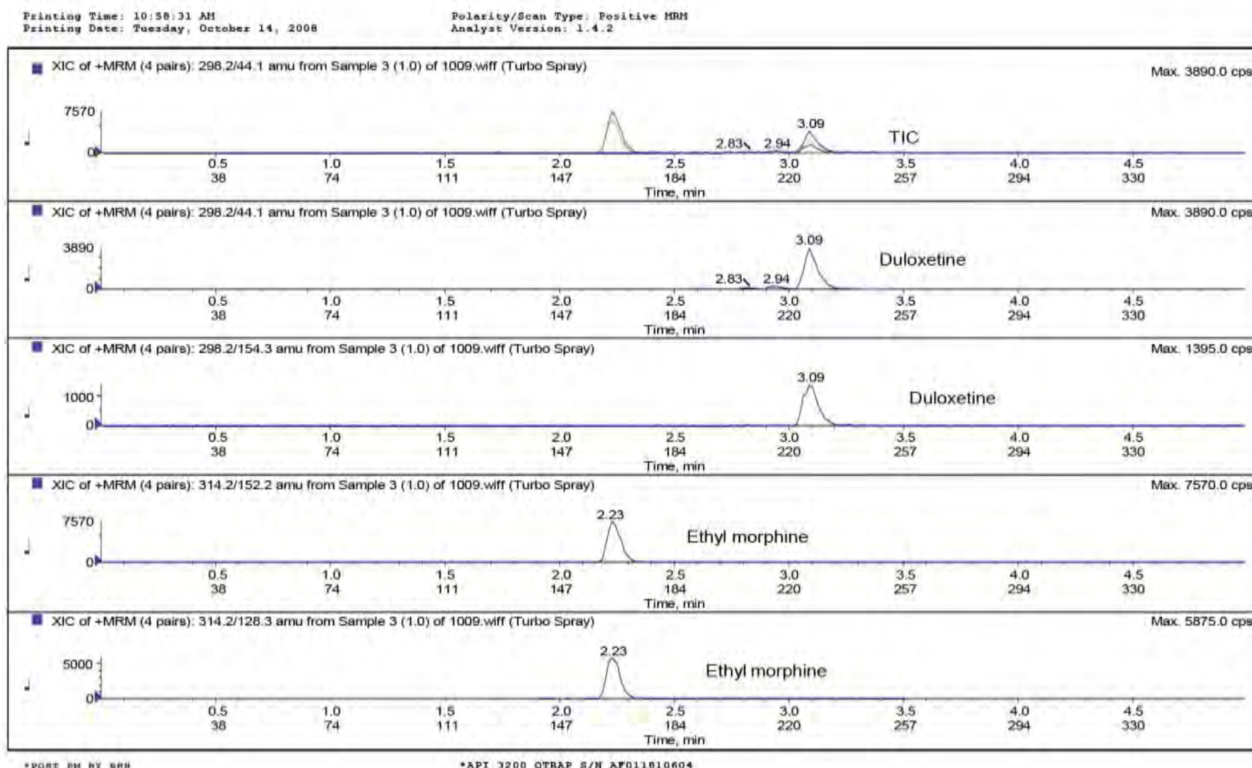
Column: 50 x 2.1 mm (5 µm) C_{18}
Mobile phase:

Time/ min	% Acetonitrile	% 0.1 % Formic Acid
0	5	95
4	90	10
4.1	5	95
5	5	95

Flowrate: 0.5 mL/minite. **Column Temperature:** ambient. **Detector:** API 3200 Q-Trap MS/MS.

Compound	MRM Transistion
* Ethyl Morphine (Internal Standard)	314.2/ 152.2
Duloxetine	298.1/44.1

Chromatogram of Ethyl Morphine and Duloxetine



*Presented at SOFT annual meeting 2008 by A.A. Elian

A SOLID PHASE METHOD FOR GAMMA-HYDROXYBUTYRATE (GHB) IN BLOOD, URINE, VITREOUS OR TISSUE WITHOUT CONVERSION TO GAMMA-BUTRYLACTONE (GBL)

Part #:
ZSGHB020 – CLEAN SCREEN® GHB 200 mg, 10mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TCMS

GHB working standard; 200 µg/mL in D.I. H_2O; prepared from Cerilliant stock 1 mg/mL.

GHB –D6working internal standard; 100 µg/mL; use as supplied Cerilliant stock (0.1 mg/mL).

Working Standard	Whole Blood	Concentration
10 µL	200 µL	10 µg/mL
25 µL	200 µL	25 µg/mL
50 µL	200 µL	50 µg/mL
100 µL	200 µL	100 µg/mL

1. Make calibration standards and pipet 200 µL of QC and unknown bloods* into appropriately labeled 1.5 mL plastic centrifuge tubes.

   NOTE: *All samples including urine, vitreous or homogenized tissues (1:4)

2. Add 25 µL of internal standard.

3. Add 1 mL of acetone; Vortex 15 seconds.

4. Centrifuge; Transfer acetone layer to culture tubes.

5. Evaporate extracts @ 70°C w/nitrogen.

6. Reconstitute the dried extracts with 200 µL of 100 mM Phosphate Buffer (pH 6.0); Vortex 15 seconds.

7. **CONDITION CLEAN SCREEN® GHB EXTRACTION COLUMN:**
   1 x 3 mL of CH_3OH.
   1 x 3 mL of D.I. H_2O.
   1 x 1 mL of 100 mM Phosphate Buffer (pH 6.0).

NOTE: Aspirate at 3 inches of Hg or less to prevent sorbent drying.

8. **APPLY SAMPLE:**
   Add sample with Eppendorf pipette.
   Aspirate at ~1 inch Hg.

9. **ELUTE GHB:**

Place clean test tubes into vacuum manifold

Add 1 mL of CH_3OH/ NH_4OH (99:1) to original sample test tube; Vortex.

Decant onto column and collect extract.

Aspirate ~1 inch Hg.

10. **CONCENTRATE:**

Remove test tube from Vacuum Manifold.

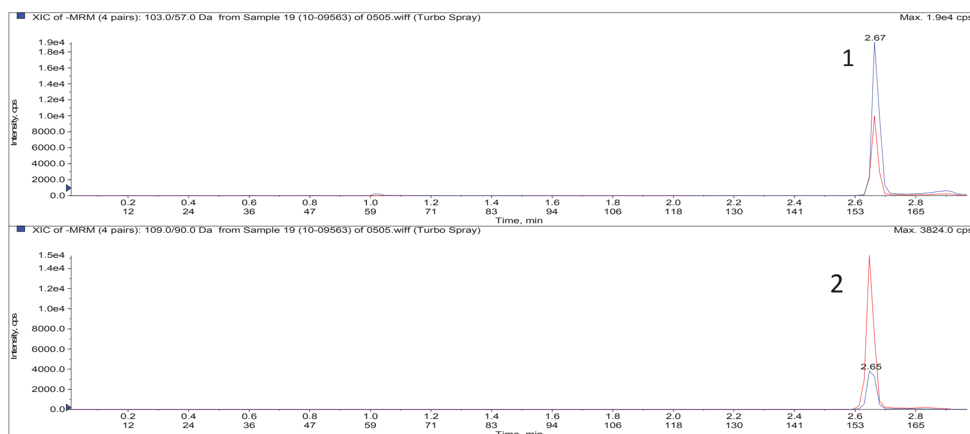
Evaporate to dryness at 70 °C using a steam of nitrogen or air.

11. **RECONSTITUTE / DERIVATIZE:**

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 20µL.
- **GC-MS:** Dissolve residue in 100 µL of Ethyl Acetate and 100 µL of BSTFA with 1% TMCS. Mix/Vortex; Heat at 70◦C for 30 minutes.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. GHB	103.02	84.9	2.67
2. GHB-D_6	109.13	90.0	2.65

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Flow Rate: 1.25mL/minute

Polarity: Negative

Reconstitute: 100µl

Injection Volume: 20µl

LC Column: Biphenyl HPLC Column 150 x 4.6mm 5µm

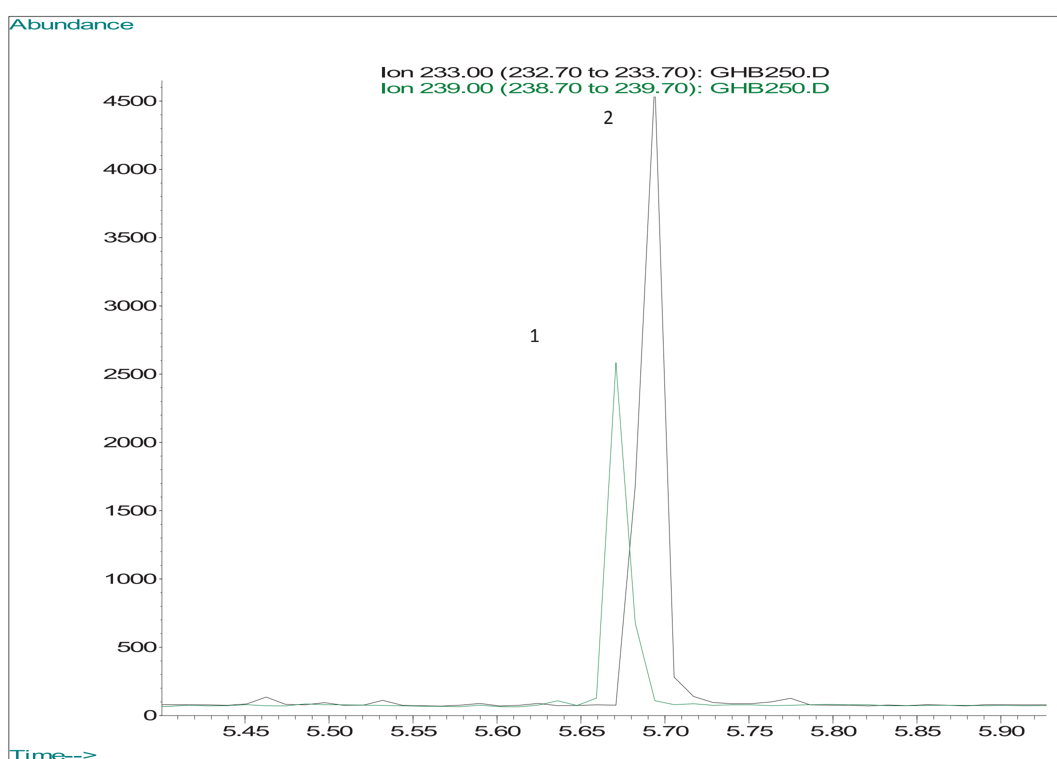
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Gradient:

Time	%A	%B
0.0	95	5
1.5	95	5
2.5	50	50
3.1	95	5
4.1	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



BSTFA-OXIME DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (minutes)
1.GHB-D_6	239	240		5.67
2.GHB	233	234	235	5.69

PARAMETERS

GC/MS: HP 5890 5972MSD GC/MS System with 7673 ALS System

GC capillary column: 30m x0.25mm (0.25um) RTX-5MS

Injector: 1µL Splitless 250°C

Oven temperature program: 70°C for 1 min; 15°C/min to 130°C, then to 300°C 50°C/min. Hold for 0.1min

Carrier gas: Helium

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

Developed by: Mr. Joseph A. Crifasi, M.A., M.T., (ASCP) Certified Toxicology Specialist, ABFT; Saint Louis University Health Sciences Center,
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GHB IN URINE AND BLOOD WITHOUT CONVERSION TO GAMMA-BUTRYLACTONE (GBL) BY LC-MS/MS OR GC-MS CLEAN SCREEN® GHB EXTRACTION COLUMN

Part #
ZSGHB020 – CLEAN SCREEN® GHB 200 mg, 10 mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

1. **PREPARE SAMPLE:**
   Blood: To 1 mL blood sample add internal standard and 0.5 mL of 100 mM phosphate buffer (pH 6.0).
   Mix/vortex.
   Rock for 10 minutes.
   Centrifuge for 10 minutes at 2700 rpm.

   Urine: To 200 µL of urine add internal standard(s) and 100 µL of 100 mM Phosphate buffer (pH 6.0)
   Mix/vortex

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**
   Place test tubes into vacuum manifold for collection
   The sample loading and elute are both collected
   Load at 1 to 2 mL/minute
   Collect elute as it is applied to the column
   After the sample is off the columns apply full vacuum for about 15 seconds to remove any residual blood/urine.

4. **ELUTE GHB:**
   Remove test tubes and set aside
   Place clean tubes into vacuum manifold for collection
   1 x 2 mL Methano/ NH_4OH (99:1) onto SPE column and collect

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

6. **SAMPLE CLEAN-UP:**
   Add 200 µL of dimethylformamide.
   Add 1 mL of hexane saturated with dimethylformamide.
   Mix by inversion for 5 minutes.
   Centrifuge at 3000 rpm for 5 minutes
   Transfer lower dimethylformamide layer to a clean test tube

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

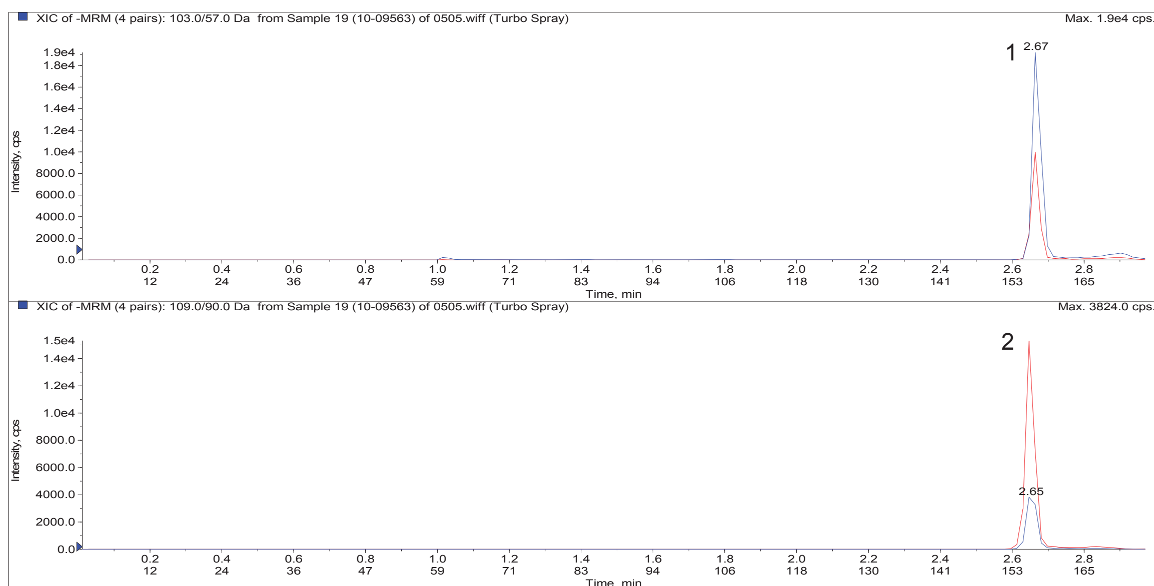
8. **RECONSTITUTE / DERIVATIZE**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL

   - **GC-MS**: **DERIVATIZE with TMS**
     Add 50 µL Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
     Overlay with N_2 and cap. Mix/vortex.
     React 30 minutes at 70 °C. Remove from heat source to cool.
     NOTE: Do not evaporate BSTFA solution

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.GHB	**103.02**	**84.9**	**2.67**
2.GHB-D_6	**109.13**	**90.0**	**2.65**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Flow Rate: 1.25 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

LC Column: Biphenyl HPLC Column 150 x 4.6 mm 5 µm

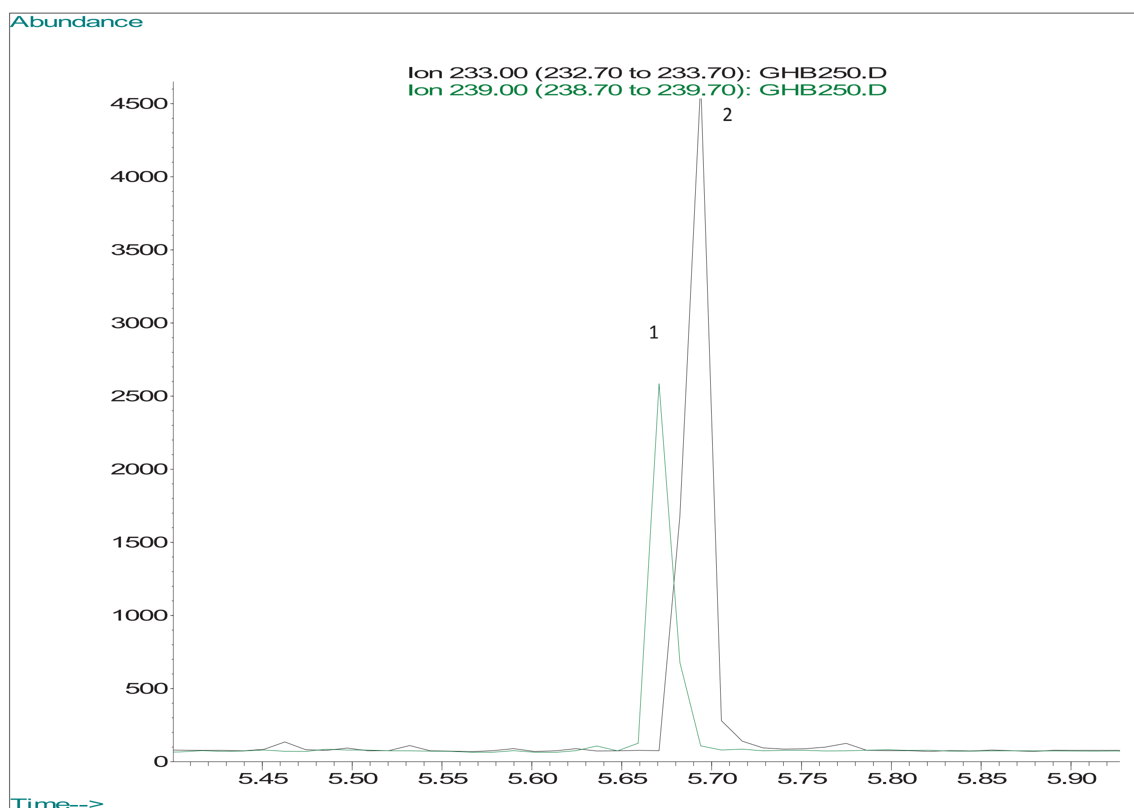
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Gradient:

Time	%A	%B
0.0	95	5
1.5	95	5
2.5	50	50
3.1	95	5
4.1	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



BSTFA-OXIME DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. GHB-D_6	239	240	241	5.67
2. GHB	233	234	235	5.69

PARAMETERS

GC/MS: HP 5890 5972MSD GC/MS System with 7673 ALS System

GC capillary column: 30 m x0.25 mm (0.25 µm) RTX-5MS

Injector: 1µL Splitless 250 °C

Oven temperature program: 70 °C for 1 min; 15 °C/min to 130 °C, then to 300 °C 50 °C/min. Hold for 0.1 min

Carrier gas: Helium

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

GHB IN URINE WITHOUT CONVERSION TO GAMMA-BUTRYLACTONE (GBL) BY LC-MS/MS OR GC-MS CLEAN-UP® QAX EXTRACTION COLUMN

Part #
CUQAX156 – CLEAN-UP® QAX 500 mg, 6 mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

1. **PREPARE SAMPLE:**
   Urine: To 50 µL of urine add internal standard(s) and 5 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 CH_3OH.
   Dry column (10 minutes at >10 inches Hg).

5. **ELUTE GHB:**
   1 x 3 mL 6% Glacial Acetic Acid/ 94% Methanol

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

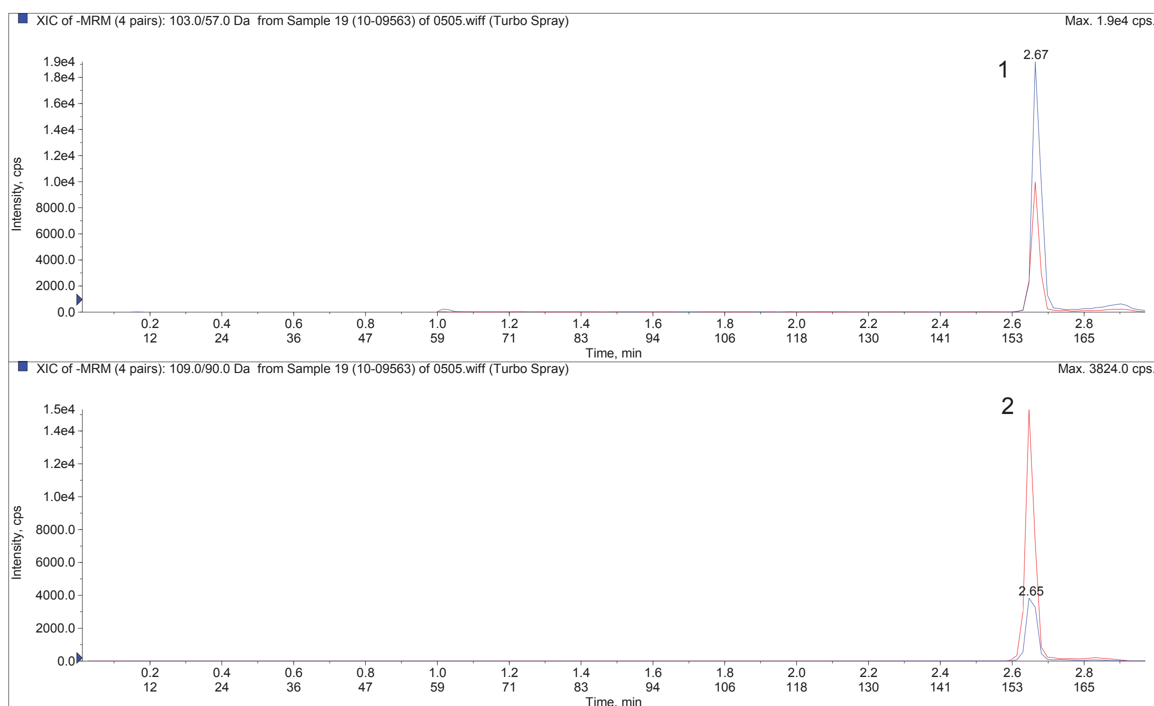
8. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL

   - **GC-MS**: **DERIVATIZE with TMS**
     Add 50 µL Ethyl Acetate and 50 µL BSTFA (with 1% TMCS)
     Overlay with N_2 and cap. Mix/vortex.
     React 30 minutes at 70°C. Remove from heat source to cool.
     NOTE: Do not evaporate BSTFA solution

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. GHB	**103.02**	**84.9**	**2.67**
2. GHB-D_6	**109.13**	**90.0**	**2.65**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Flow Rate: 1.25 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

LC Column: Biphenyl HPLC Column 150 x 4.6 mm 5 µm

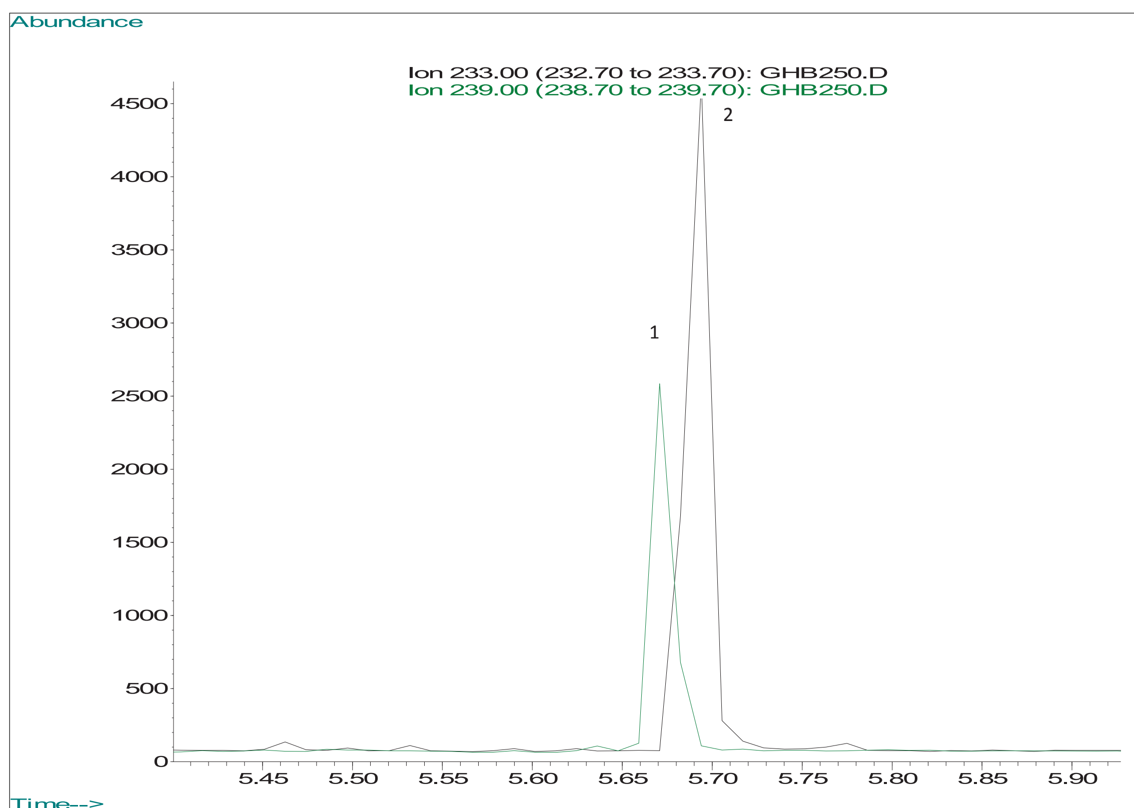
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Gradient:

Time	%A	%B
0.0	95	5
1.5	95	5
2.5	50	50
3.1	95	5
4.1	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



BSTFA-OXIME DERIVATIVES

Analyte	Quantify Ion	Qualifer Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. GHB-D_6	239	240		5.67
2. GHB	233	234	235	5.69

PARAMETERS

GC/MS: HP 5890 5972MSD GC/MS System with 7673 ALS System

GC capillary column: 30 m x 0.25 mm (0.25 µm) RTX-5MS

Injector: 1 µL Splitless 250 °C

Oven temperature program: 70 °C for 1 min; 15 °C/min to 130 °C, then to 300 °C 50 °C/min. Hold for 0.1 min

Carrier gas: Helium

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

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

Part #
CSDAU020 – CLEAN SCREEN® DAU 200 mg, 10mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA100ID21-3UM – Selectra® DA 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 1 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 acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE LSD AND METABOLITES:**
   1 x 3 mL Ethyl Acetate/ Acetonitrile: NH_4OH (78: 20: 2 v/v)

   or

   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2 v/v) Collect eluate at 1-2 mL /minute.

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

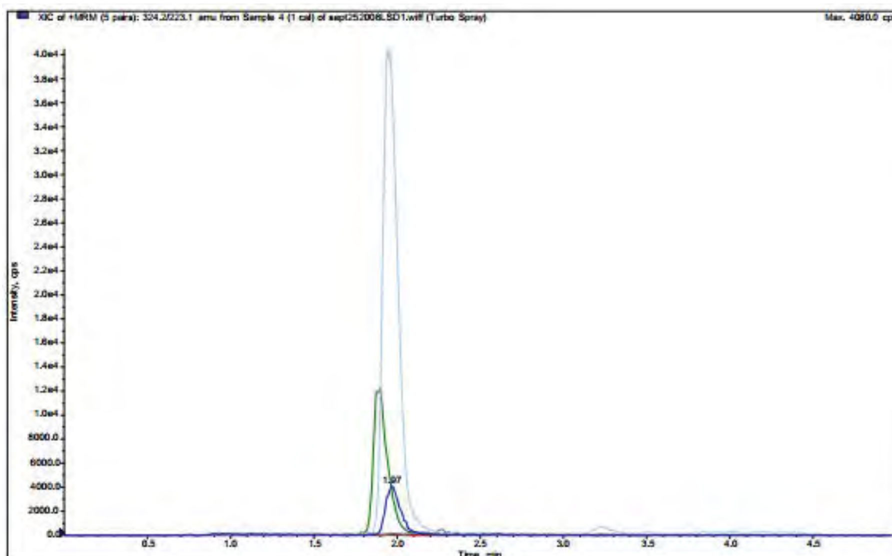
6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

7. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of BSTFA w/ 1% TMCS and react at 70 °C for 30 minutes; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions	
	Q1	Q3
1. LSD	**324.2**	**223.1**
2. Iso-LSD	**324.2**	**281.1**
3. Nor-LSD	**310.2**	**209.1**
4. OH-LSD	**356.2**	**338.1**
5. LSD-D_3	**327.2**	**226.1**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 5 µL

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

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

Gradient:

Time	%A	%B
0.00	30	70
3.00	90	10
3.01	30	70
5.00	30	70
5.01	STOP	

INSTRUMENT CONDITIONS (GC-MS):

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion
1. LSD-D_3-TMS	**298**	**296**	**271**
2. LSD-TMS	**395**	**293**	**268**

NARCOTICS/METABOLITES PANEL 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® 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 3 mL 100 mM acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE NARCOTICS/METABOLITES:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2 v/v)
   Collect eluate at 1 to 2 mL/minute
   or
   1 x 3mL Ethyl Acetate/ IPA/ NH_4OH (78:20:2 v/v)

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

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

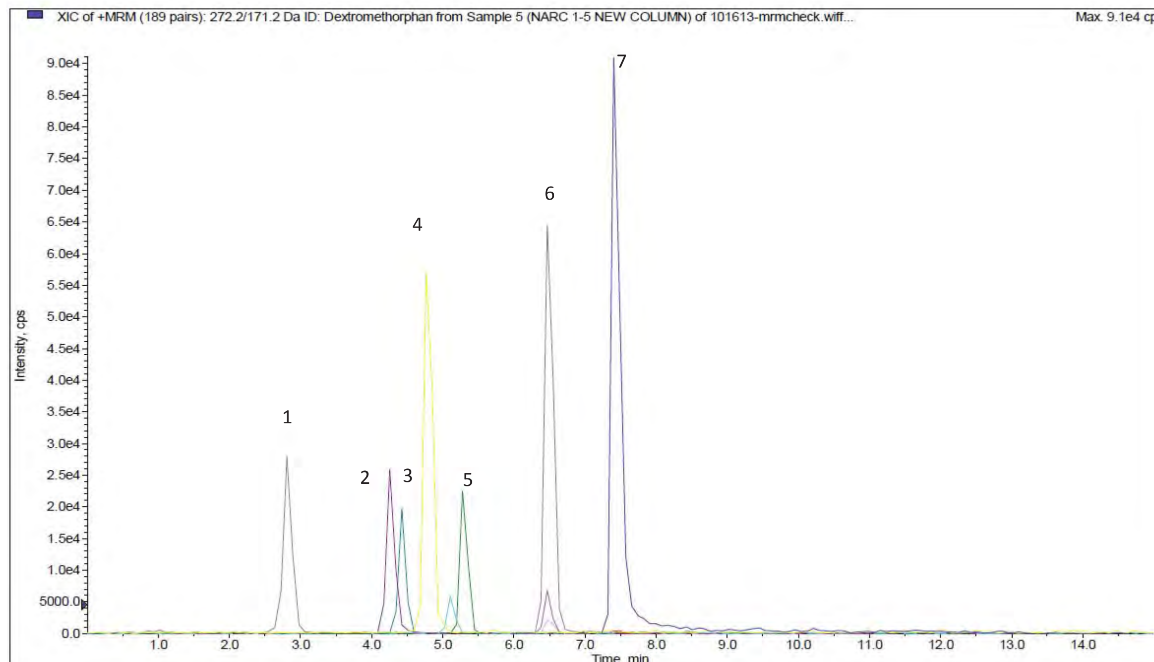
7. **RECONSTITUTE / DERIVATIZE:**
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.
   - **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

     Alternate Derivatization

     Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and react at 70 °C for 30 minutes; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Naloxone	328.2	310.2	2.80
2. Norketamine	224.1	207.1	4.25
3. NorFentanyl	233.2	84.1	4.45
4. Tramadol	264.2	58.0	4.80
5. Normeperdine	234.1	91.2	5.10
6. Norbuprenorphine	414.2	187.1	6.50
7. DXM	272.2	171.2	7.42

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 20 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	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

PAROXETINE 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
SLDA50ID21-3UM - SELECTRA® DA HPLC Column 50 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 acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at > 10 inches Hg).

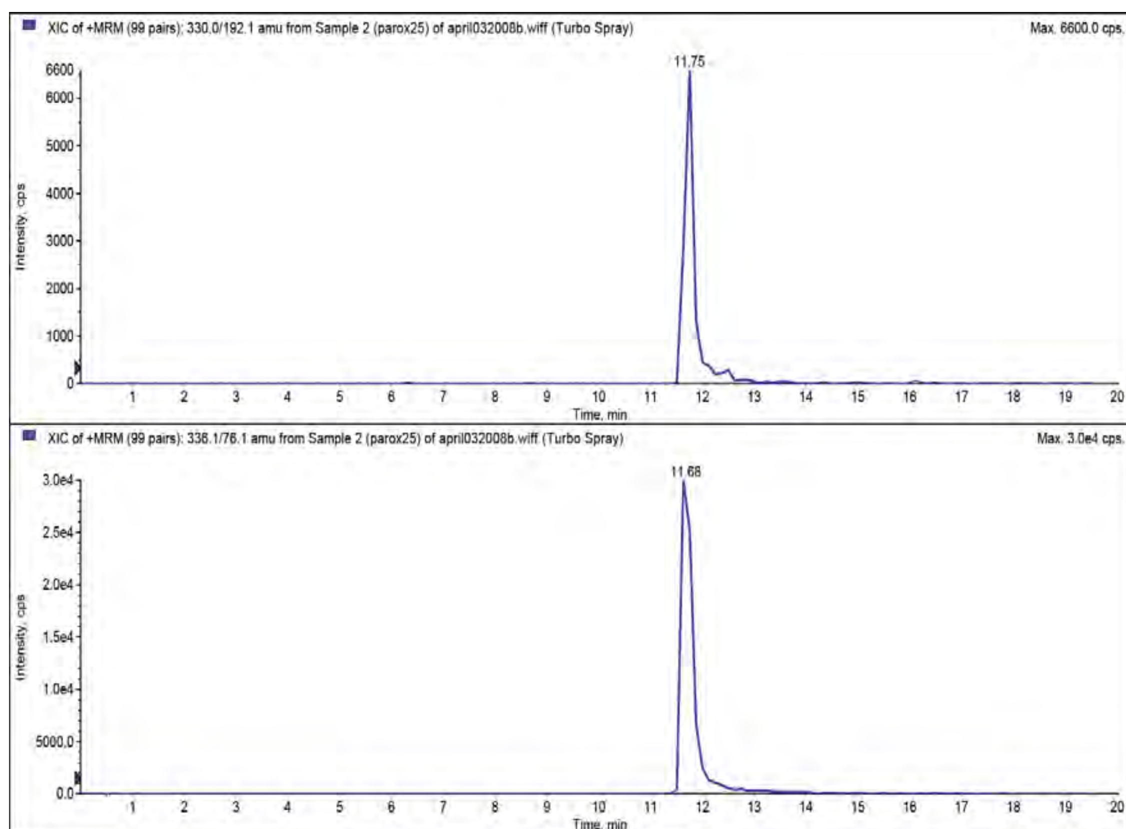
5. **ELUTE PAROXETINE:**
   1 x 3 mL Ethyl Acetate/ Acetonitrile/ NH_4OH (78:20:2)
   Collect eluate at 1-2 mL / minute.

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

7. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 μL of Methanol
     Inject 5 μL.
   - **GC-MS**: Dissolve residue in 100 μL of Ethyl Acetate
     Inject 1 to 2 μL onto gas chromatograph.

INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions	
	Q1	Q3
Paroxetine	**330.0**	**190.1**
Paroxetine-D_6	**336.0**	**76.1**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Flow Rate: 0.35 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 5 µL

LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 3 µm

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

Gradient:

Time	%A	%B
0.0	90	10
15.0	50	50
16.0	90	10
20.0	90	10
20.5	STOP	

PHENCYCLIDINE IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS STYRE SCREEN® DBX EXTRACTION COLUMN

Part #

SSDBX033 – STYRE SCREEN® DBX 30 mg 3 mL Tube

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

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. **APPLY SAMPLE:**

   Load at 1 to 2 mL/minute

3. **WASH COLUMN:**

   1 x 1 mL D.I. H_2O

   1 x 1 mL 100 mM acetic acid

   1 x 1 mL CH_3OH

   Dry column (5 minutes at full vacuum or pressure)

4. **ELUTE PHENCYCLIDINE:**

   2 x 0.5 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)

   Collect eluate at 1 to 2 mL/minute

   NOTE: Prepare elution solvent daily

   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

5. **DRY ELUATE:**

   Add 1 drop 1% HCl in Methanol to eluate before evaporating.

   Evaporate to dryness at < 40 °C

6. **RECONSTITUTE / DERIVATIZE:**

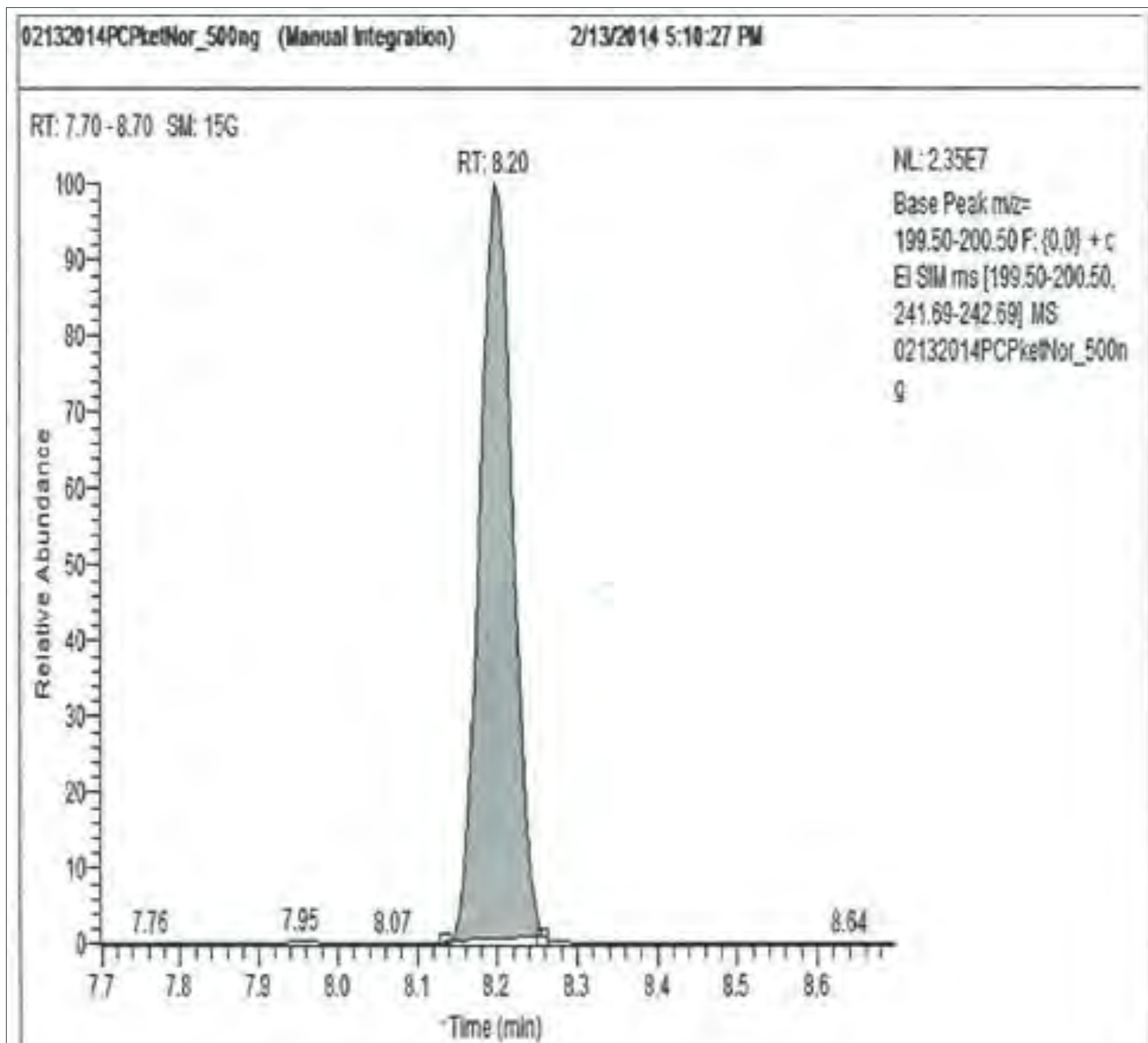
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 20 µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

LC-MS PCP TRANSITIONS

Analyte	MRM Transitions	
	Q1	Q3
Phencyclidine	**244.2**	**86.1**
Phencyclidine-D_5	**249.2**	**164.2**

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Retention Time (min)
1. **Phencyclidine**	**200**	**91**	**242**	**8.20**
2. **Phencyclidine-D_5**	**205**	**96**	**247**	**8.18**

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

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

Injector: 1μL Splitless, 250°C

Oven temperature program: 50 °C (0.5) to 320 °C (30 °C/minute): hold (5 minutes)

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

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

PSILOCIN IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS STYRE SCREEN® DBX EXTRACTION COLUMN

Part #

SSDBX206 – STYRE SCREEN® DBX, 200 mg, 6 mL Tube

BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase

SLDA50ID21-3UM – SELECTRA® DA HPLC Column 50 x 2.1mm, 3µm

1. **PREPARE SAMPLE:**

   Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
   Add 1-2 mL of blood, plasma/ serum, 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.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet

   Urine: PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:
   To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 1-2 hours at 65 °C.
   Allow sample to cool
   Do not adjust pH~ sample is ready to be added to the extraction column.

2. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute.

3. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM acetic acid
   1 x 3 mL of CH_3OH
   Dry column (5 minutes at > 10 inches Hg).

4. **ELUTE PSILOCIN:**
   1 x 3 mL Ethyl Acetate/ Acetonitrile/ NH_4OH (78: 20: 2 v/v)
   Or
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2 v/v)
   Collect eluate at 1-2 mL /minute.

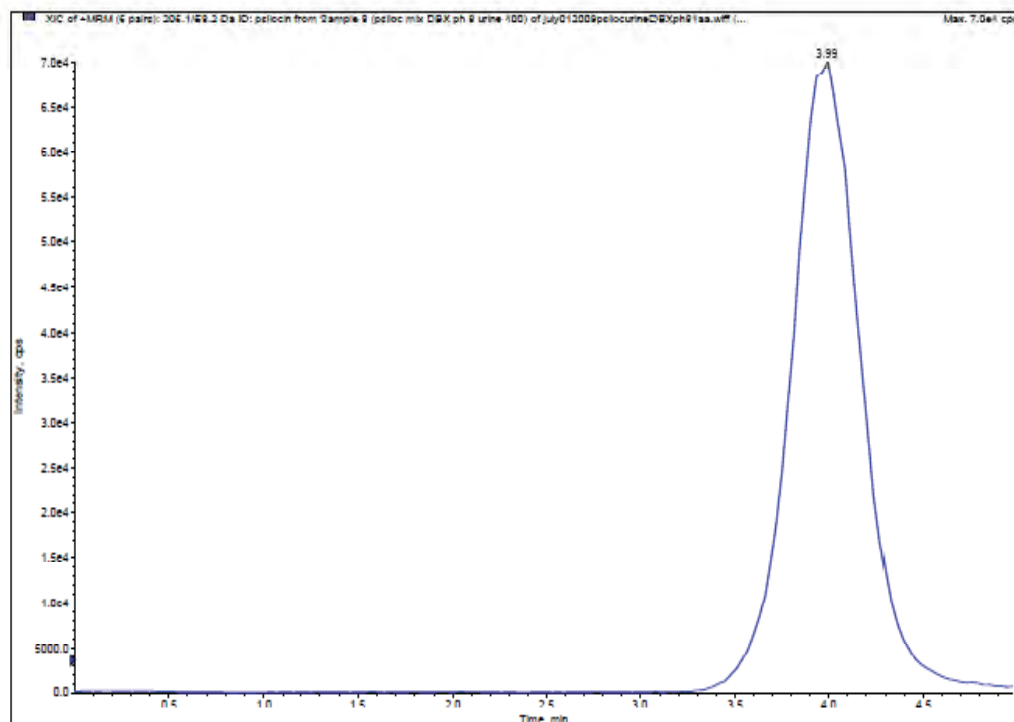
5. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

6. **RECONSTITUTE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of Methanol
     Inject 10 µL.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time
	Q1	Q3	(minutes)
Psilocin	**205.2**	**58.2**	**3.99**
Psilocin-D_{10}	**215.2**	**68.2**	**-**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Flow Rate: 0.3 mL/minute

Polarity: Positive

Injection Volume: 10 µL

LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 3 µm

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

Isocratic Flow:

Time	%A	%B
0.00	20	80
5.00	STOP	

PSILOCIN AND PSILOCYBIN IN URINE BY LC/MS/MS CLEAN SCREEN® EXTRACTION COLUMN

Part #
CSDAU206 – CLEAN SCREEN® DAU, 200 mg, 6 mL Tube
BETA-GLUC-10 - Selectrazyme® Beta-glucuronidase

1. **PREPARE SAMPLE:**
   To 1-2 mL of urine sample, add 500 µL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase.
   Optionally, add 500 µL of acetate buffer and 25 µL of concentrated β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C.
   Allow sample to cool
   Do not adjust pH~ sample is ready to be added to the extraction plate.

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 of CH_3OH

   Aspirate at full vacuum or pressure for 5 minutes

5. **ELUTE PSILOCIN:**
   1 x 3 mL Ethyl Acetate containing 2% NH_4OH
   Collect eluate at 1-2 mL /minute

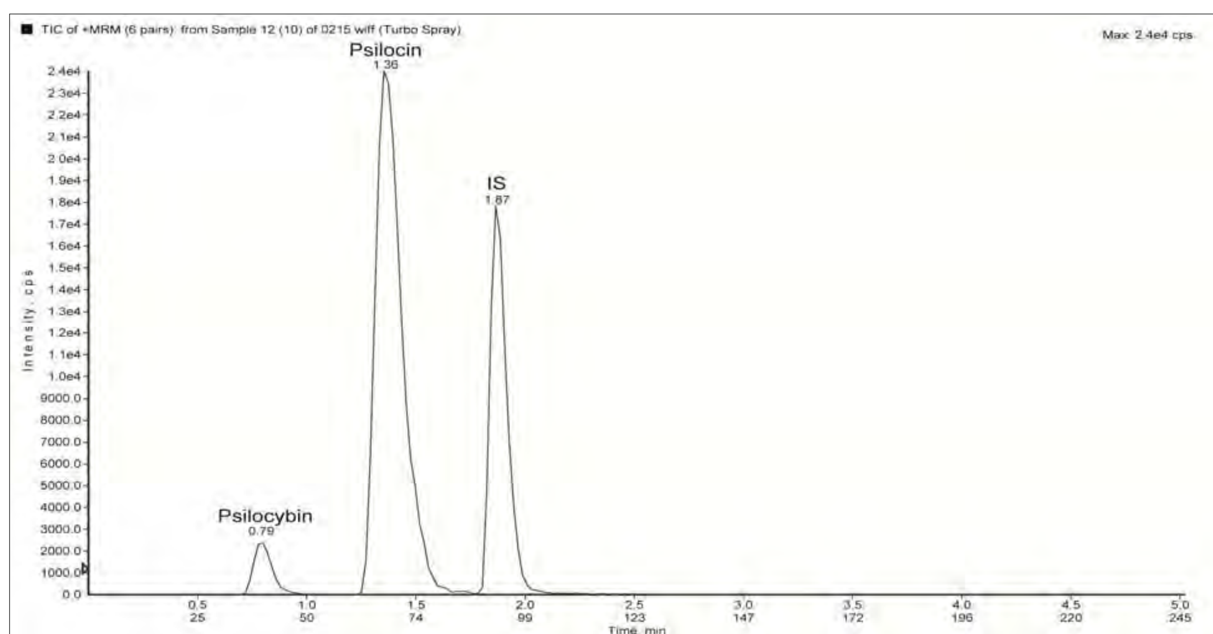
5b. ELUTE PSILOCYBIN:
   1 x 3 mL CH_3OH containing 2% NH_4OH
   Collect eluate at 1-2 mL /minute

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

7. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of Methanol
     Inject 10 µL.

CHROMATOGRAM



PARAMETERS

*Time = Dwell Time: *DP= Declustering Potential; *EP= Exit Potential: *CXP= Collision Cell Exit Potential: * CE=Collision Energy

Compound	Q1	Q2	Time/ms	DP/volts	SP/volts	CXP/volts	CE/volts
Psilocybin (1)	284.97	205.2	200	36	4.5	16	23
Psilocybin (2)	284.97	240.0	200	36	4.5	16	25
Psilocin (1)	205.081	58.1	200	26	8.5	14	23
Psilocin (2)	205.081	160.2	200	26	8.5	14	23
Ethyl morphine (1)	314.203	152.2	200	51	4	14	85
Ethylmorphine (2)	314.203	128.3	200	51	4	14	81

Mobile Phase A: 1% Formic Acid in D.I. H_2O

Mobile Phase B: 1% Formic Acid in Acetonitrile

Instrument: API 3200 QTrap MS/MS Compound MRM Transition

LC Column: 50 x 2.0 mm (3 µm) C_{18}

Flow Rate: 0.5 mL/minute

Injection Volume: 10 µL

Gradient:

Time	%A	%B
0	95	5
4	5	95
5.1	5	95

CLINICAL

FORENSICS

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Amphetamines

CPS analitica for Chemistry

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

Part #
CSDAU – CLEAN SCREEN® DAU
PFAA-0-1 – SELECTR-SIL® PFAA
SPFPOH-1 – SELECTRA-SIL® PFPOH
SHFAA-0-1– SELECTRA-SIL® HFAA
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA50ID21-5UM – SELECTRA® DA HPLC Column, 50 x 2.1 mm, 5 μm
or
SLPFPP50ID21-5UM – SELECTRA® PFPP 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 3 mL 100 mM Acetic Acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE AMPHETAMINES:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

6. **DRY ELUATE:**
   Add 100 μL of 1% HCl in Methanol to each test tube
   Evaporate to dryness at < 40 °C

7. **RECONSTITUTE / DERIVATIZE:**
   - **LC-MS/MS:** Reconstitute sample in 100 μL of mobile phase
     Inject 10-20 μL.

   - **GC-MS**: Fluoroacylate with PFPA (PFAA)
     Add 50 μL PFPA. Over lay with N_2 and cap
     *Improved derivatization by addition of PFPOH
     React 20 minutes at 70 °C. Evaporate to dryness < 40 °C
     Reconstitute with 100 μL Ethyl Acetate

Alternate Derivatization

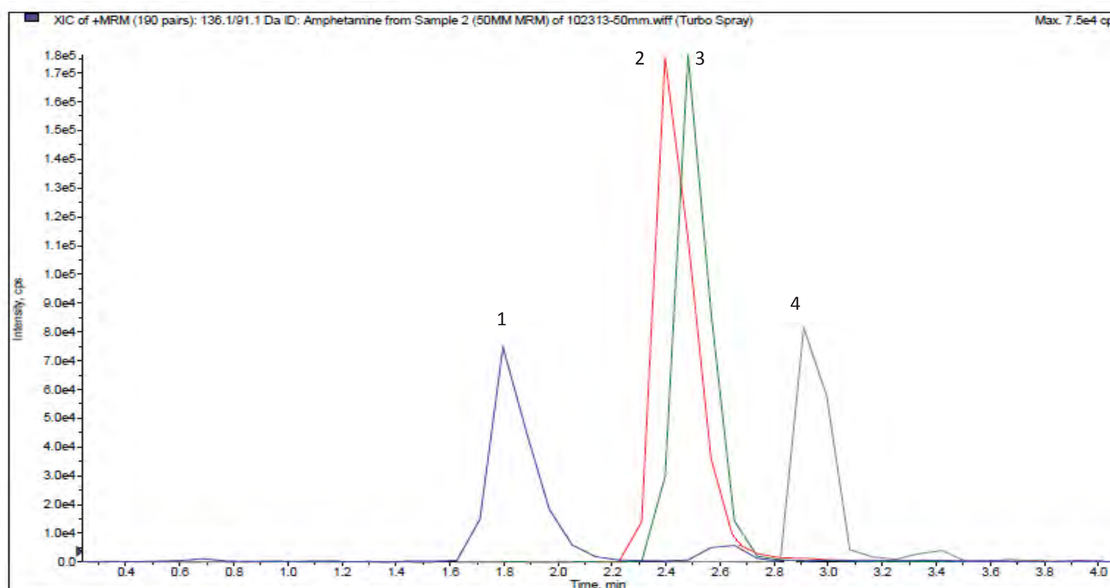
1. Fluoroacylate with HFPA (HFAA)
   Add 50 µL HFPA. Over lay with N_2 and cap
   *Improved derivatization by addition of PFPOH
   React 20 minutes at 70 °C. Evaporate to dryness < 40 °C
   Reconstitute with 100 µL Ethyl Acetate

2. Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate;
   React 45 minutes at 70 °C

Note: Sodium periodate can be added to sample during preparation if oxidation is preferred.
To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard(s). Add 2 mL of urine and 1 mL 0.35 M sodium periodate.
Mix/vortex
Incubate at room temp.for 20 min.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
Sample is now ready to be added to the extraction column

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	136.1	91.1	1.18
2. Methamphetamine	150.1	91.1	2.40
3. MDA	180.1	105.0	2.45
4. MDMA	194.1	105.1	2.95

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 20 µL

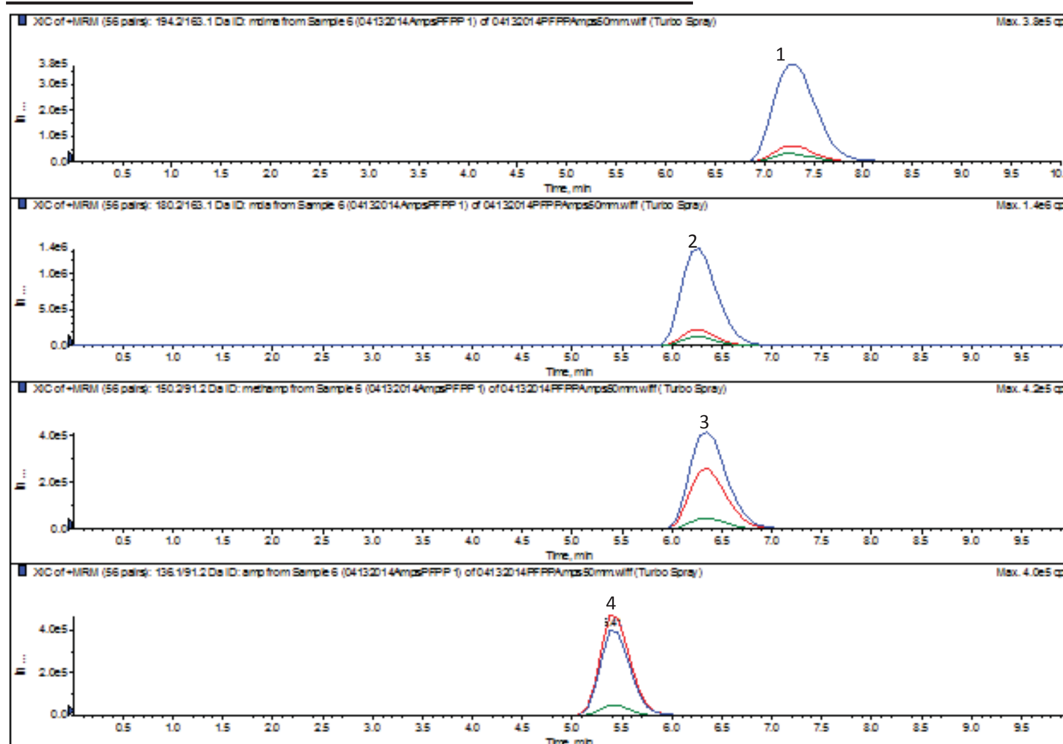
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Gradient:

Time	%A	%B
0.0	80	20
0.5	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	136.1	91.2	5.41
2. MDA	180.2	163.1	6.24
3. Methamphetamine	150.1	91.2	6.35
4. MDMA	194.2	163.1	7.29

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® PFPP HPLC Column 50 x 2.1 mm 5 µm

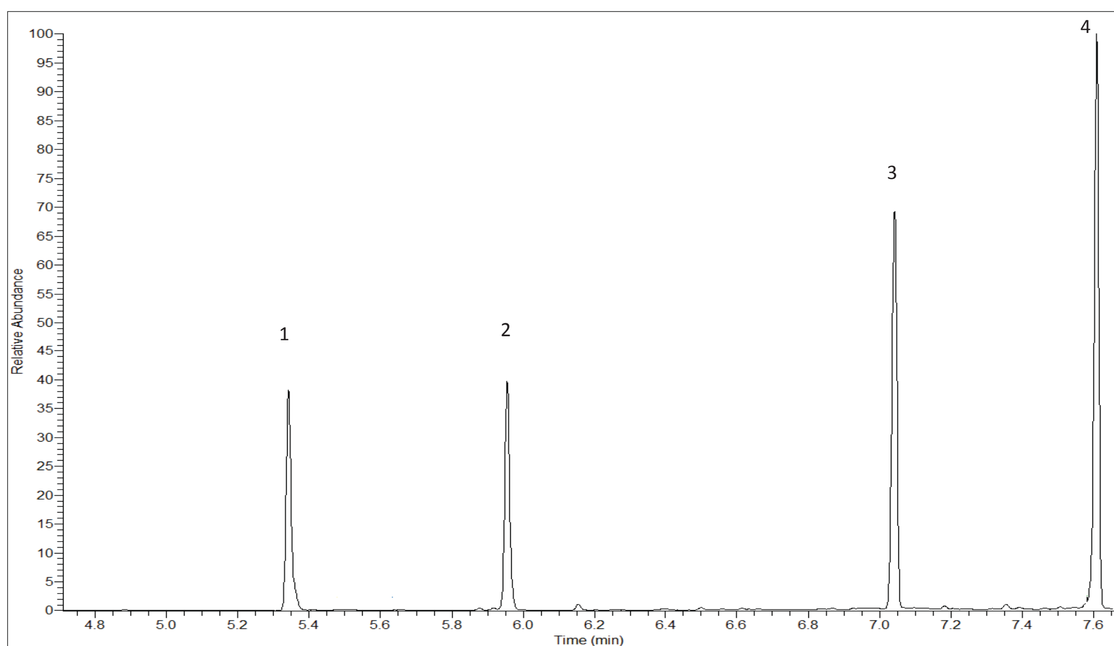
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	30	70
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

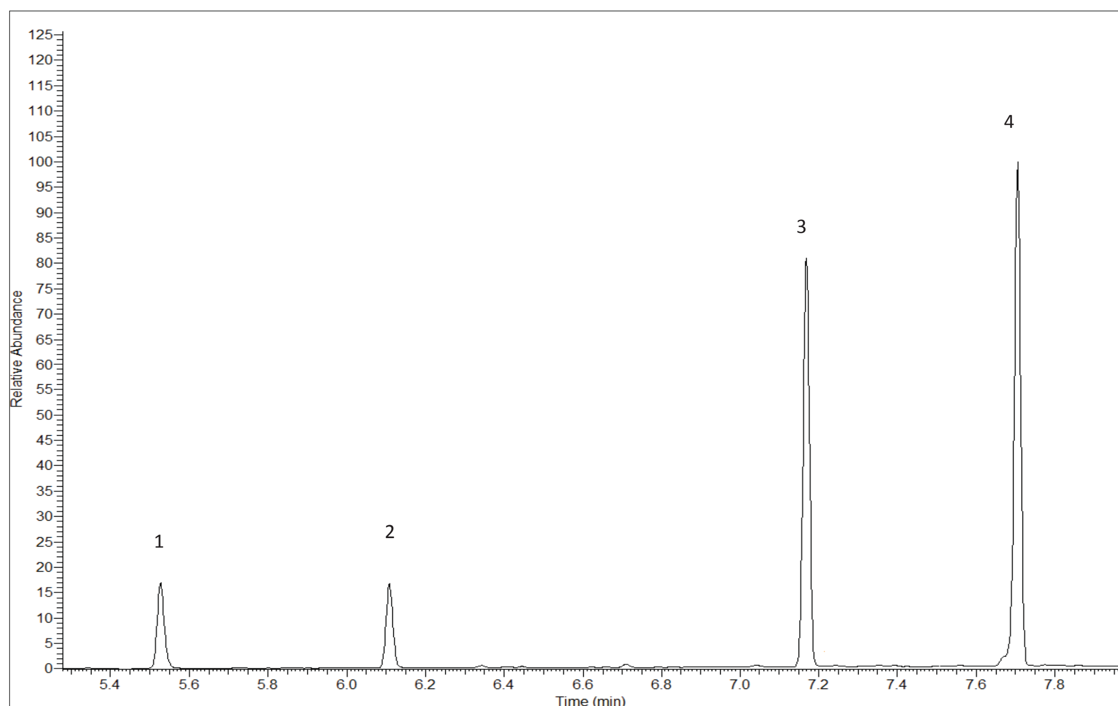
CHROMATOGRAM



Fluoroacylate with PFPA (PFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Amphetamine	190	118	91	5.34
2. Methamphetamine	204	160	118	5.95
3. MDA	162	325	135	7.61
4. MDMA	162	204	135	7.04

CHROMATOGRAM



Fluoroacylate with HFPA (HFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Amphetamine	240	91	118	5.53
2. Methamphetamine	254	210	118	6.11
3. MDA	375	162	135	7.17
4. MDMA	254	210	162	7.71

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

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

Injector: 1μL Splitless, 250 °C

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

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

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

AMPHETAMINES IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS STYRE SCREEN® DBX EXTRACTION COLUMN

Part #
SSDBX033 – STYRE SCREEN® DBX 30 mg, 3 mL Tube
PFAA-0-1 – SELECTRA-SIL® PFAA
SPFPOH-1 – SELECTRA-SIL® PFPOH
SHFAA-0-1– SELECTRA-SIL® HFAA
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA50ID21-5UM – SELECTRA® DA HPLC Column, 50 x 2.1 mm, 5 µm
or
SLPFPP50ID21-5UM – SELECTRA® PFPP 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. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute

3. **WASH COLUMN:**
   1 x 1 mL D.I. H_2O
   1 x 1 mL 100 mM Acetic Acid
   1 x 1 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

4. **ELUTE AMPHETAMINES:**
   2 x 0.5 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

5. **DRY ELUATE:**
   Add 100 µL of 1% HCl in Methanol to each test tube
   Evaporate to dryness at < 40 °C

6. **RECONSTITUTE / DERIVATIZE:**
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10-20 µL.
   - **GC-MS**: Fluoroacylate with PFPA (PFAA)
     Add 50 µL PFPA. Over lay with N_2 and cap
     *Improved derivatization by addition of PFPOH
     React 20 minutes at 70 °C. Evaporate to dryness <40 °C
     Reconstitute with 100 µL Ethyl Acetate

Alternate Derivatization

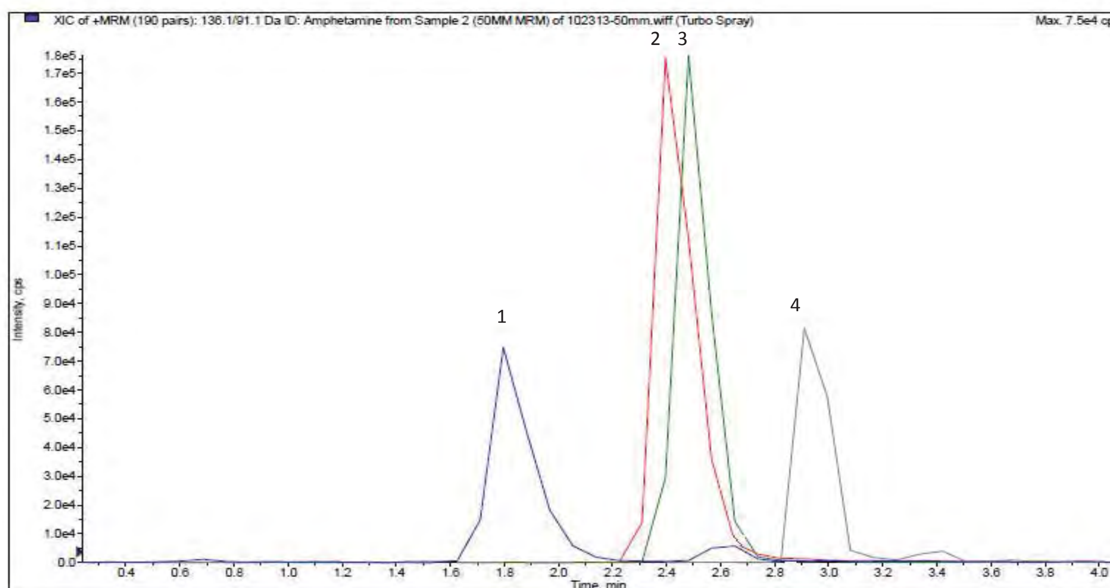
3. Fluoroacylate with HFPA (HFAA)
   Add 50 µL HFPA. Over lay with N_2 and cap
   *Improved derivatization by addition of PFPOH
   React 20 minutes at 70 °C. Evaporate to dryness <40 °C
   Reconstitute with 100 µL Ethyl Acetate

4. Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate;
   React 45 minutes at 70 °C

Note: Sodium periodate can be added to sample during preparation if oxidation is preferred.
To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard(s). Add 2 mL of urine and 1 mL 0.35 M sodium periodate.
Mix/vortex
Incubate at room temp.for 20 min.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
Sample is now ready to be added to the extraction column

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.Amphetamine	136.1	91.1	1.18
2.Methamphetamine	150.1	91.1	2.40
3.MDA	180.1	105.0	2.45
4.MDMA	194.1	105.1	2.95

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 20 µL

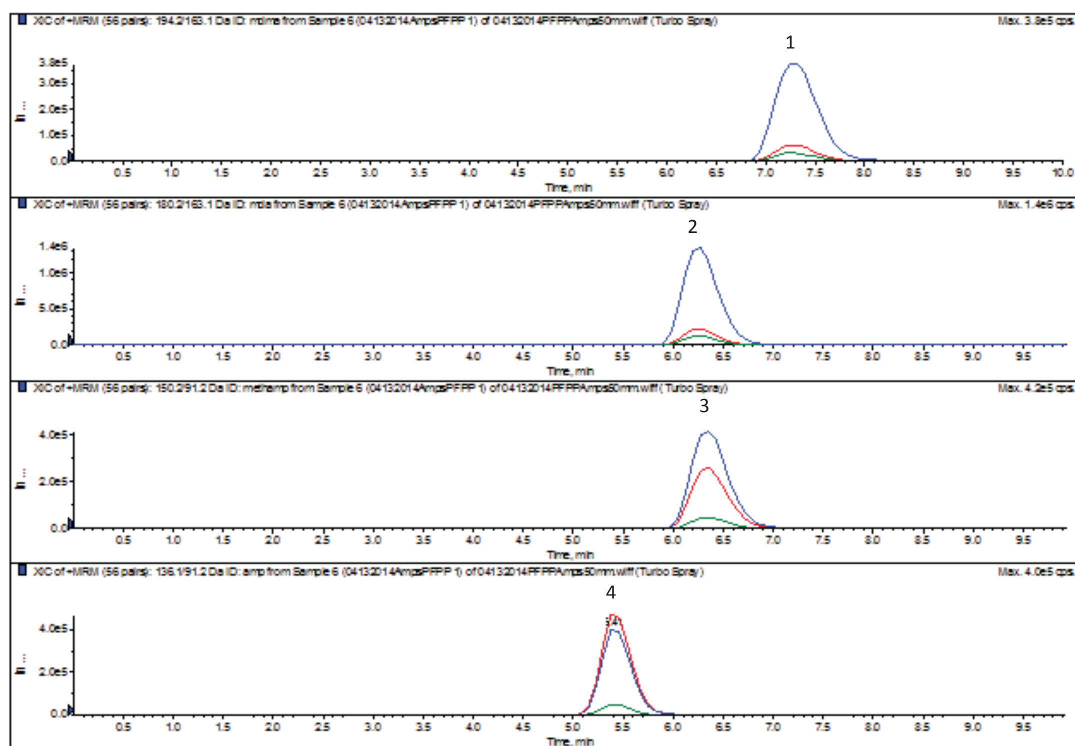
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Gradient:

Time	%A	%B
0.0	80	20
0.5	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	**136.1**	**91.2**	**5.41**
2. MDA	**180.2**	**163.1**	**6.24**
3. Methamphetamine	**150.1**	**91.2**	**6.35**
4. MDMA	**194.2**	**163.1**	**7.29**

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® PFPP HPLC Column 50 x 2.1 mm 5 µm

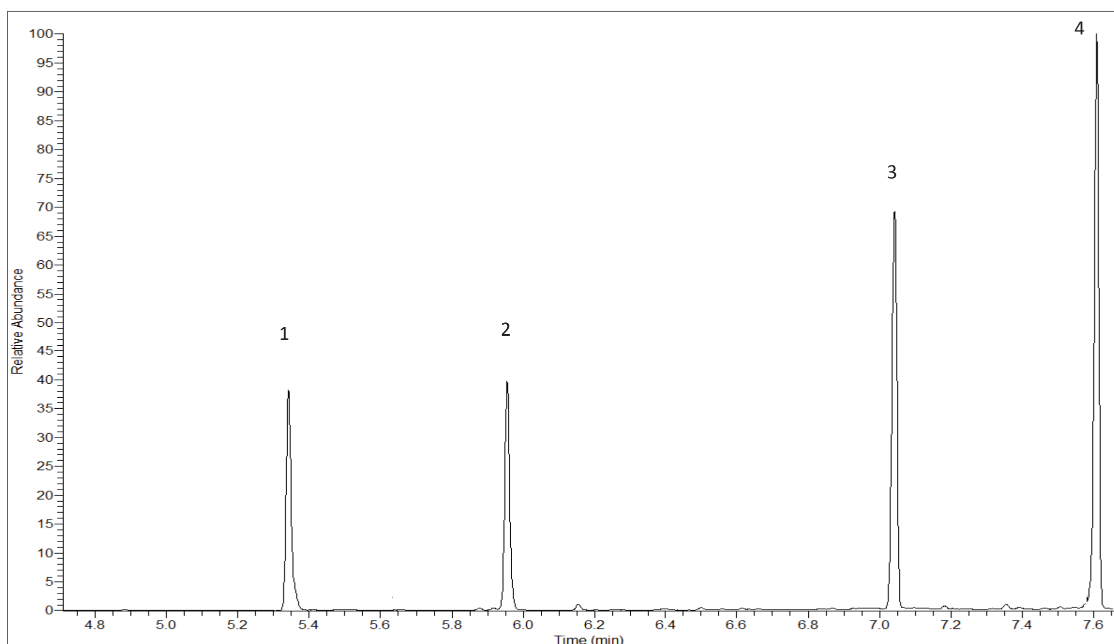
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	30	70
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

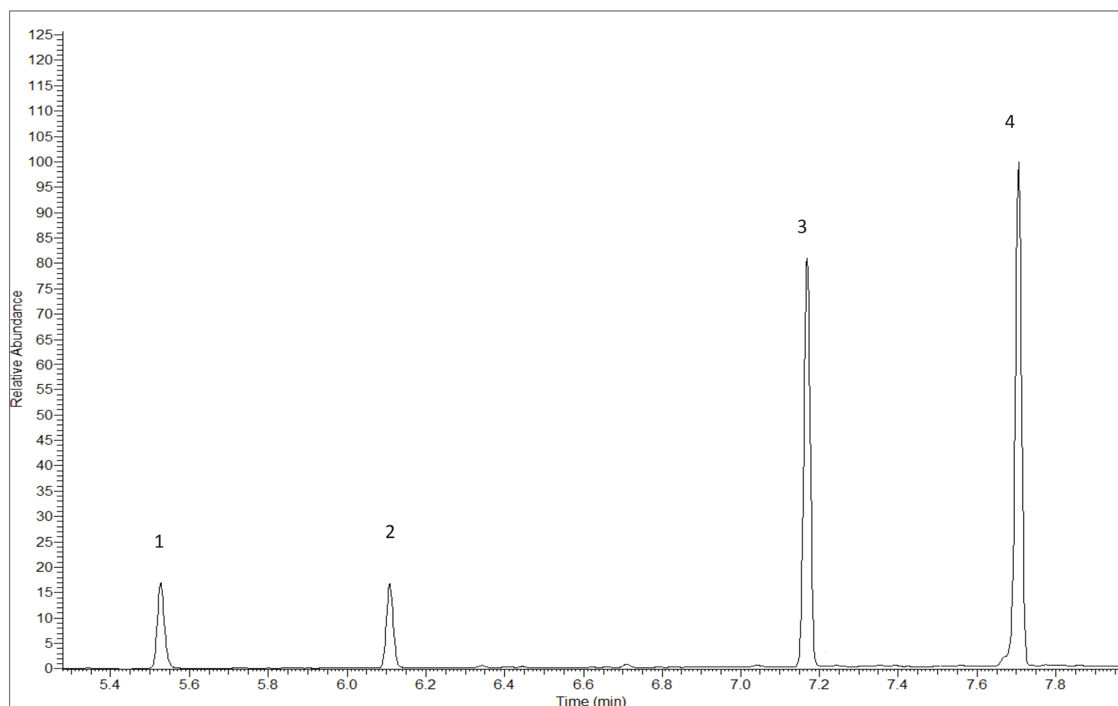
CHROMATOGRAM



Fluoroacylate with PFPA (PFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Amphetamine	190	118	91	5.34
2. Methamphetamine	204	160	118	5.95
3. MDA	162	325	135	7.61
4. MDMA	162	204	135	7.04

CHROMATOGRAM



Fluoroacylate with HFPA (HFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Amphetamine	240	91	118	5.53
2. Methamphetamine	254	210	118	6.11
3. MDA	375	162	135	7.17
4. MDMA	254	210	162	7.71

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: 70 °C (0.5) to 320 °C (25 °C/ minute): hold (2 minutes)

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

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

AMPHETAMINES IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN XCEL® I EXTRACTION COLUMN

Part #
CSXCE111 – CLEAN SCREEN XCEL® 130 mg, 1 mL Tube
PFAA-0-1 – SELECTR-SIL® PFAA
SPFPOH-1 – SELECTRA-SIL® PFPOH
SHFAA-0-1– SELECTRA-SIL® HFAA
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA100ID21-5UM – SELECTRA® DA HPLC Column, 100 x 2.1 mm, 5 µm
or
SLPFPP50ID21-5UM – SELECTRA® PFPP 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. APPLY SAMPLE

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. WASH

1 x 3 mL 98% Methanol: 2% Acetic Acid
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. ELUTION

1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. DRY ELUTE

Add 50 µL of 1% HCl in CH_3OH to each tube
Evaporate fraction to complete dryness under stream of dry air or nitrogen at~ 35 °C.

NOTE: A 1% HCl in CH_3OH solution has been used to prevent volatization by the formation of the hydrochloric salt of the drugs.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 5-20 µL.
- **GC-MS**: Fluoroacylate with PFPA (PFAA)
  Add 50 µL PFPA. Over lay with N_2 and cap
  *Improved derivatization by addition of PFPOH
  React 20 minutes at 70 °C. Evaporate to dryness <40 °C
  Reconstitute with 100 µL Ethyl Acetate

NOTES: *(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis, if being used. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity*

Alternate Derivatization

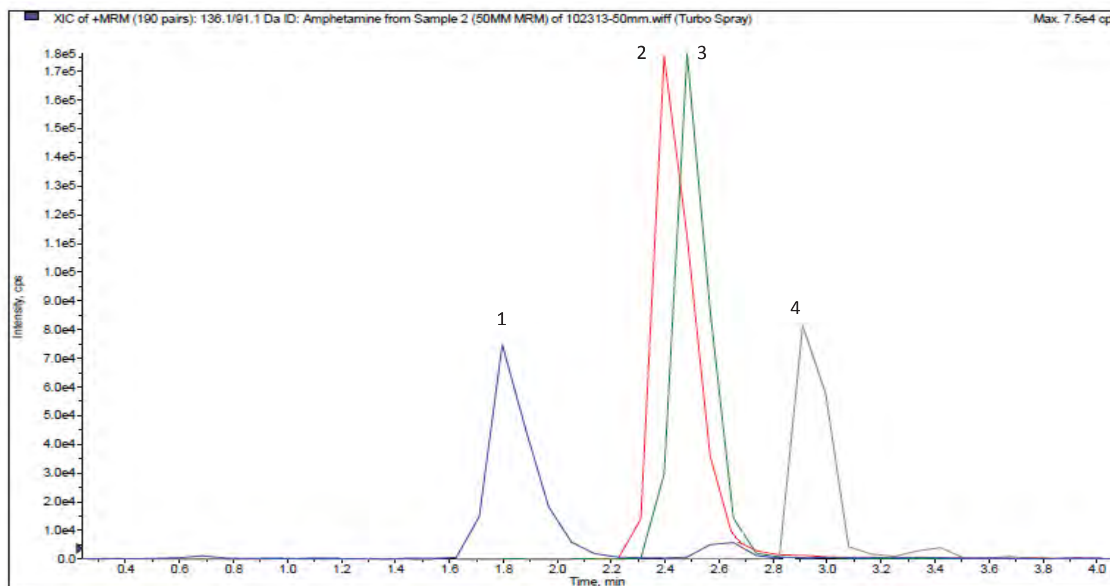
5. Fluoroacylate with HFPA (HFAA)
   Add 50 µL HFPA. Over lay with N_2 and cap
   *Improved derivatization by addition of PFPOH
   React 20 minutes at 70 °C. Evaporate to dryness <40 °C
   Reconstitute with 100 µL Ethyl Acetate

6. Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate;
   React 45 minutes at 70 °C

<u>**Note**</u>: Sodium periodate can be added to sample during preparation if oxidation is preferred.
To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard(s). Add 2 mL of urine and 1 mL 0.35 M sodium periodate.
Mix/vortex
Incubate at room temp.for 20 min.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
Sample is now ready to be added to the extraction column

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	136.1	91.1	1.18
2. Methamphetamine	150.1	91.1	2.40
3. MDA	180.1	105.0	2.45
4. MDMA	194.1	105.1	2.95

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 20 µL

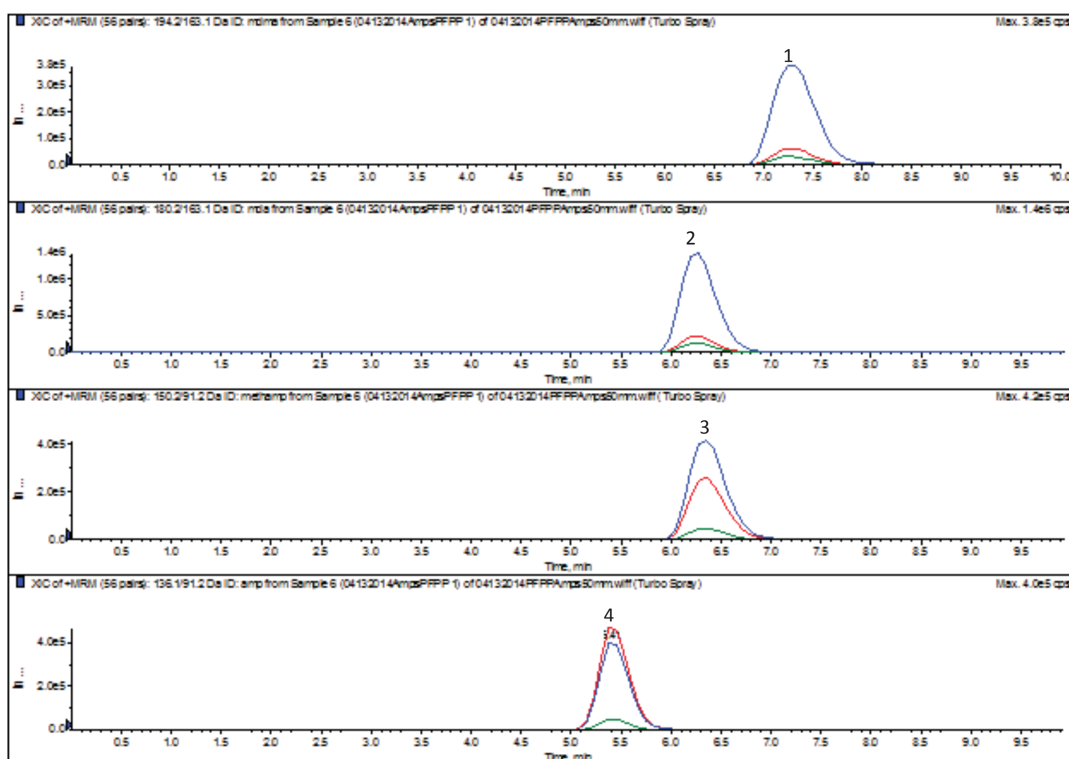
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Gradient:

Time	%A	%B
0.0	80	20
0.5	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	**136.1**	**91.2**	**5.41**
2. MDA	**180.2**	**163.1**	**6.24**
3. Methamphetamine	**150.1**	**91.2**	**6.35**
4. MDMA	**194.2**	**163.1**	**7.29**

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® PFPP HPLC Column 50 x 2.1 mm 5 µm

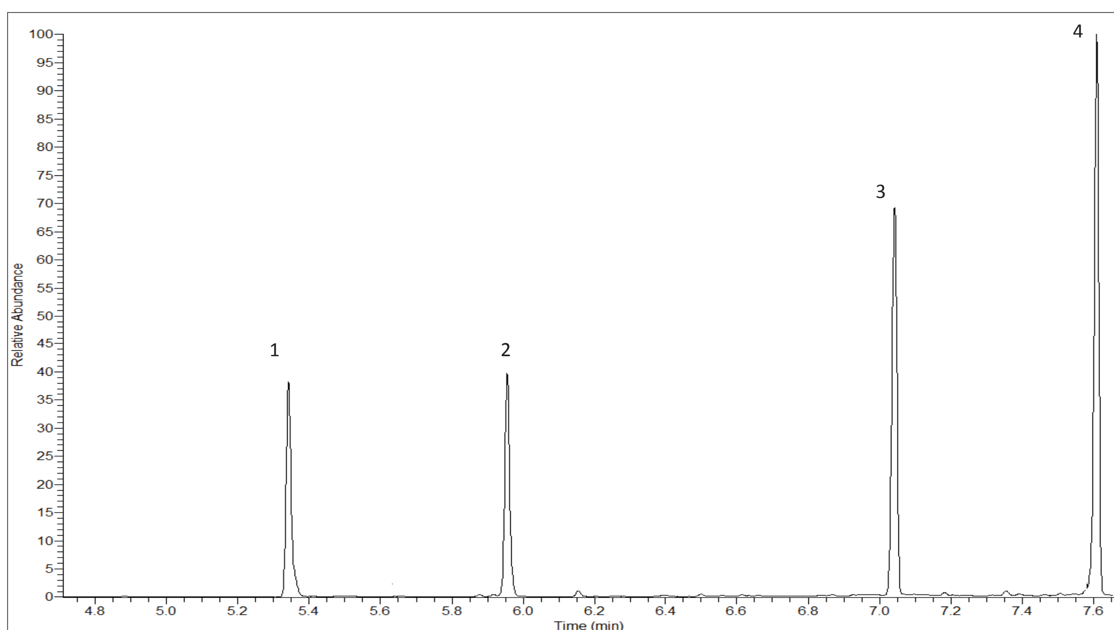
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	30	70
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

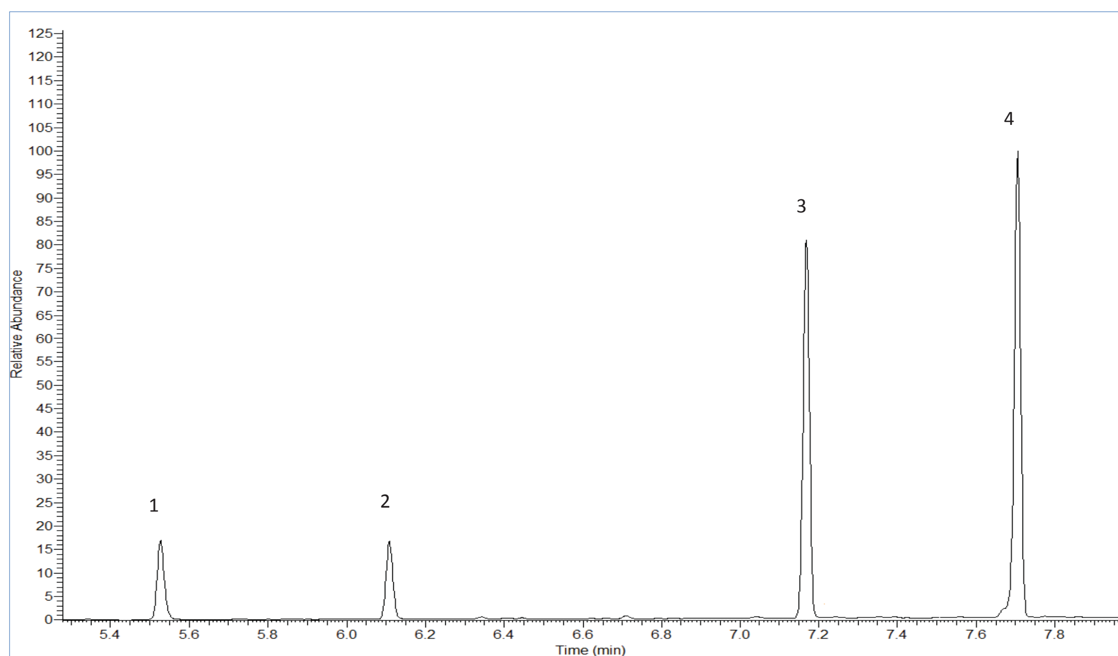
CHROMATOGRAM



Fluoroacylate with PFPA (PFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Amphetamine	190	118	91	5.34
2. Methamphetamine	204	160	118	5.95
3. MDA	162	325	135	7.61
4. MDMA	162	204	135	7.04

CHROMATOGRAM



Fluoroacylate with HFPA (HFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Amphetamine	240	91	118	5.53
2. Methamphetamine	254	210	118	6.11
3. MDA	375	162	135	7.17
4. MDMA	254	210	162	7.71

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: 70 °C (0.5) to 320 °C (25 °C/ minute): hold (2 minutes)

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

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

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

Part #
CSDAU – CLEAN SCREEN® DAU
PFAA-0-1 – SELECTRA-SIL® PFAA
SPFPOH-1 – SELECTRA-SIL® PFPOH
SLDA100ID21-5UM – SELECTRA® DA HPLC Column, 100 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 3 mL 100 mM Acetic Acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE BATH SALTS:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute

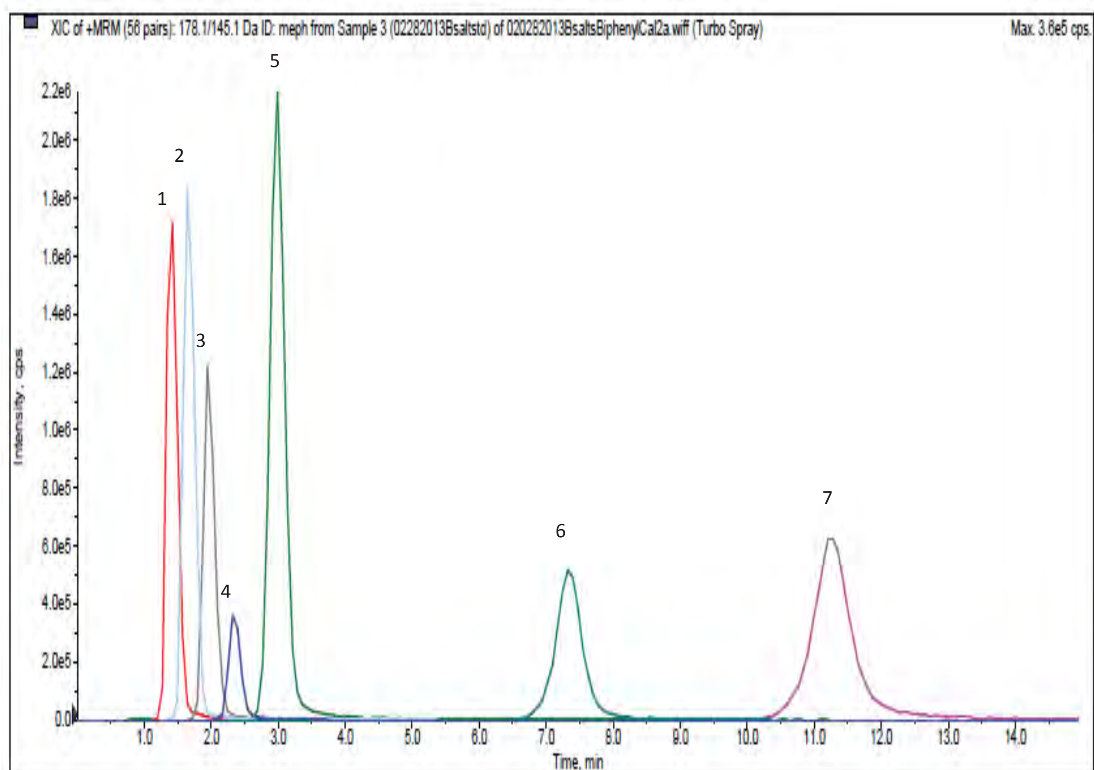
   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

6. **DRY ELUATE:**
   Add 50 µL of 1% HCl in Methanol to each test tube
   Evaporate to dryness at < 40 °C

NOTE: A 1% HCl in CH_3OH solution has been used to prevent volatization by the formation of The hydrochloric salt of the drugs

7. **RECONSTITUTE:**
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 5 µL.
   - **GC-MS**: Fluoroacylate with PFPA (PFAA)
     Add 50 µL PFPA. Over lay with N_2 and cap
     *Improved derivatization by addition of PFPOH
     React 20 minutes at 70 °C. Evaporate to dryness <40 °C
     Reconstitute with 100 µL Ethyl Acetate

INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1.Flephedrone	**182.1**	**164.2**	**1.41**
2.Methylone	**208.1**	**160.1**	**1.66**
3.Methadrone	**194.1**	**161.1**	**1.96**
4.Methedrone	**178.1**	**145.1**	**2.34**
5.Methethcathinone	**192.2**	**174.0**	**2.98**
6.MDPV	**276.2**	**126.1**	**7.34**
7.Pyravalerone	**246.2**	**105.2**	**11.24**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 5 µL

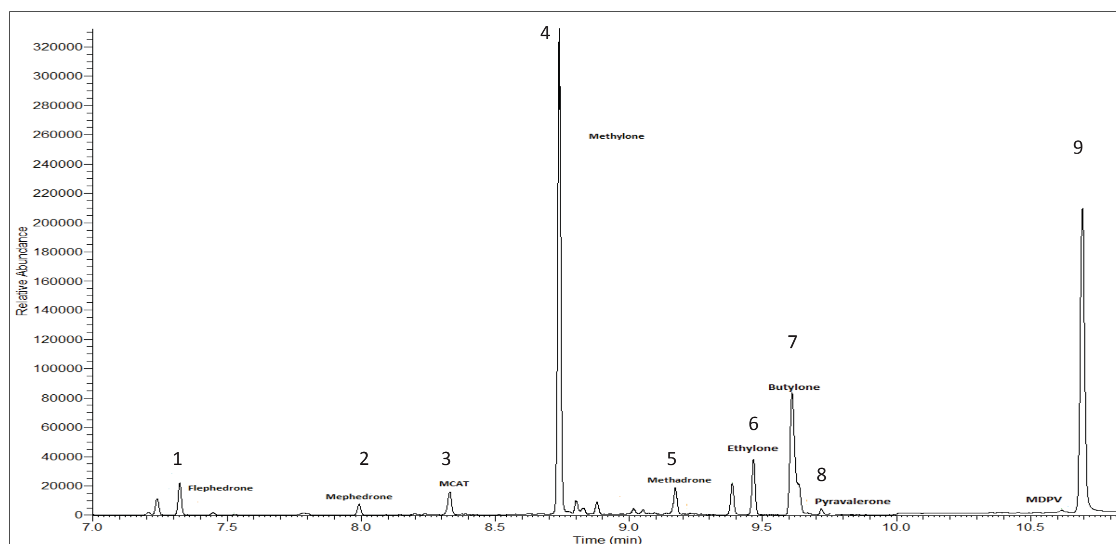
LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

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

Isocratic:

Time	%A	%B
0.00	70	30
15.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):



Fluoroacylate with PFPA (PFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1.Flephedrone	123	204	160	7.32
2.Mephedrone	204	160	149	7.99
3.MCAT	218	174	91	8.33
4.Methylone	353	204	160	8.74
5.Methadrone	135	160	204	9.17
6.Ethylone	218	190	367	9.47
7.Butylone	218	160	367	9.61
8.Pyravalerone	126	84	91	9.72
9.MDPV	126	96	84	10.62

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: 50 °C (1) to 310 °C (25 °C/ minute): hold (3.6 minute)

Carrier gas: Helium (1.2 mL/ minute)

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

Reference:
Comprehensive Forensic Toxicological Analysis of Designer Drugs; NIJ Grant
Author(s): Anthony P. DeCaprio, W. Lee Hearn, Madeleine J. Swortwood
Document No.: 244233
Date Received: December 2013

BATH SALTS IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN XCEL® I EXTRACTION COLUMN

Part #:
CSXCE111 – CLEAN SCREEN XCEL® 130 mg, 1 mL Tube
PFAA-0-1 – SELECTRA-SIL® PFAA
SPFPOH-1 – SELECTRA-SIL® PFPOH
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. **APPLY SAMPLE**
   Load sample directly to column without any preconditioning.
   Pull sample through at a rate of 1-2 mL/ minute.
   Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. **WASH**
   1 x 3 mL 98% Methanol: 2% Acetic Acid
   Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. **ELUTION**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. **DRY ELUTE**

   Add 50 µL of 1% HCl in CH_3OH to each tube
   Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

   NOTE: A 1% HCl in CH_3OH solution has been used to prevent volatization by the formation of the hydrochloric salt of the drugs.

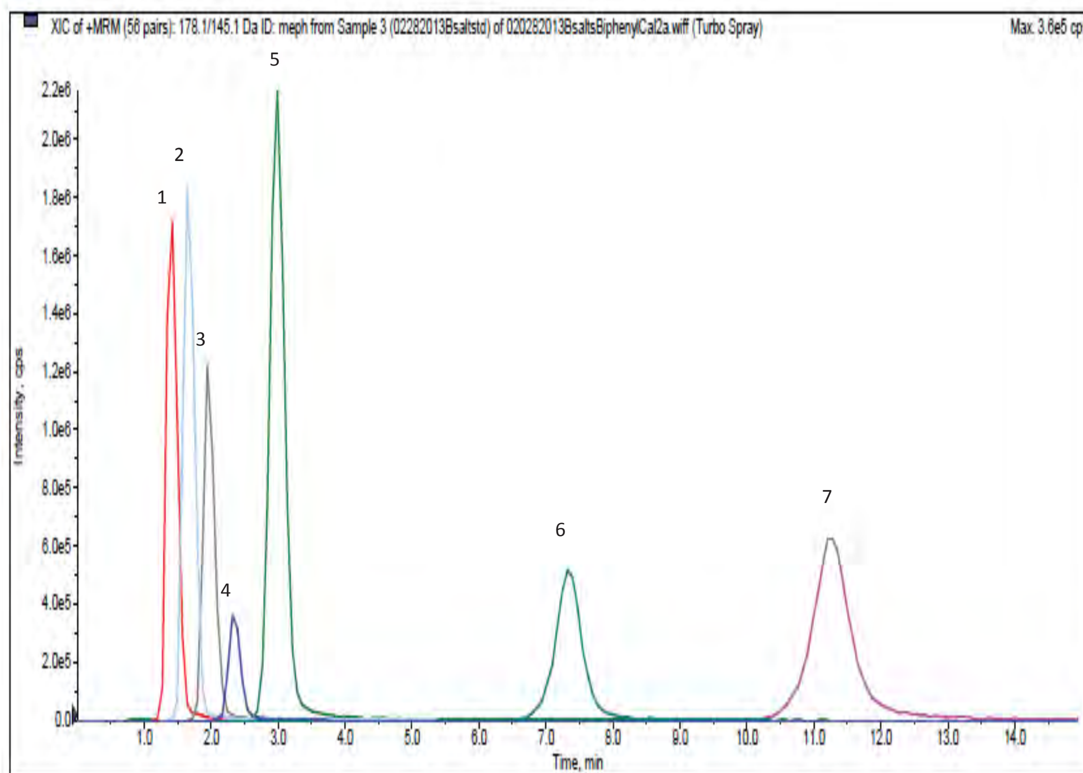
6. **RECONSTITUTE / DERIVATIZE**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 5 µL.

   - **GC-MS**: Fluoroacylate with PFPA (PFAA)
     Add 50 µL PFPA. Over lay with N_2 and cap
     *Improved derivatization by addition of PFPOH
     React 20 minutes at 70 °C. Evaporate to dryness <40 °C
     Reconstitute with 100 µL Ethyl Acetate

NOTES: *(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis, if being used. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.*

INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Flephedrone	**182.1**	**164.2**	**1.41**
2. Methylone	**208.1**	**160.1**	**1.66**
3. Methadrone	**194.1**	**161.1**	**1.96**
4. Methedrone	**178.1**	**145.1**	**2.34**
5. Methethcathinone	**192.2**	**174.0**	**2.98**
6. MDPV	**276.2**	**126.1**	**7.34**
7. Pyravalerone	**246.2**	**105.2**	**11.24**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 5 µL

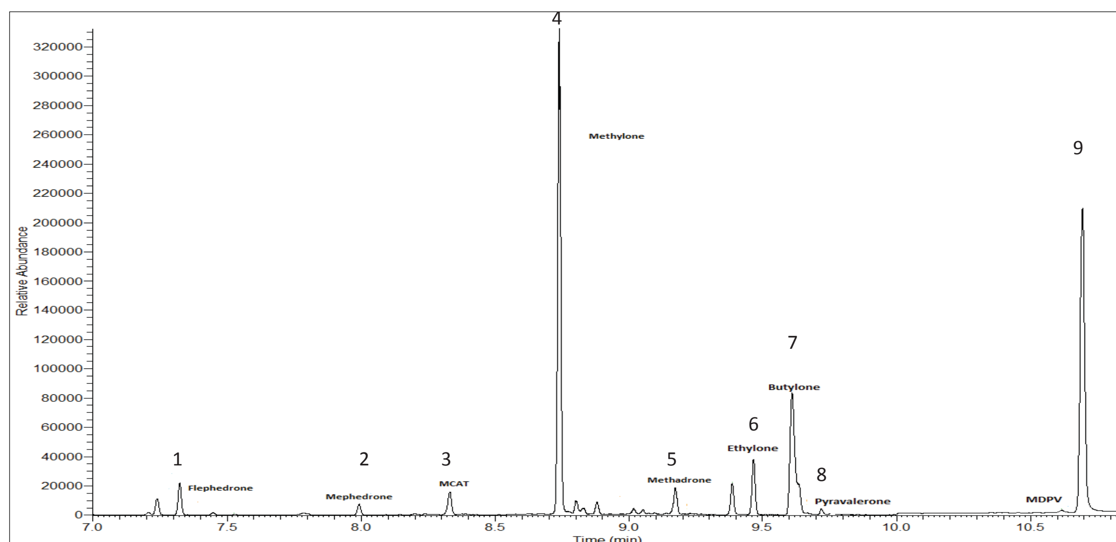
LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

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

Isocratic:

Time	%A	%B
0.00	70	30
15.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):



Fluoroacylate with PFPA (PFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1.Flephedrone	123	204	160	7.32
2.Mephedrone	204	160	149	7.99
3.MCAT	218	174	91	8.33
4.Methylone	353	204	160	8.74
5.Methadrone	135	160	204	9.17
6.Ethylone	218	190	367	9.47
7.Butylone	218	160	367	9.61
8.Pyravalerone	126	84	91	9.72
9.MDPV	126	96	84	10.62

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: 50 °C (1) to 310 °C (25 °C/ minute): hold (3.6 minute)

Carrier gas: Helium (1.2 mL/ minute)

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

Reference:

Comprehensive Forensic Toxicological Analysis of Designer Drugs; NIJ Grant
Author(s): Anthony P. DeCaprio, W. Lee Hearn, Madeleine J. Swortwood
Document No.: 244233
Date Received: December 2013

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

Part #
CSDAU – CLEAN SCREEN® DAU
PFAA-0-1 – SELECTRA-SIL® PFAA
SPFPOH-1 – SELECTRA-SIL® PFPOH
SHFAA-0-1– SELECTRA-SIL® HFAA
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
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 3 mL 100 mM Acetic Acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE SMA'S:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

6. **DRY ELUATE:**
   Add 100 µL of 1% HCl in Methanol to each test tube
   Evaporate to dryness at < 40 °C

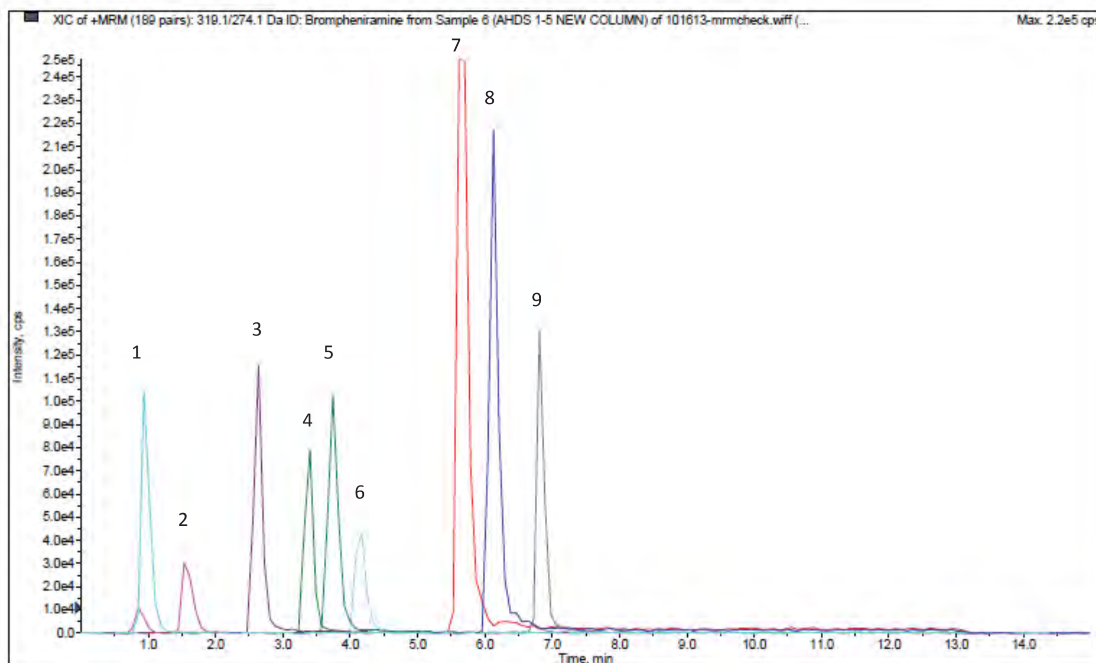
7. **RECONSTITUTE / DERIVATIZE:**
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.
   - **GC-MS**: Fluoroacylate with PFPA (PFAA)
     Add 50 µL PFPA. Over lay with N_2 and cap
     *Improved derivatization by addition of PFPOH
     React 20 minutes at 70 °C. Evaporate to dryness <40 °C
     Reconstitute with 100 µL Ethyl Acetate

Alternate Derivatization

1. Fluoroacylate with HFPA (HFAA)
   Add 50 µL HFPA. Over lay with N_2 and cap
   *Improved derivatization by addition of PFPOH
   React 20 minutes at 70 °C. Evaporate to dryness <40 °C
   Reconstitute with 100 µL Ethyl Acetate

2. Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate;
   React 45 minutes at 70 °C

INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Phenylpropanolamine	152.2	134.2	0.95
2. Ephedrine	166.2	148.3	1.55
3. Phentermine	150.2	91.20	2.60
4. Diethylproprion	206.2	100.2	3.40
5. Pheniramine	241.2	167.2	3.75
6. Doxylamine	271.2	167.2	4.15
7. Chlorpheniramine	275.1	230.1	5.65
8. Brompheniramine	319.1	274.1	6.13
9. Diphenhydramine	256.1	152.1	6.82

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.5 mL/minute
Polarity: Positive
Reconstitute: 100 µL
Injection Volume: 20 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
0.0	80	20
0.5	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

PFPA DERIVATIZATION

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Amphetamine	190	91	118
Amphetamine-D_5	194	92	123
Methamphetamine	204	91	160
Methamphetamine-D_5	208	92	163
MDA	135	162	325
MDMA	204	162	339
Pseudoephedrine	204	160	119
Phenylephrine	190	119	267
Ephedrine	204	160	119

HFPA DERIVATIZATION

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Amphetamine	240	118	91
Amphetamine-D_5	244	123	122
Methamphetamine	254	118	91
Methamphetamine-D_5	258	213	120
MDA	375	162	135
MDMA	254	210	162
MDEA	268	240	162
Pseudoephedrine	344	254	210
Ephedrine	344	254	169

BSTFA DERIVATIZATION

Analyte (TMS)	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Amphetamine	116	192	91
Amphetamine-D_5	120	197	92
Amphetamine-D_6	120	198	93
Amphetamine-D_{10}	120	202	97
Amphetamine-D_{12}	120	203	98
Methamphetamine	130	206	91
Methamphetamine-D_5	134	211	92
Methamphetamine-D_8	137	214	92
Methamphetamine-D_9	137	215	93
MDA	116	236	135
MDMA	130	250	131
Pseudoephedrine	130	147	294
Ephedrine	130	147	294

Benzodiazepines

CPS analitica for Chemistry

BENZODIAZEPINES IN BLOOD, PLASMA/SERUM, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® BNZ EXTRACTION COLUMN

Part #
ZSBNZ030 – CLEAN SCREEN® BNZ 300 mg, 10 mL Tube
SMTBSTFA-1-1 – SELECTRA- SIL® MTBSTFA w/ 1% TBDMCS
SLDA50ID21-5UM – SELECTRA® DA HPLC Column, 50 x 2.1 mm, 5 µm
or
SLPFPP100ID21-5UM – SELECTRA® PFPP HPLC Column, 100 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, 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 5% Acetonitrile in 100 mM phosphate buffer (pH 6.0).
   Dry column (5 minutes at full vacuum or pressure).
   1 x 3 mL Hexane.

5. **ELUTE BENZODIAZEPINES:**
   2 x 3 mL Ethyl Acetate containing 2% NH_4OH
   Collect eluate at 1 to 2 mL/minute.

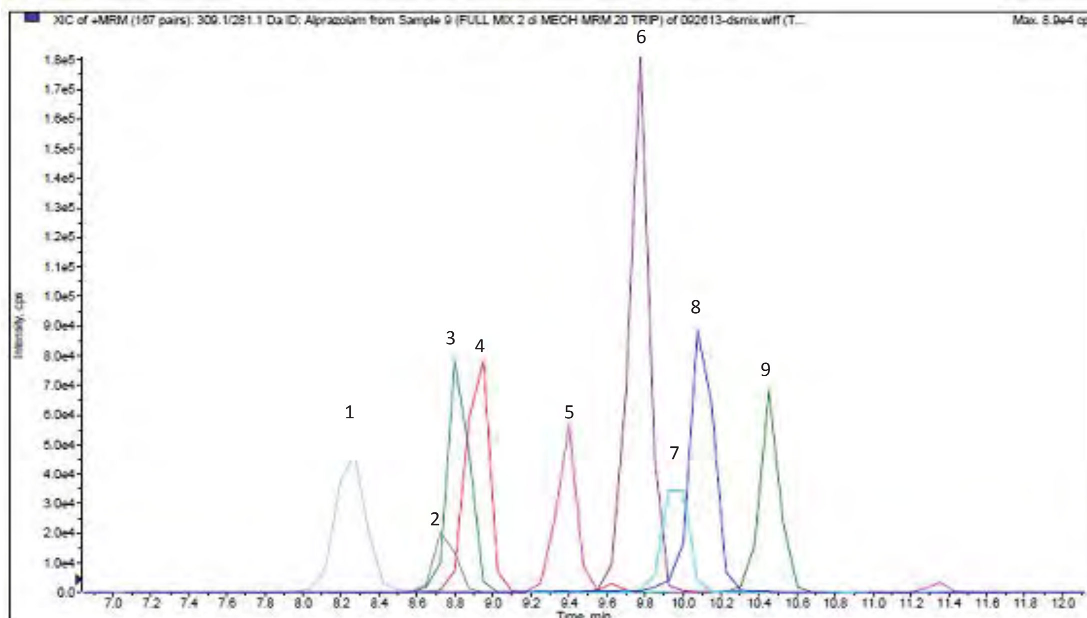
   NOTE: Prepare elution solvent daily.

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

7. **RECONSTITUTE / DERIVATIZE:**
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10-20 µL.
   - **GC-MS**: Dissolve residue in 50 µL of ACN and 50 µL MTBSTFA w/ 1% TBDMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70° C; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Midazolam	326.1	291.1	8.25
2. Lorazepam	321.0	229.1	8.70
3. Oxazepam	287.1	241.1	8.80
4. Clonazepam	316.1	270.1	8.95
5. Nordiazepam	271.1	140.1	9.40
6. Temazepam	301.1	255.1	9.75
7. Triazolam	343.0	239.0	10.0
8. Alprazolam	309.1	281.1	10.1
9. Diazepam	285.1	193.1	10.5

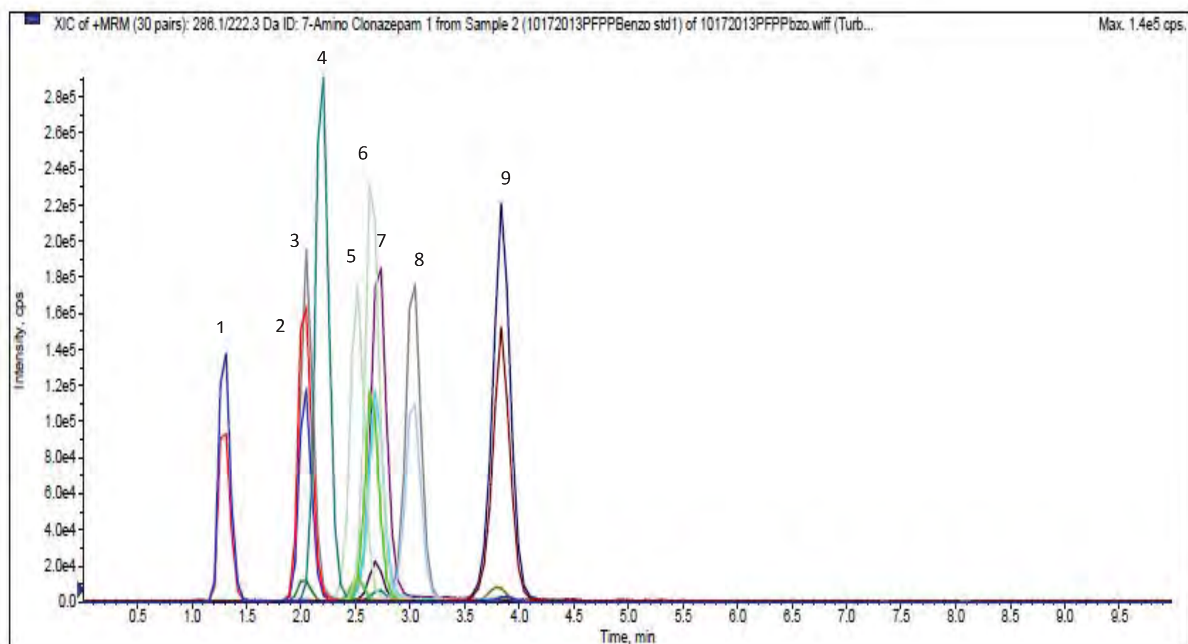
PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.5 mL/minute
Polarity: Positive
Reconstitute: 100 µL
Injection Volume: 20 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
0.0	80	20
0.5	80	20
12	10	90
12.01	80	20
15.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. 7-Amino Clonazepam	286.09	222.3	1.30
2. Lorazepam	321.06	303.3	2.04
3. Alpha- Hydroxy- Alprazolam	325.18	297.1	2.05
4. Oxazepam	287.09	241.3	2.19
5. Clonazepam	316.13	270.2	2.51
6. Temazepam	301.12	255.2	2.65
7. Alprazolam	309.16	205.3	2.71
8. Nordiazepam	271.09	140.1	3.03
9. Diazepam	285.1	193.1	3.84

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

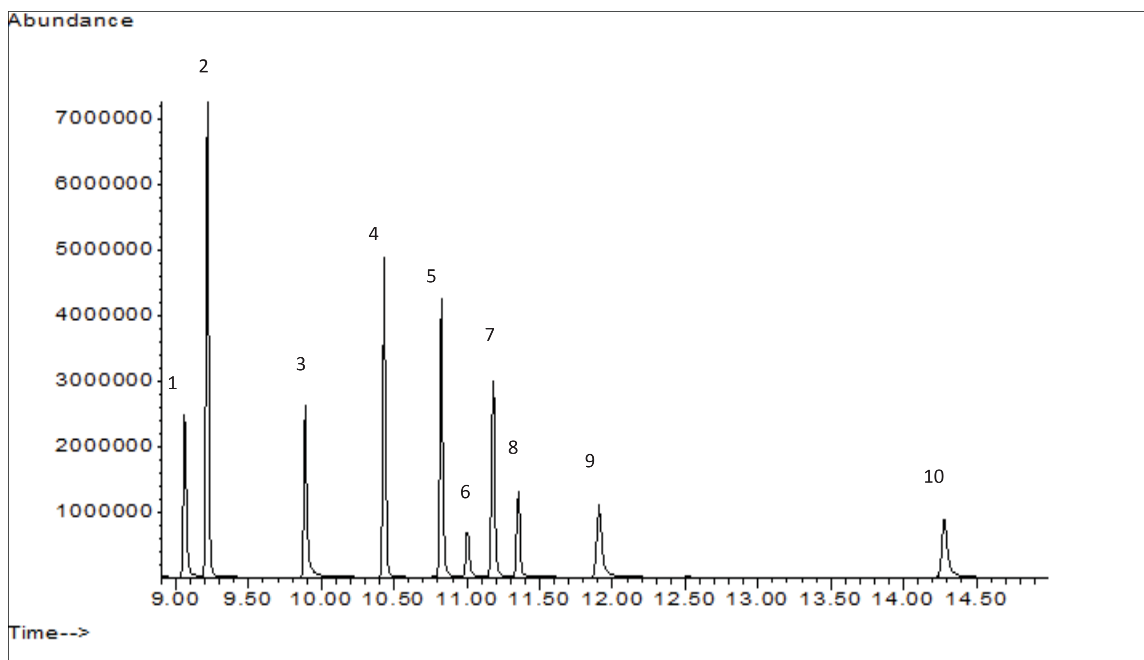
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic Flow:

Time	%A	%B
0.00	40	60
10.0	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



TBDMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Diazepam	256.0	283.0	221.0	9.06
2. Nordiazepam TBDMS	327.0	383.1	369.0	9.22
3. Midiazolam	310.0	325.0	297.0	9.89
4. Oxazepam 2TBDMS	457.1	513.2	383.1	10.43
5. Temazepam TBDMS	357.0	283.0	385.1	10.82
6. 7-Amino Clonazepam TBDMS	342.0	399.1	328.0	11.00
7. Lorazepam 2TBDMS	491.1	513.2	533.1	11.18
8. Clonazepam TBDMS	372.0	326.0	429.0	11.36
9. Alprazolam	279.0	204.0	308.0	11.91
10. Alpha-Hydroxy Alprazolam TBDMS	381.0	423.1	346.0	14.28

PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30 m x 0.25 mm, 0.25 µm

Injector: 1 µL Splitless 250 °C

Oven temperature program: 160 °C for 0.5 min; 15 °C/min to 310 °C for 4.50 minutes

Carrier gas: Helium

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

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

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube
SMTBSTFA-1-1 – SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS
SLDA50ID21-5UM – SELECTRA® DA HPLC Column, 50 x 2.1 mm, 5 µm
SLPFPP100ID21-5UM - SELECTRA® PFPP HPLC Column, 100 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, 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 5% Acetonitrile in 100 mM phosphate buffer (pH 6.0).
   Dry column (5 minutes at full vacuum or pressure).
   1 x 2 mL Hexane.

5. **ELUTE BENZODIAZEPINES:**
   1 x 3 mL Ethyl Acetate containing 2% NH_4OH
   Collect eluate at 1 to 2 mL/minute.

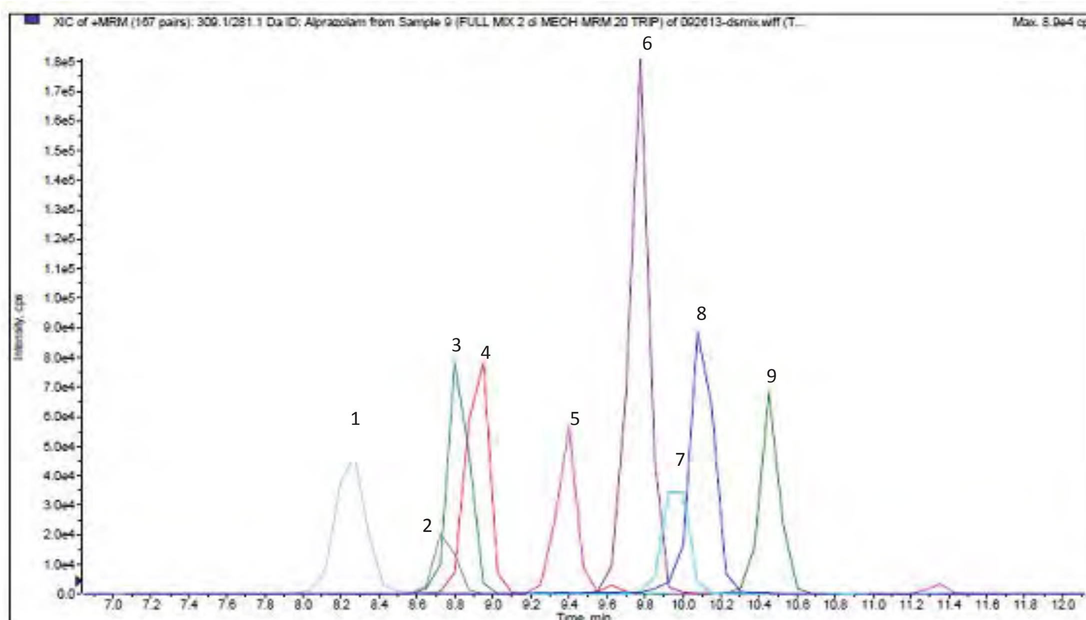
   NOTE: Prepare elution solvent daily.

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

7. **RECONSTITUTE / DERIVATIZE:**
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10-20 µL.
   - **GC-MS**: Dissolve residue in 50 µL of ACN and 50 µL MTBSTFA w/ 1%TBDMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70°C; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Midazolam	326.1	291.1	8.25
2. Lorazepam	321.0	229.1	8.70
3. Oxazepam	287.1	241.1	8.80
4. Clonazepam	316.1	270.1	8.95
5. Nordiazepam	271.1	140.1	9.40
6. Temazepam	301.1	255.1	9.75
7. Triazolam	343.0	239.0	10.0
8. Alprazolam	309.1	281.1	10.1
9. Diazepam	285.1	193.1	10.5

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 20 µL

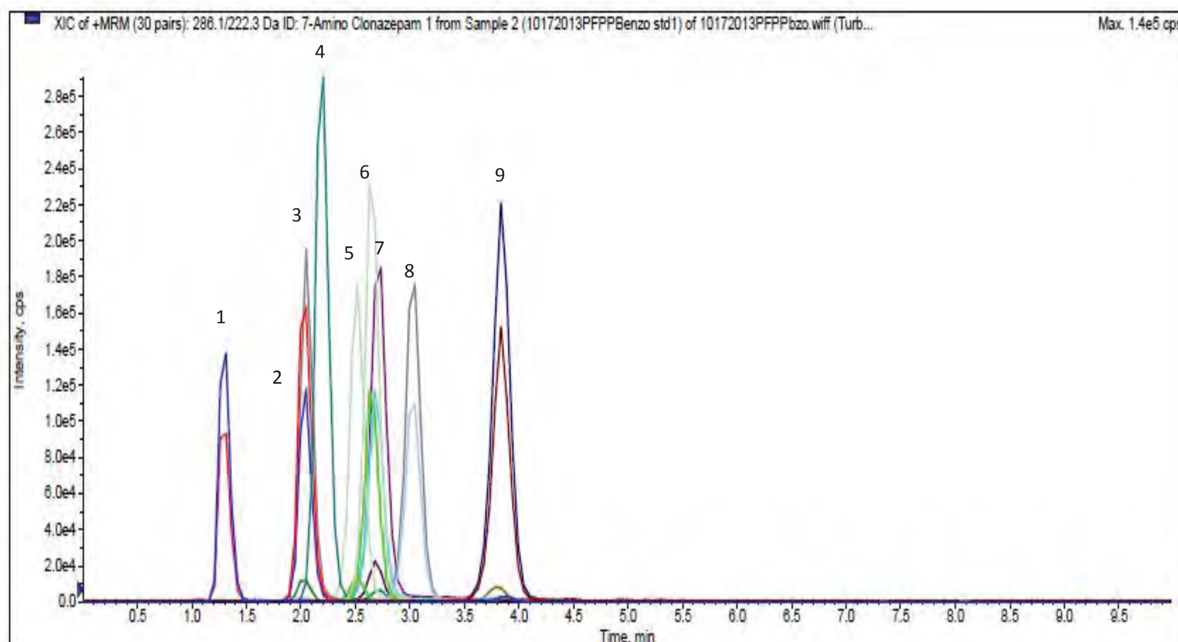
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Gradient:

Time	%A	%B
0.0	80	20
0.5	80	20
12	10	90
12.01	80	20
15.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. 7-Amino Clonazepam	**286.09**	**222.3**	**1.30**
2. Lorazepam	**321.06**	**303.3**	**2.04**
3. Alpha- Hydroxy- Alprazolam	**325.18**	**297.1**	**2.05**
4. Oxazepam	**287.09**	**241.3**	**2.19**
5. Clonazepam	**316.13**	**270.2**	**2.51**
6. Temazepam	**301.12**	**255.2**	**2.65**
7. Alprazolam	**309.16**	**205.3**	**2.71**
8. Nordiazepam	**271.09**	**140.1**	**3.03**
9. Diazepam	**285.1**	**193.1**	**3.84**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

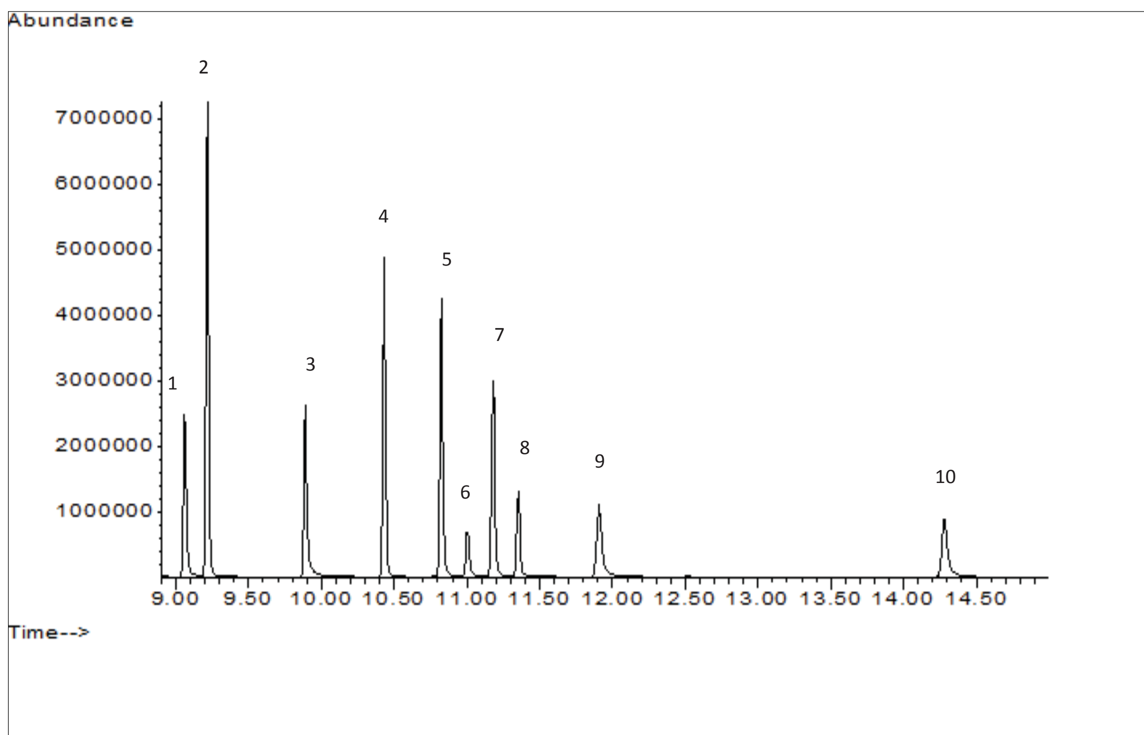
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic Flow:

Time	%A	%B
0.00	40	60
10.0	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



TBDMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Diazepam	256.0	283.0	221.0	9.06
2. Nordiazepam TBDMS	327.0	383.1	369.0	9.22
3. Midiazolam	310.0	325.0	297.0	9.89
4. Oxazepam 2TBDMS	457.1	513.2	383.1	10.43
5. Temazepam TBDMS	357.0	283.0	385.1	10.82
6. 7-Amino Clonazepam TBDMS	342.0	399.1	328.0	11.00
7. Lorazepam 2TBDMS	491.1	513.2	533.1	11.18
8. Clonazepam TBDMS	372.0	326.0	429.0	11.36
9. Alprazolam	279.0	204.0	308.0	11.91
10. Alpha-Hydroxy Alprazolam TBDMS	381.0	423.1	346.0	14.28

PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30m x 0.25 mm, 0.25 µm

Injector: 1 µL Splitless 250 °C

Oven temperature program: 160 °C for 0.5min; 15 °C/min to 310 °C for 4.50 minutes

Carrier gas: Helium

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

EXTRACTION OF BENZODIAZEPINES FROM BLOOD, PLASMA/SERUM, TISSUE BY LC-MS/MS OR GC-MS

Part #
CSXCE106 CLEAN SCREEN XCEL® I 130 mg, 6 mL Tube
SMTBSTFA-1-1 – SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm
SLPFPP100ID21-5UM - Selectra® PFPP HPLC Column, 100 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, 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. APPLY SAMPLE

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. WASH

1 x 3mL 100 mM phosphate buffer (pH6).
1 x 3 mL CH_2Cl_2
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5-10 minutes.

4. ELUTION

1 x 3 mL Ethyl Acetate:NH_4OH (98:2)
Collect eluate at 1 to 2 mL/minute.
NOTE: Prepare elution solvent daily.

5. DRY ELUTE

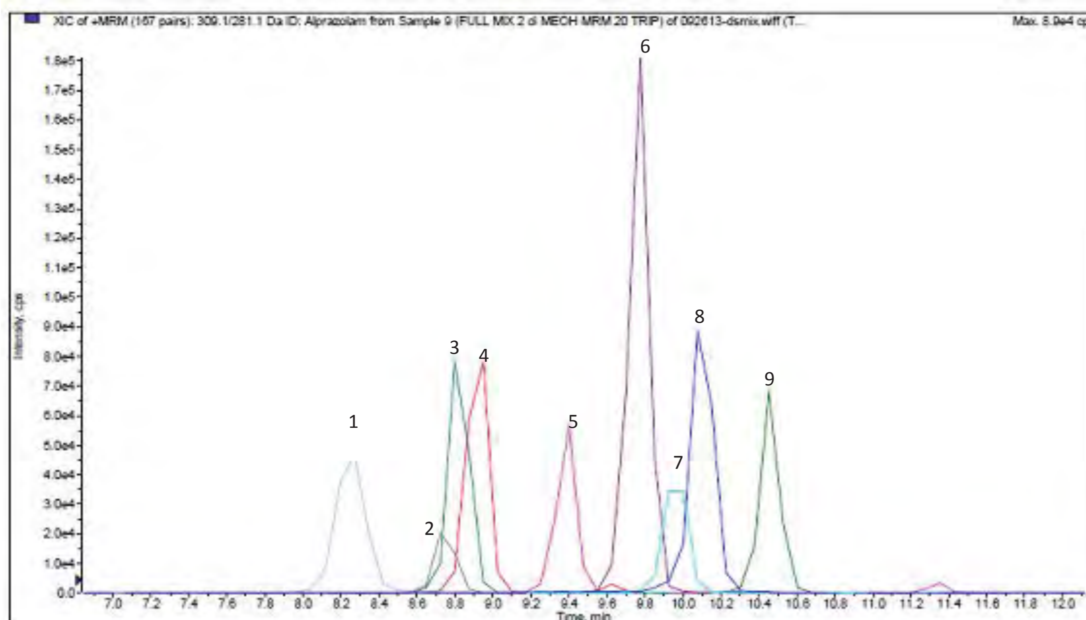
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10-20 µL.
- **GC-MS**: Dissolve residue in 50 µL of ACN and 50 µL MTBSTFA w/ 1%TBDMCS
  Overlay with N_2 and cap. Mix/vortex
  React 30 minutes at 70 °C; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Midazolam	326.1	291.1	8.25
2. Lorazepam	321.0	229.1	8.70
3. Oxazepam	287.1	241.1	8.80
4. Clonazepam	316.1	270.1	8.95
5. Nordiazepam	271.1	140.1	9.40
6. Temazepam	301.1	255.1	9.75
7. Triazolam	343.0	239.0	10.0
8. Alprazolam	309.1	281.1	10.1
9. Diazepam	285.1	193.1	10.5

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 20 µL

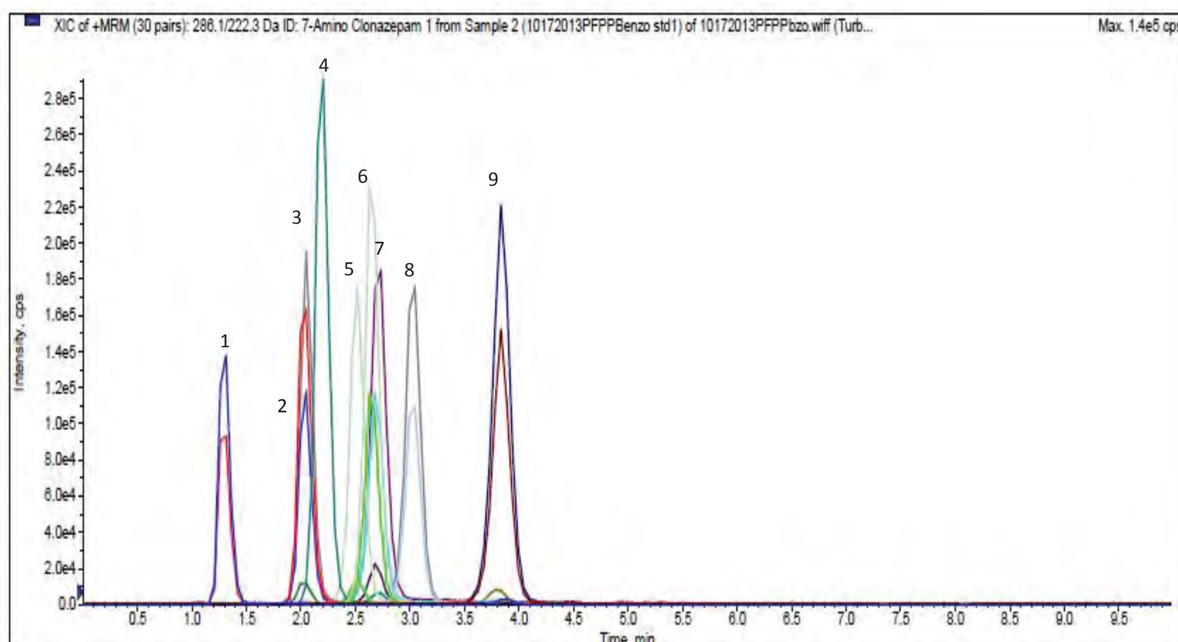
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Gradient:

Time	%A	%B
0.0	80	20
0.5	80	20
12	10	90
12.01	80	20
15.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. 7-Amino Clonazepam	286.09	222.3	1.30
2. Lorazepam	321.06	303.3	2.04
3. Alpha- Hydroxy- Alprazolam	325.18	297.1	2.05
4. Oxazepam	287.09	241.3	2.19
5. Clonazepam	316.13	270.2	2.51
6. Temazepam	301.12	255.2	2.65
7. Alprazolam	309.16	205.3	2.71
8. Nordiazepam	271.09	140.1	3.03
9. Diazepam	285.1	193.1	3.84

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

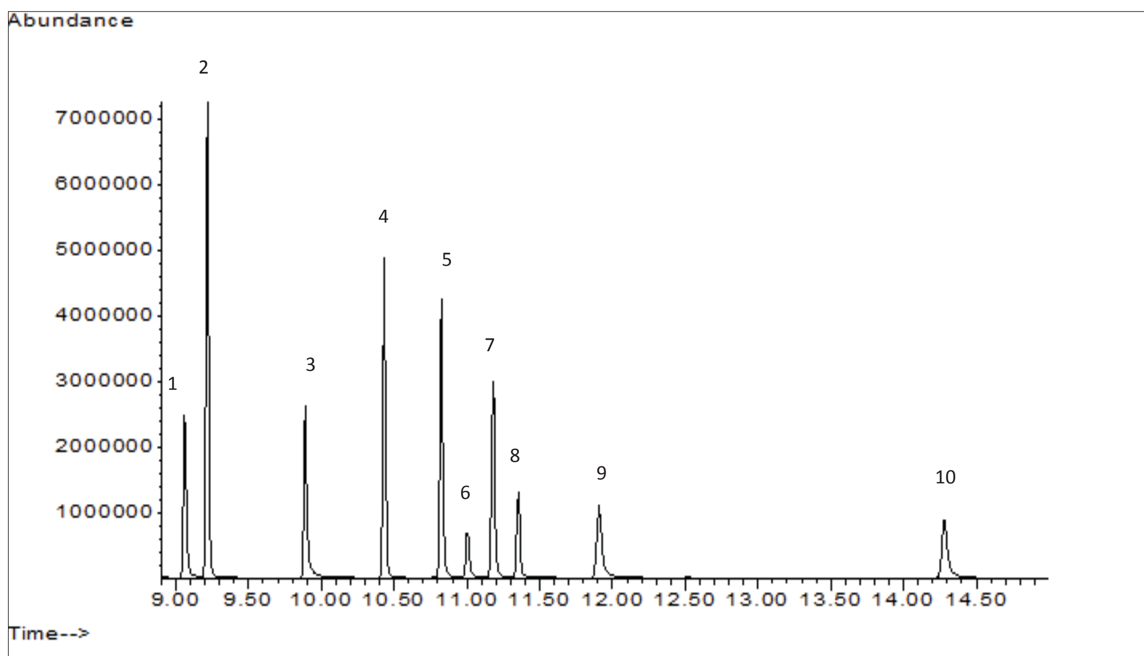
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic Flow:

Time	%A	%B
0.00	40	60
10.0	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



TBDMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Diazepam	256.0	283.0	221.0	9.06
2. Nordiazepam TBDMS	327.0	383.1	369.0	9.22
3. Midiazolam	310.0	325.0	297.0	9.89
4. Oxazepam 2TBDMS	457.1	513.2	383.1	10.43
5. Temazepam TBDMS	357.0	283.0	385.1	10.82
6. 7-Amino Clonazepam TBDMS	342.0	399.1	328.0	11.00
7. Lorazepam 2TBDMS	491.1	513.2	533.1	11.18
8. Clonazepam TBDMS	372.0	326.0	429.0	11.36
9. Alprazolam	279.0	204.0	308.0	11.91
10. Alpha-Hydroxy Alprazolam TBDMS	381.0	423.1	346.0	14.28

PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30 m x 0.25 mm, 0.25 µm

Injector: 1 µL Splitless 250 °C

Oven temperature program: 160°C for 0.5min; 15°C/min to 310°C for 4.50 minutes

Carrier gas: Helium

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

BENZODIAZEPINES IN URINE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #
ZSDAU020 CLEAN SCREEN® DAU 200 mg, 10 mL Tube
BETA-GLUC-10 – SELECTRAZYME® Beta-glucuronidase
SMTBSTFA-1-1 – SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS
SLDA50ID21-5UM – SELECTRA® DA HPLC Column, 50 x 2.1 mm, 5 µm
SLPFPP100ID21-5UM - SELECTRA® PFPP HPLC Column, 100 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:**
   To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 1-2 hours at 65 °C.
   Allow sample to cool
   Do not adjust pH~ sample is ready to be added to the extraction column.

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 5% Acetonitrile in 100 mM phosphate buffer (pH 6.0).
   Dry column (5 minutes at full vacuum or pressure).
   1 x 2 mL Hexane.

5. **ELUTE BENZODIAZEPINES:**
   1 x 3 mL Ethyl Acetate containing 2% NH_4OH
   collect eluate at 1 to 2 mL/minute.

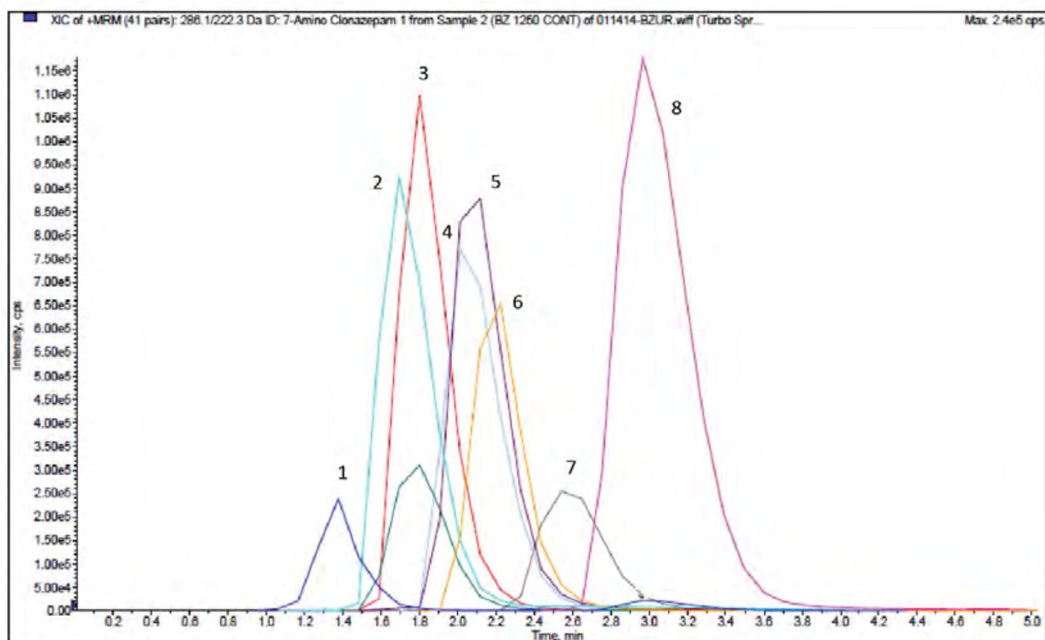
   NOTE: Prepare elution solvent daily.

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 50 µL of ACN and 50 µL MTBSTFA w/ 1% TBDMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1 -2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



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8. Diazepam	285.1	193.1	3.00

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.1 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

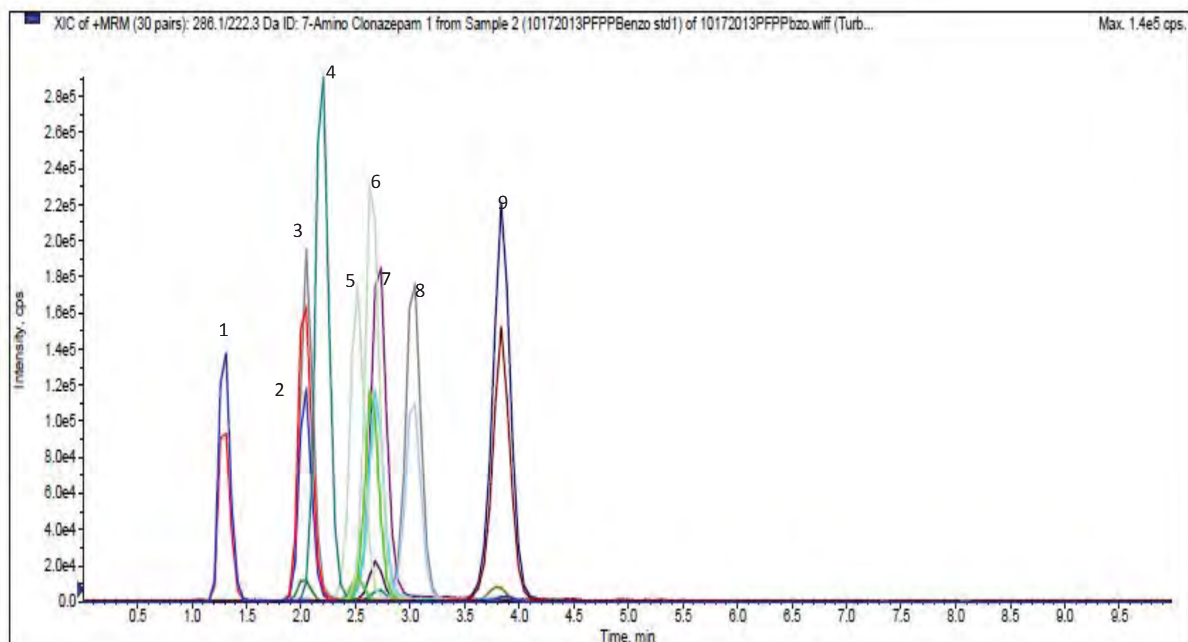
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Isocratic Flow:

Time	%A	%B
0.00	50	50
7.50	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



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Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

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

Isocratic Flow:

Time	%A	%B
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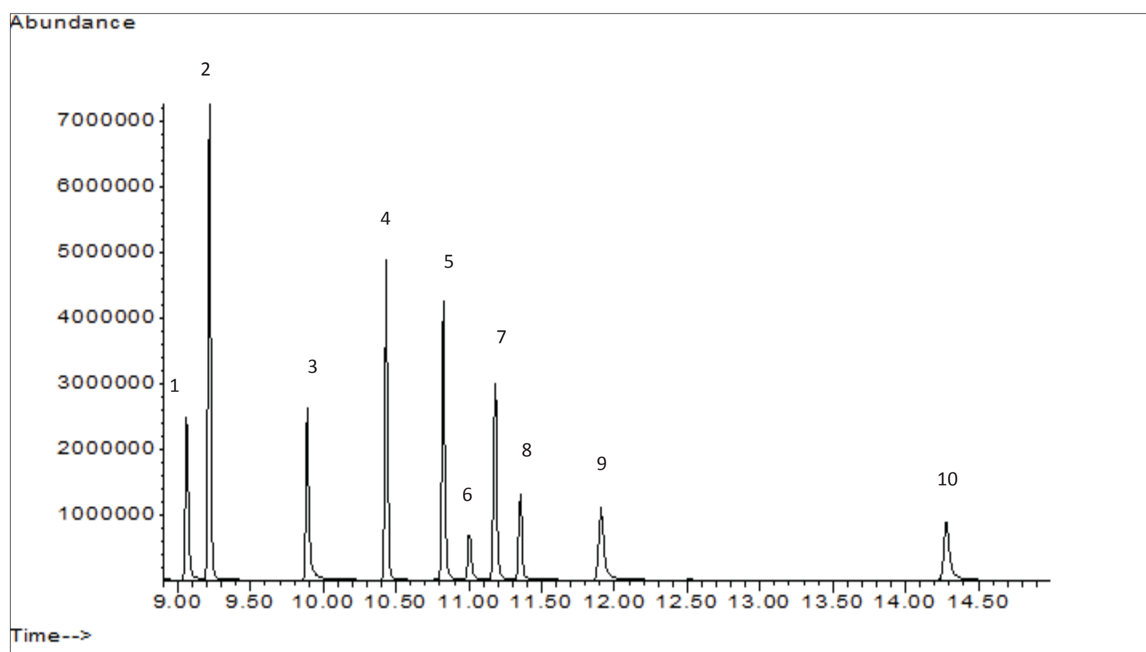
10.0	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



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9. Alprazolam	279.0	204.0	308.0	11.91
10. Alpha-Hydroxy Alprazolam TBDMS	381.0	423.1	346.0	14.28

PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30m x 0.25 mm, 0.25 µm

Injector: 1 µL Splitless 250 °C

Oven temperature program: 160 °C for 0.5min; 15 °C/min to 310 °C for 4.50 minutes

Carrier gas: Helium

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

EXTRACTION OF BENZODIAZEPINES FROM URINE BY CLEAN SCREEN XCEL® I COLUMN AND ANALYSIS BY LC-MS/MS OR GC-MS

Part #
CSXCE106 – CLEAN SCREEN XCEL® I 130 mg, 6 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SMTBSTFA-1-1 – SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm
SLPFPP100ID21-5UM - Selectra® PFPP HPLC Column, 100 x 2.1 mm, 5 µm

2. PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES

To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
Vortex and heat for 1-2 hours at 65°C.
Allow sample to cool
Do not adjust pH~ sample is ready to be added to the extraction column.

2. APPLY SAMPLE

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. WASH

1 x 3 mL 100 mM phosphate buffer (pH 6.0).
1 x 3 mL CH_2Cl_2
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5-10 minutes.

4. ELUTION

1 x 3 mL Ethyl Acetate:NH_4OH (98:2)
Collect eluate at 1 to 2 mL/minute.
NOTE: Prepare elution solvent daily.

5. DRY ELUTE

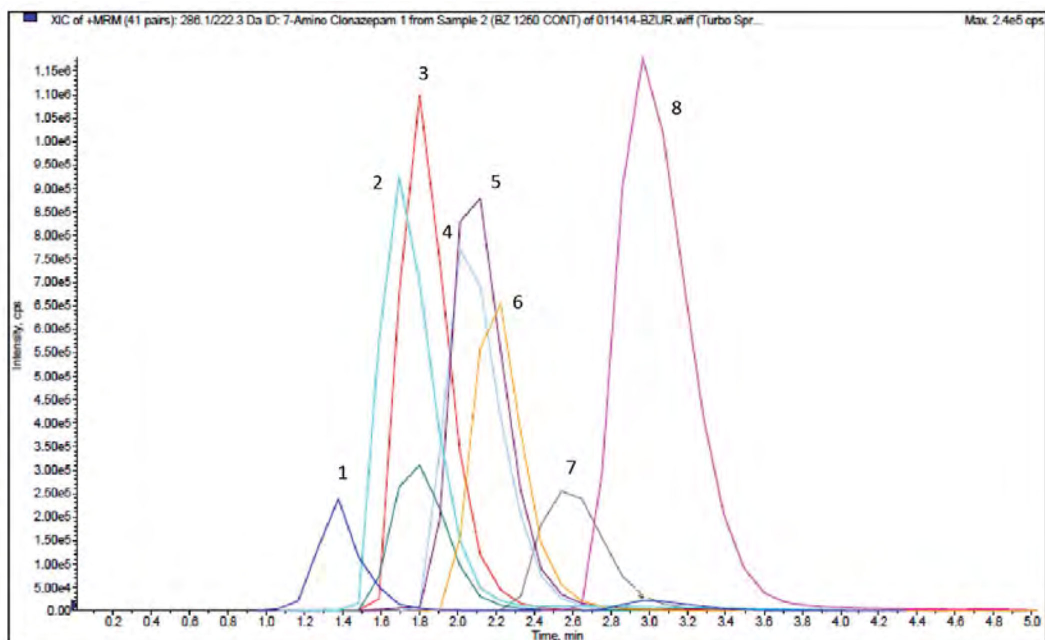
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10 µL.
- **GC-MS**: Dissolve residue in 50 µL of ACN and 50 µL MTBSTFA w/ 1% TBDMCS
  Overlay with N_2 and cap. Mix/vortex
  React 30 minutes at 70 °C; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
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7. Alprazolam	309.16	205.3	2.60
8. Diazepam	285.1	193.1	3.00

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.1 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

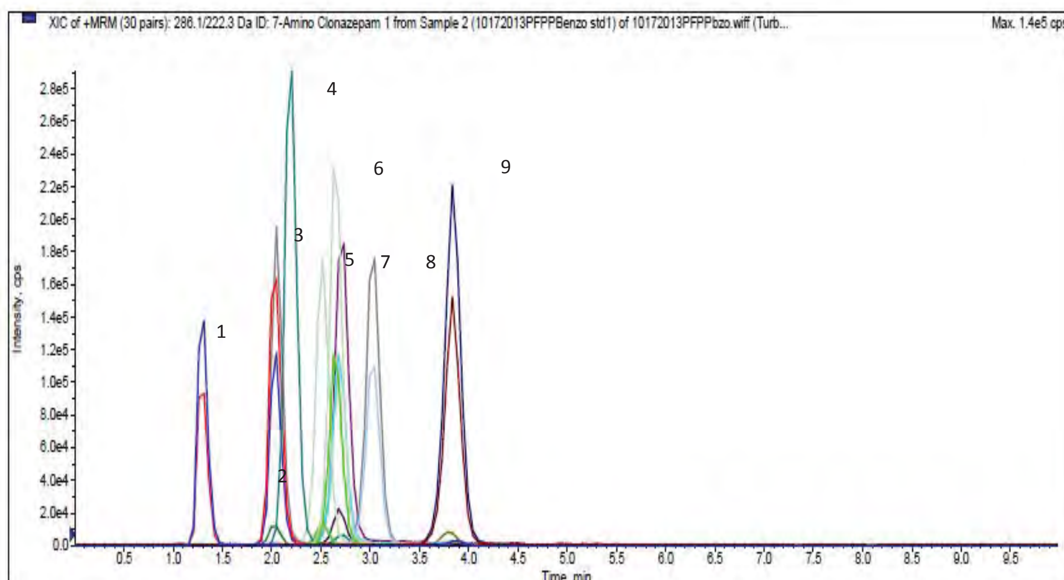
LC Column: Selectra® DA HPLC Column 50 x 2.1mm 5µm

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

Isocratic Flow:

Time	%A	%B
0.00	50	50
7.50	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



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PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

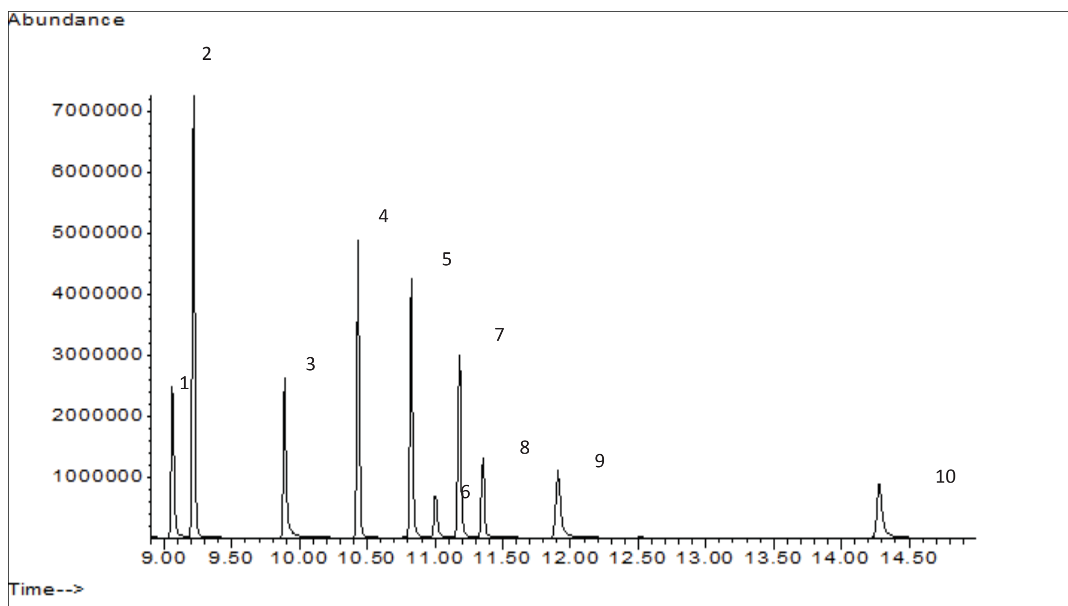
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic Flow:

Time	%A	%B
0.00	40	60
10.0	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



TBDMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Diazepam	256.0	283.0	221.0	9.06
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4. Oxazepam 2TBDMS	457.1	513.2	383.1	10.43
5. Temazepam TBDMS	357.0	283.0	385.1	10.82
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8. Clonazepam TBDMS	372.0	326.0	429.0	11.36
9. Alprazolam	279.0	204.0	308.0	11.91
10. Alpha-Hydroxy Alprazolam TBDMS	381.0	423.1	346.0	14.28

PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30 m x 0.25 mm, 0.25 µm

Injector: 1 µL Splitless 250 °C

Oven temperature program: 160 °C for 0.5min; 15 °C/min to 310 °C for 4.50 minutes

Carrier gas: Helium

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

BENZODIAZEPINES IN BLOOD, PLASMA/SERUM, URINE BY LC-MS/MS OR GC-MS CLEAN SCREEN XCEL® I 96 WELLPLATE

Part #
WSH96EXE11 – CLEAN SCREEN XCEL® I 130 mg, 96 well plate
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidadse
SMTBSTFA-1-1 – SELECTRA- SIL® MTBSTFA w/ 1% TBDMCS
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm
or
SLPFPP100ID21-5UM – Selectra® PFPP HPLC Column, 100 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE**
   To 1-2 mL whole blood, plasma/ serum add 500 µL100mM phosphate buffer (pH 6.0)
   Add appropriate volume and concentration of internal standard.
   Note: See Hydrolysis step if required

   Hydrolysis: To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 1-2 hours at 65°C.
   Allow sample to cool
   Do not adjust pH~ sample is ready to be added to the extraction column.

2. **APPLY SAMPLE**

   Load sample directly to column without any preconditioning.
   Pull sample through at a rate of 1-2 mL/ minute.
   Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. **WASH**
   1 x 1 mL CH_2Cl_2
   Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. **ELUTION**
   1 x 1 mL Ethyl Acetate/NH_4OH (98:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.

5. **DRY ELUTE**

   Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. **RECONSTITUTE / DERIVATIZE**

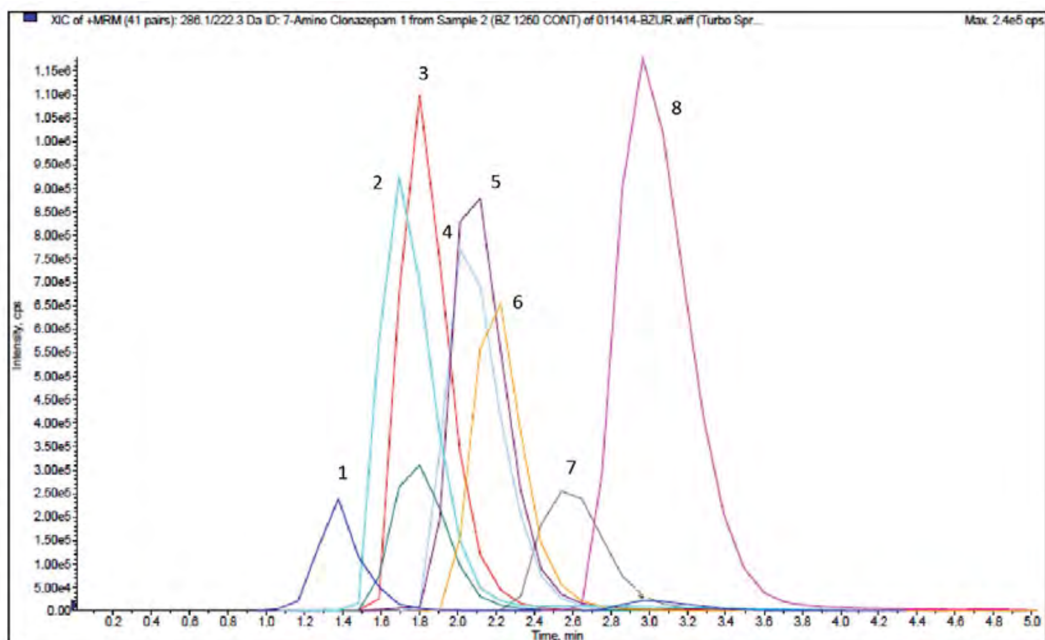
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL.
   - **GC-MS**: Dissolve residue in 50 µL of ACN and 50 µL MTBSTFA w/ 1% TBDMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1-2 µL

NOTES

(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis, if being used. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



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Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.1 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

Isocratic Flow:

Time	%A	%B
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7.50	STOP	

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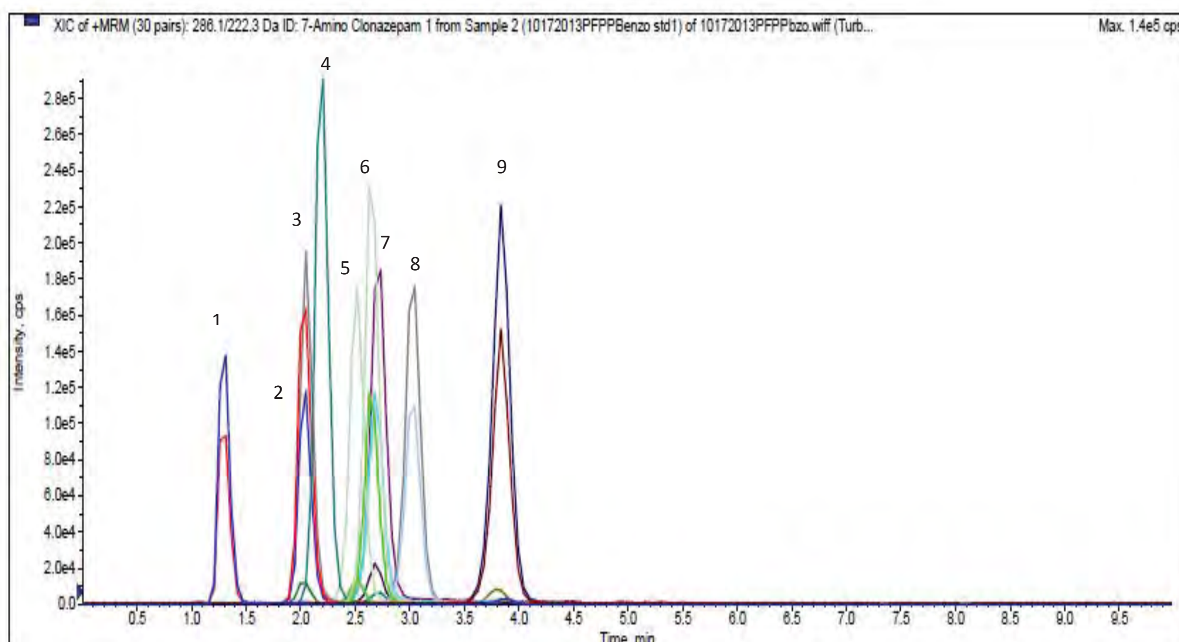

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Reconstitute: 100 µL

Injection Volume: 10 µL

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

Isocratic Flow:

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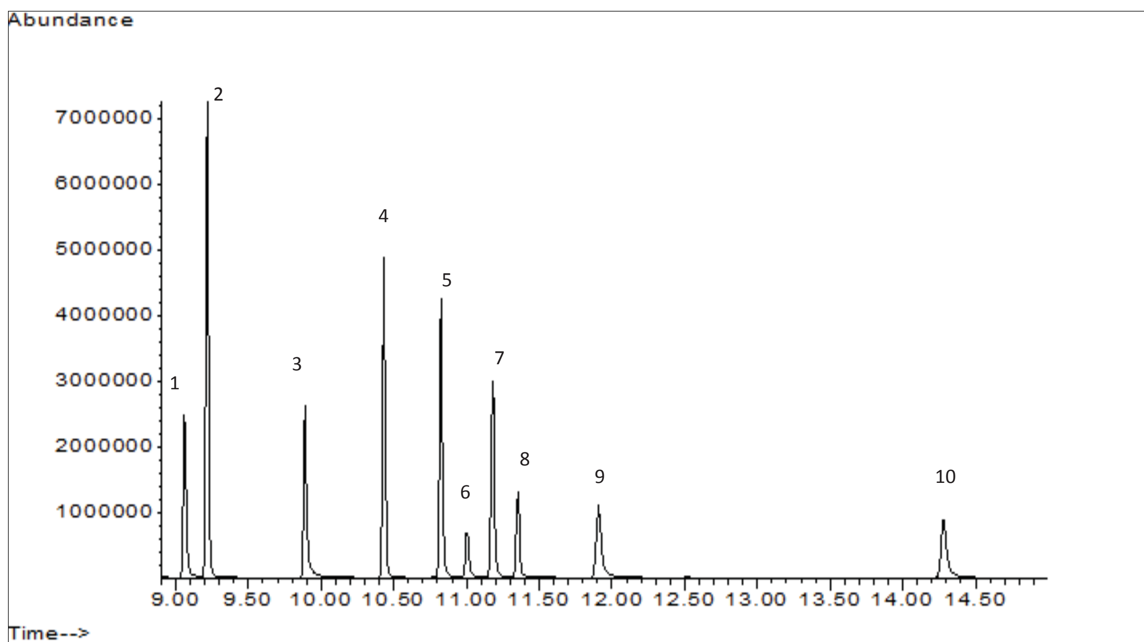
INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



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PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30 m x 0.25 mm, 0.25 µm

Injector: 1 µL Splitless 250 °C

Oven temperature program: 160 °C for 0.5 min; 15 °C/min to 310 °C for 4.50 minutes

Carrier gas: Helium

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

Cannabinoids

CPS analitica for Chemistry

CARBOXY-THC IN URINE BY LC-MS/MS OR GC-MS USING CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #
ZSTHC020 – CLEAN SCREEN® THC 200 mg 10 mL Tube
or
CSDAU206 – CLEAN SCREEN® DAU 200 mg 6 mL Tube
SMSTFA-1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE-BASE HYDROLYSIS OF GLUCURONIDES:**
   To 2 mL of urine add internal standard and 100 µL of 10 M NaOH.
   Mix/vortex.
   Hydrolyze for 20 minutes at 60 °C. Cool before proceeding.
   Adjust sample pH to 3.0 with approx. 1.0 mL Glacial Acetic Acid. (pH should be ~3.0)

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL Acetate buffer (pH 3.0)
   NOTE: Aspirate at full vacuum or pressure

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

4. **WASH COLUMN:**
   1 x 2 mL D.I. H_2O
   1 x 2 mL 100 mM HCl/Acetonitrile (95:5)
   Dry column (10 minutes at full vacuum or pressure)
   1 x 200 µL Hexane
   Aspirate at full vacuum or pressure (Additional step to remove any residual moisture)

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

NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency

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

7. **RECONSTITUTE / DERIVATIZE:**

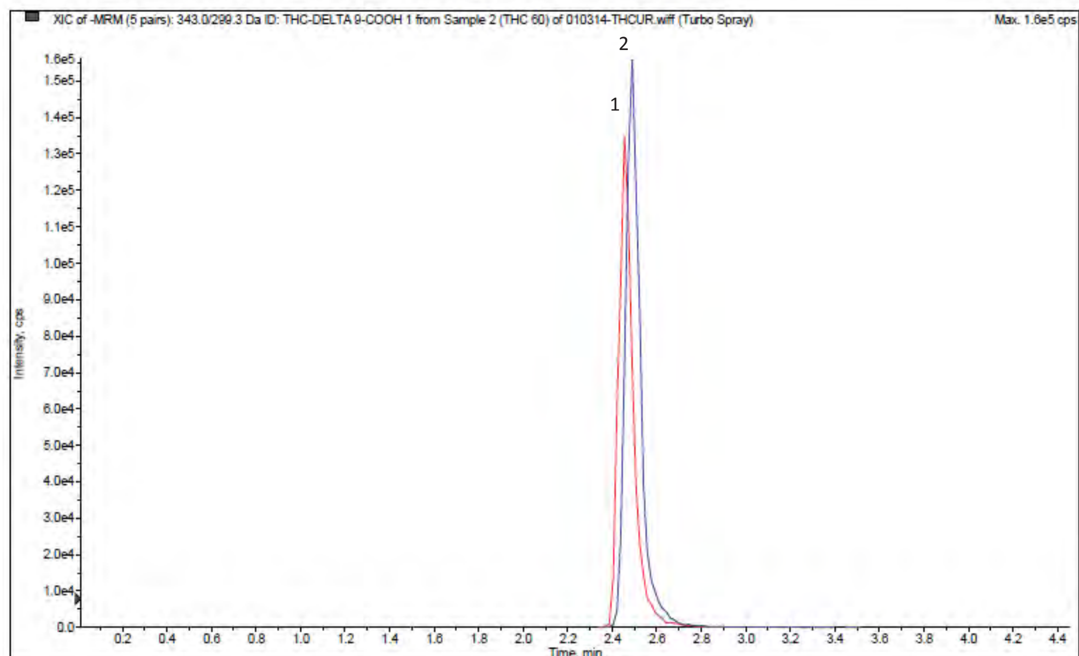
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and
     50 µL MSTFA w/1%TMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1 µL

   Alternate Derivatization

   1. Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate; React 45 minutes at 70 °C

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. THC-DELTA 9-COOH D_9	352	308	2.44
2. THC-DELTA 9-COOH	343	299	2.49

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

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

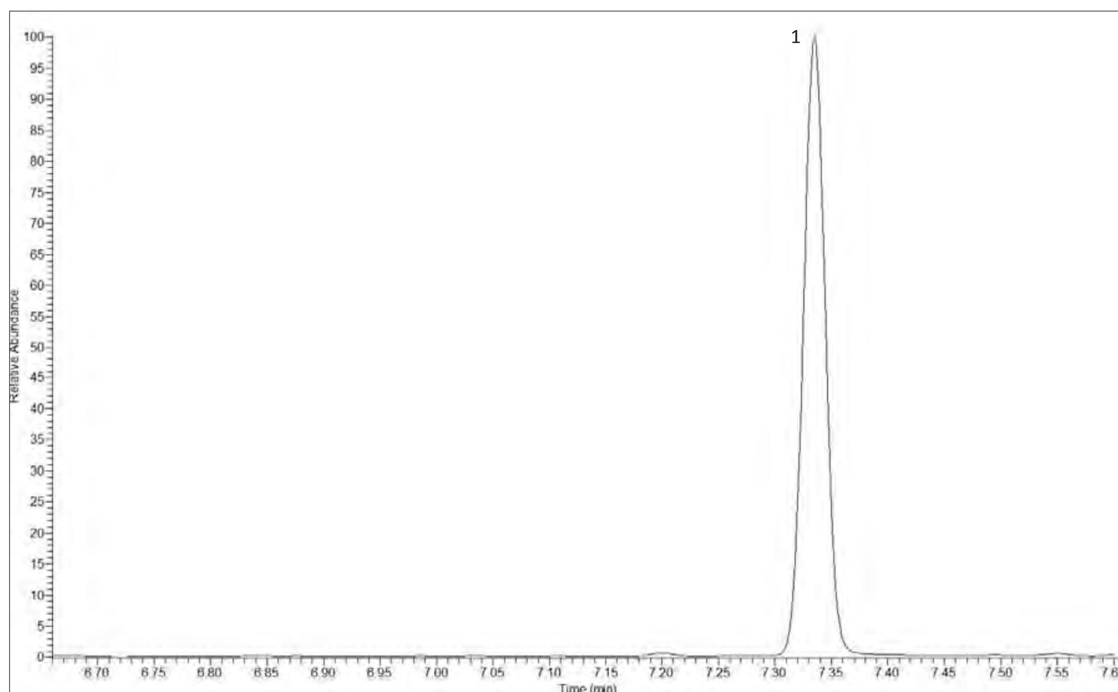
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	60	40
2.00	30	70
2.50	10	90
2.51	60	40
4.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



MSTFA/BSTFA TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
THC-COOH	371	473	488	7.34
THC-COOH D_3	374	476	491	7.31

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: 170 °C (1) to 310 °C (30 °C/ minute): hold (5 minutes)

Carrier gas: Helium (1.2 mL/ minute)

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

CARBOXY-THC IN URINE BY LC-MS/MS OR GC-MS USING 30mg STYRE SCREEN® DBX EXTRACTION COLUMN

Part #:

SSDBX033 without Tips - Styre Screen® DBX 30 mg 3 mL Tube

or

SCDBX033 with CLEAN-THRU® Tips – Syre Screen® DBX w/ CLEAN THRU® Tips 30 mg 3 mL Tube

SMSTFA-1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS

SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

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

1. PREPARE SAMPLE-BASE HYDROLYSIS OF GLUCURONIDES:
To 2 mL of urine add internal standard and 100 µL 10M NaOH.
Mix/vortex. Hydrolyze for 20 mins at 60 °C. Cool before proceeding.
Adjust sample pH to 3.5 ± 0.5 with 1.0 mL glacial acetic acid.

2. APPLY SAMPLE TO DBX COLUMN:
Load at a rate of 1 to 2 mL/min.

3. WASH COLUMN:
1 x 1 mL D.I. H_2O.
1 x 1 mL 0.1M HCl/Acetonitrile (70/30).
Dry column (3 mins at > 10 inches Hg).
1 x 200 µL Hexane.

4. ELUTE CARBOXY-THC:
2 x 0.5 mL Hexane/ Ethyl Acetate (50:50); Collect eluate at 1 to 2 mL/min.
Evaporate eluate to dryness at < 40 °C.

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

6. RECONSTITUTE / DERIVATIZE:

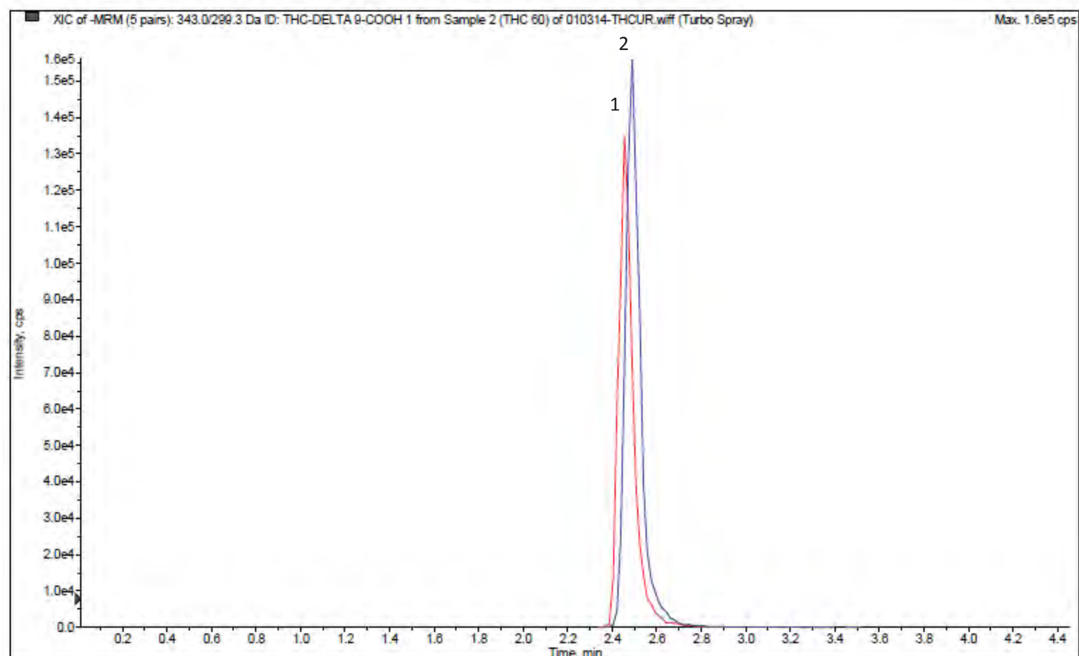
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 20 µL.
- **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL MSTFA w/ 1%TMCS
  Overlay with N_2 and cap. Mix/vortex
  React 30 minutes at 70 °C; Cool and inject 1 µL

Alternate Derivatization

1. Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate; React 45 minutes at 70 °C

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. THC-DELTA 9-COOH D_9	352	308	2.44
2. THC-DELTA 9-COOH	343	299	2.49

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

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

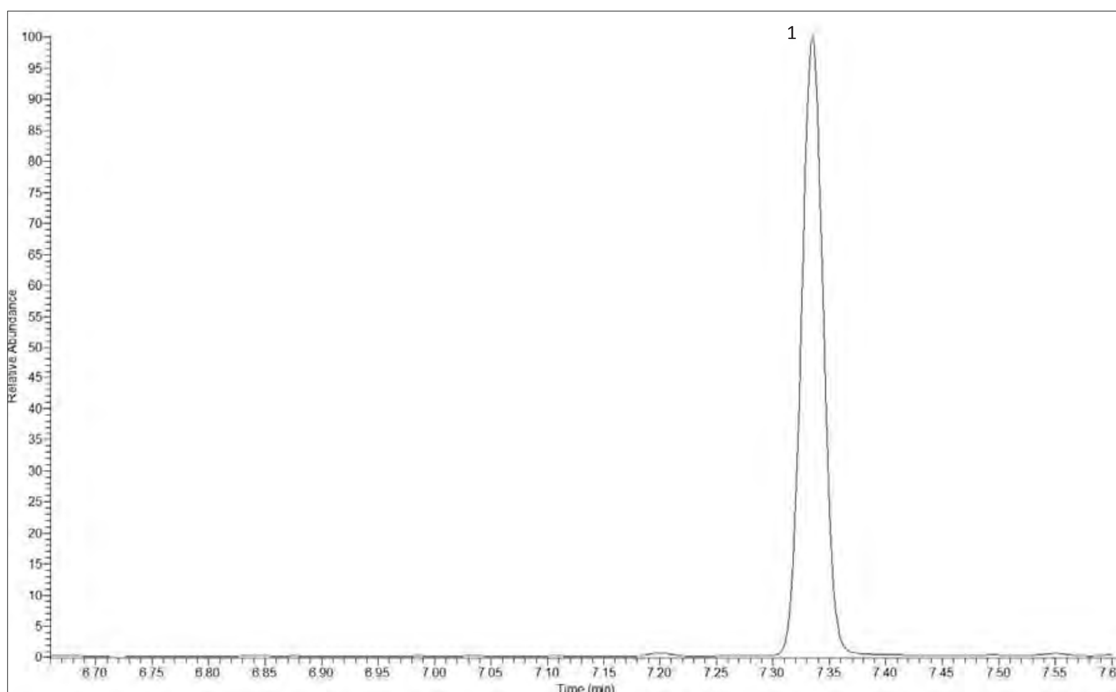
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	60	40
2.00	30	70
2.50	10	90
2.51	60	40
4.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



MSTFA/BSTFA TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. THC-COOH	371	473	488	7.34
THC-COOH D_3	374	476	491	7.31

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: 170 °C (1) to 310 °C (30 °C/ minute): hold (5 minutes)

Carrier gas: Helium (1.2 mL/ minute)

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

CARBOXY-THC IN URINE BY LC-MS/MS OR GC-MS USING CLEAN SCREEN XCEL® II EXTRACTION COLUMN

Part #
CSXCE2106 – CLEAN SCREEN XCEL® II 130 mg 6 mL Tube
SMSTFA-1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA50ID21-5UM - Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE-BASE HYDROLYSIS OF GLUCURONIDES:**
   To 2 mL of urine add internal standard and 50 µL of 10 M NaOH
   Mix/vortex
   Hydrolyze for 15 minutes at 60-70 °C. Cool before proceeding
   Adjust sample pH to 7.0 with 50 µL of 1:1 H_2O: Glacial Acetic Acid.
   Add 200 µL pH 7.0 100mM Phosphate Buffer
   (pH should be ~7.0)

2. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute
   Dry column (2 minutes at full vacuum or pressure)

3. **WASH COLUMN:**
   1 x 2 mL Hexane
   Dry Column at full vacuum or pressure for 10 minutes

4. **ELUTE ANALYTE:**
   1 x 3 mL Hexane/ Ethyl Acetate/ Glacial Acetic Acid (49:49:2)
   Collect eluate at 1 to 2 mL/minute

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

6. **RECONSTITUTE / DERIVATIZE:**

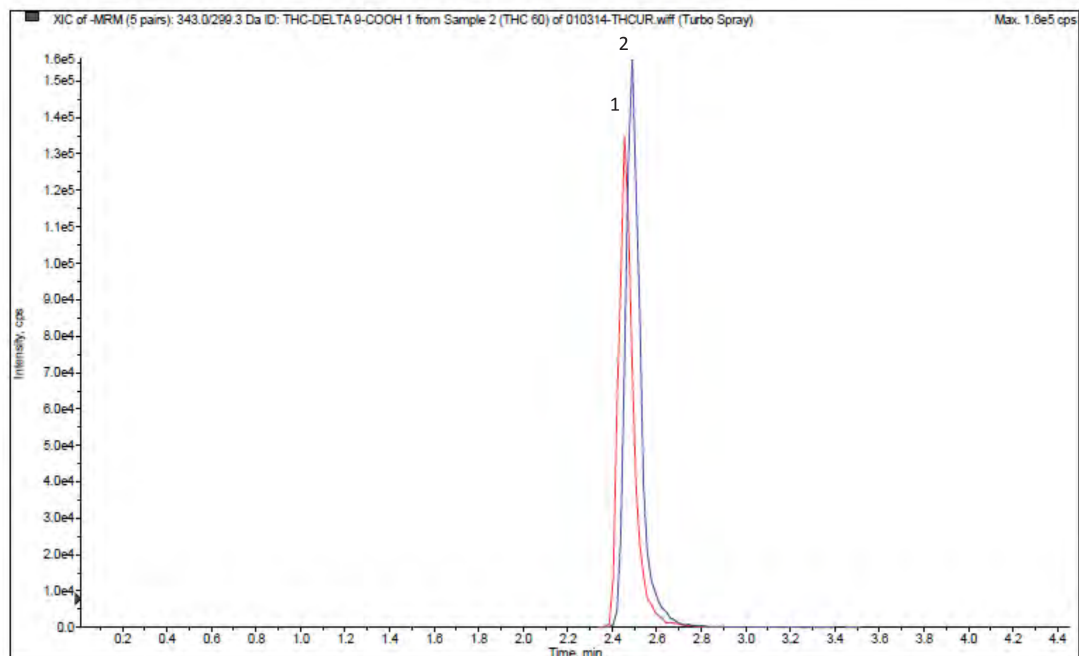
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL MSTFA (with 1%TMCS)
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1 µL

Alternate Derivatization

1. Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate; React 45 minutes at 70 °C

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. THC-DELTA 9-COOH D_9	**352**	**308**	**2.44**
2. THC-DELTA 9-COOH	**343**	**299**	**2.49**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

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

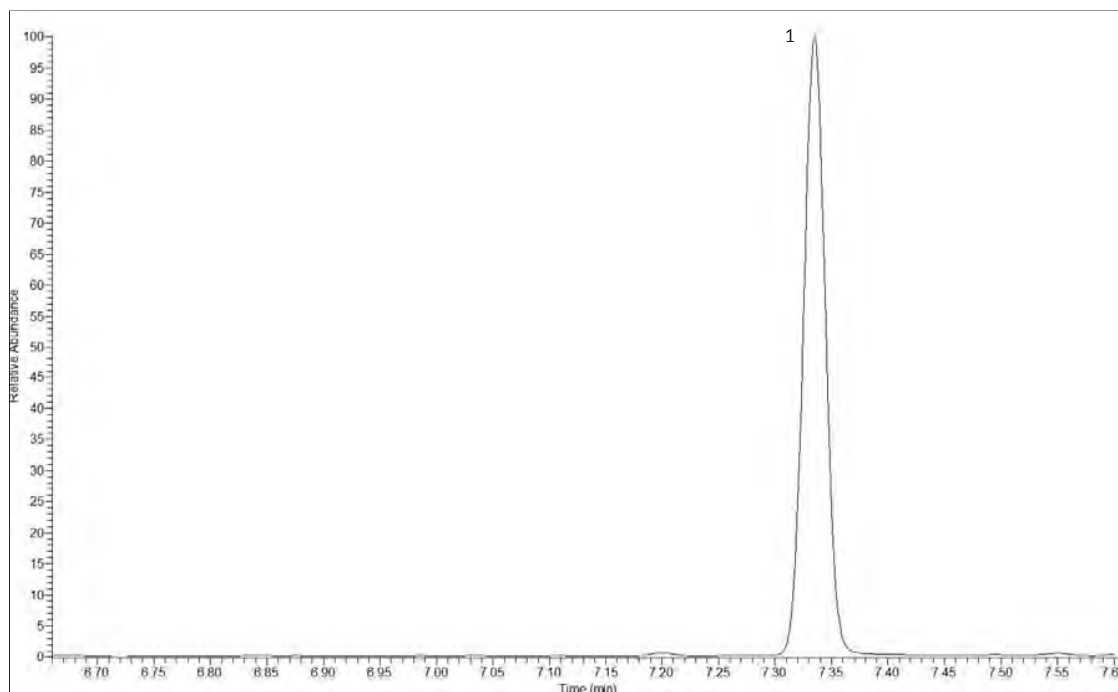
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	60	40
2.00	30	70
2.50	10	90
2.51	60	40
4.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



MSTFA/BSTFA TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
THC-COOH	371	473	488	7.34
THC-COOH D_3	374	476	491	7.31

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: 170 °C (1) to 310 °C (30 °C/ minute): hold (5 minutes)

Carrier gas: Helium (1.2 mL/ minute)

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

THC, THC-OH, and THC-COOH IN WHOLE BLOOD BY LC-MS/MS or GC-MS USING CLEAN SCREEN® EXTRACTION COLUMN

Part #

CSTHC206 – CLEAN SCREEN® THC 200 mg 6 mL Tube

or

CSDAU206 – CLEAN SCREEN® DAU 200 mg 6 mL Tube

SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

SMTBSTFA-1-1 - SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS

SPYR-0-50 - SELECTRA-SIL® Pyridine

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

1. **PREPARE SAMPLE:**
   To 1-2 mL of whole blood add internal standard(s)
   Mix/vortex
   Add dropwise while vortexing approximately 2.5 mL of ***Ice Cold*** acetonitrile
   Centrifuge and transfer acetonitrile to a clean test tube
   Adjust sample pH to 3.0± 0.5 with approx. 2 mL of 100 mM Sodium Acetate buffer
   (Check pH of buffer to insure that the pH value is ~ 3.0)
   Mix/Vortex

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL Acetate buffer (pH 3.0).

   NOTE: Aspirate at full vacuum or pressure

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

4. **WASH COLUMN:**
   1 x 2 mL D.I. H_2O
   1 x 2 mL of 100 mM HCl/Acetonitrile (95:5)
   Aspirate at full vacuum or pressure for 5 minutes
   1 x 200 µL Hexane
   Aspirate at full vacuum or pressure for 5 minutes

   Optional: Dry column (5 minutes at greater than 10 inches HG/ Full Flow for Positive Pressure Manifold

   Note: The delta-9-THC (parent) will elute in hexane so special attention must be paid to not use more than 200 µL hexane in the wash/dry step. The 200 µL hexane in the wash step can be eliminated if the column is allowed to dry longer under vacuum or by positive pressure gas flow.

5. **ELUTE THC (metabolites):**
   1 x 2 mL Hexane (optional, contains delta-9-THC)
   1 x 3 mL Ethyl Acetate/ Hexane (50:50)
   Collect eluate at 1-2 mL /minute
   Note: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency.

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

7. **RECONSTITUTE / DERIVATIZE:**

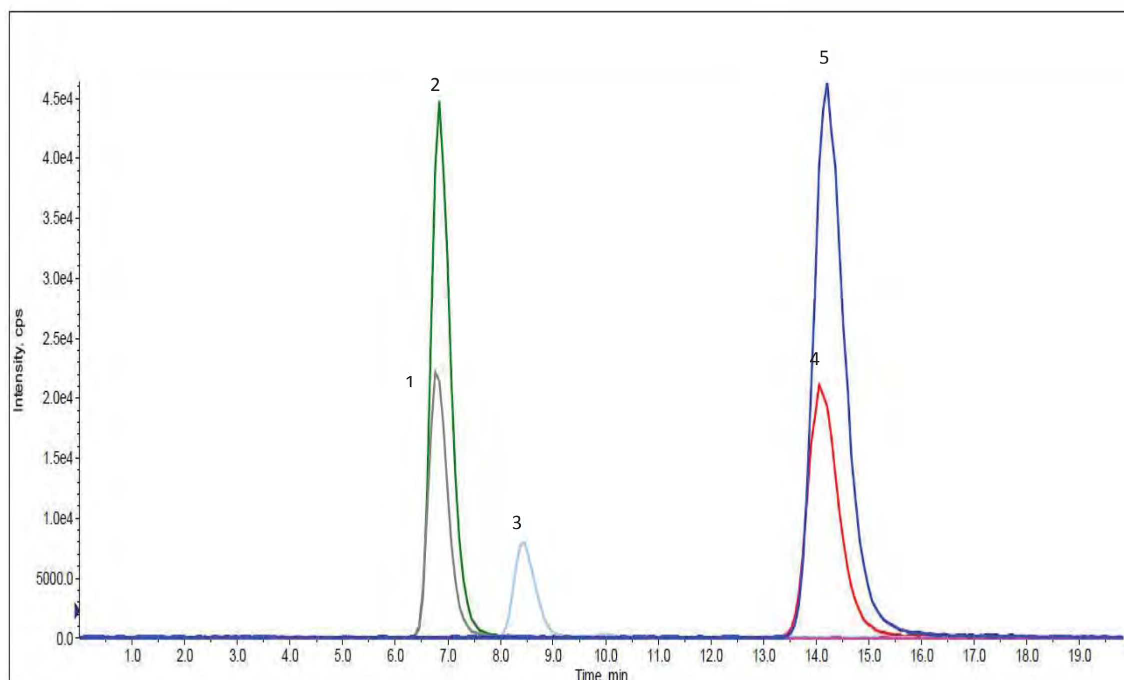
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 5 µL
- **GC-MS**: Dissolve residue in 50 µL of pyridine and
  50 µL BSTFA w/1%TMCS
  Overlay with N_2 and cap. Mix/vortex
  React 30 minutes at 70°C; Cool and inject 2 µL

Alternate Derivatization

1. **Derivatize with MTBSTFA (with 1% TBDMCS):**
   Dissolve residue in 50 µL of pyridine and 50 µL MTBSTFA w/ 1%TBDMCS
   Overlay with N_2 and cap. Mix/vortex
   React 30 minutes at 70 °C; Cool and inject 2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. HYDROXY DELTA 9-THC D_3	334.0	316.2	6.80
2. HYDROXY DELTA 9-THC	330.9	313.2	6.88
3. CARBOXY DELTA 9-THC	343.0	299.1	8.47
CARBOXY DELTA 9-THC D_3*	343.1	302.1	-
4. DELTA 9-THC D_3	318.2	196.2	14.20
5. DELTA 9-THC	315.2	193.2	14.31

*ion data provided for informational purposes only; not in run

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative/Positive

Reconstitute: 100 µL

Injection Volume: 5 µL

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

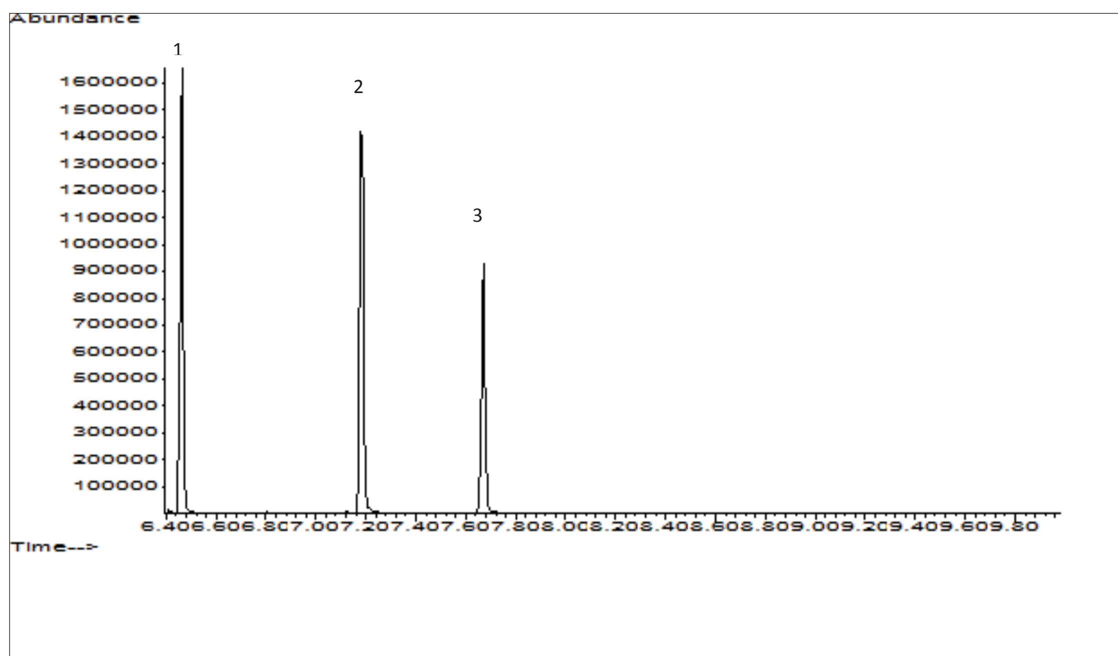
LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

Isocratic:

Time	%A	%B
0.00	25	75
20.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

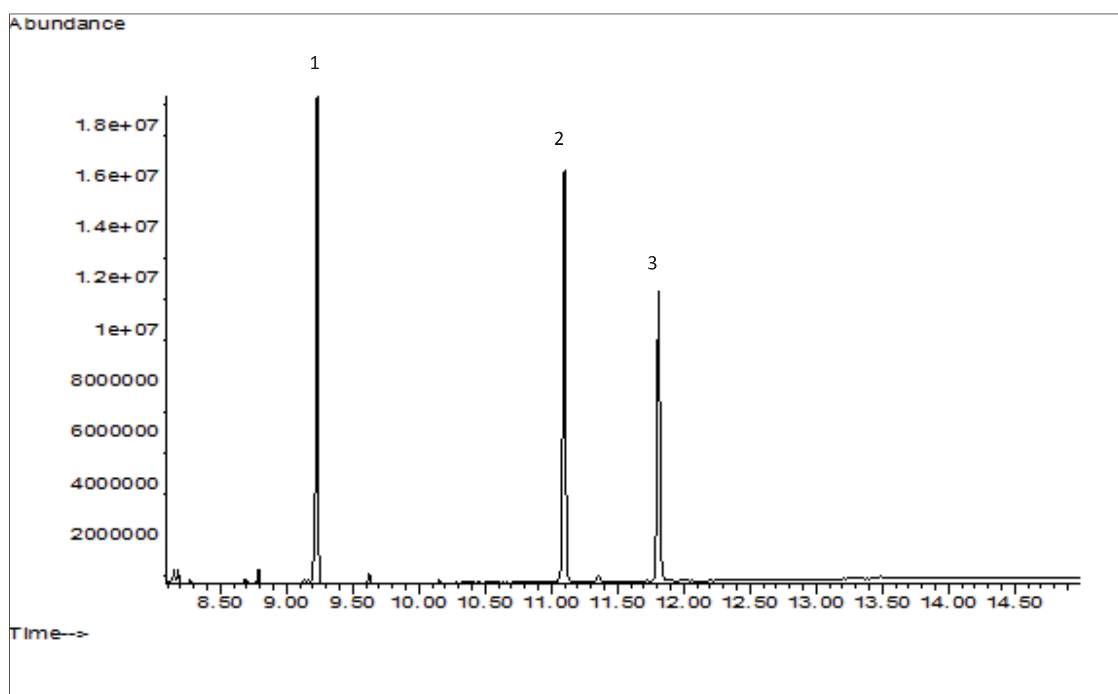
CHROMATOGRAM



TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
THC-D_3	389.25	374.3		
1. THC	386.30	371.3	303.15	6.463
THC-OH D_3	374.30	477.4		
2. THC-OH	371.3	474.4	459.3	7.178
THC-COOH D_3	374.3	491.4		
3. THC-COOH	371.2	488.4	473.3	7.670

CHROMATOGRAM



TBDMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
$THC\text{-}D_3$	374.2	431.3		
1. THC	371.2	428.3	372.2	9.209
THC-OH D_3	416.3	417.3		
2. THC-OH	413.3	369.2	414.3	11.075
THC-COOH D_3	416.3	518.3		
3. THC-COOH	413.3	515.3	572.4	11.783

PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30m X 0.25 mm, 0.25 µm

Injector: 2 µL Splitless, 250 °C

Oven temperature program: 100 °C for 1 min; 40 °C/min to 280 °C; 10 °C/min to 310 °C for 1.5 min

Carrier gas: Helium

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

THC, THC-OH, and THC-COOH CONFIRMATIONS IN WHOLE BLOOD BY LC-MS/MS or GC-MS USING 100 mg STYRESCREEN® SSTHC

Part #:
SSTHC116 – Styre Screen® THC 100 mg 6 mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SMTBSTFA-1-1 - SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS
SPYR-0-50 - SELECTRA-SIL® Pyridine
SLDA100ID21-5UM – Selectra® DA HPLC Column, 100 x 2.1mm, 5 µm

1. **PREPARE SAMPLE:**

   To 1-2 mL whole blood add appropriate internal standards prepared in alcohol
   Add drop-wise 2.5 mL ***Ice Cold*** acetonitrile
   Mix thoroughly and centrifuge
   Decant acetonitrile into a clean tube.
   Evaporate acetonitrile under a stream or air or nitrogen to ~ 200 uL
   Add 2 mL D.I. H_2O (pH of H_2O must be~6.0-7.0)

2. **APPLY SAMPLE:**

   Load at 1 to 2 mL/minute.

4. **WASH COLUMN:**

   Wash with 2 mL (84: 15: 1) D.I. H_2O: Acetonitrile: NH_4OH (made fresh daily)
   Dry column under full vacuum or pressure for 10-15 minutes

5. **ELUTE THC & metabolites:**

   1 x 3 mL Hexane/ Ethyl Acetate/ Glacial Acetic Acid (49: 49:2)
   Collect at 1-2 mL/ minute.

6. **DRY ELUATE:**

   Evaporate to dryness at < 40 °C.

7. **RECONSTITUTE / DERIVATIZE:**

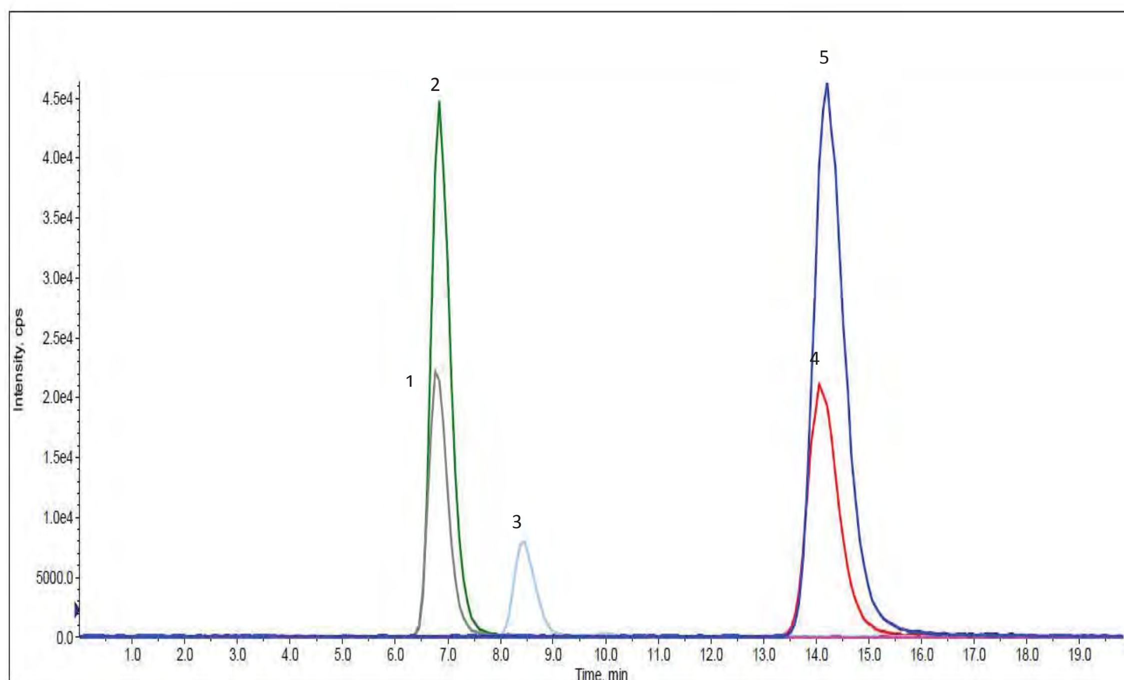
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 5 µL.
   - **GC-MS**: Dissolve residue in 50 µL of pyridine and
     50 µL BSTFA w/ 1%TMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 2 µL

Alternate Derivatization

1. **Derivatize with MTBSTFA w/ 1% TBDMCS:**
   Dissolve residue in 50 µL of pyridine and 50 µL MTBSTFA w/ 1%TBDMCS
   Overlay with N_2 and cap. Mix/vortex
   React 30 minutes at 70°C; Cool and inject 2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. HYDROXY DELTA 9-THC D_3	334.0	316.2	6.80
2. HYDROXY DELTA 9-THC	330.9	313.2	6.88
3. CARBOXY DELTA 9-THC	334.0	316.2	8.47
CARBOXY DELTA 9-THC D_3	348.3	303.0	-
4. DELTA 9-THC D_3	318.2	196.2	14.20
5. DELTA 9-THC	315.2	193.2	14.31

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative/Positive

Reconstitute: 100 µL

Injection Volume: 5 µL

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

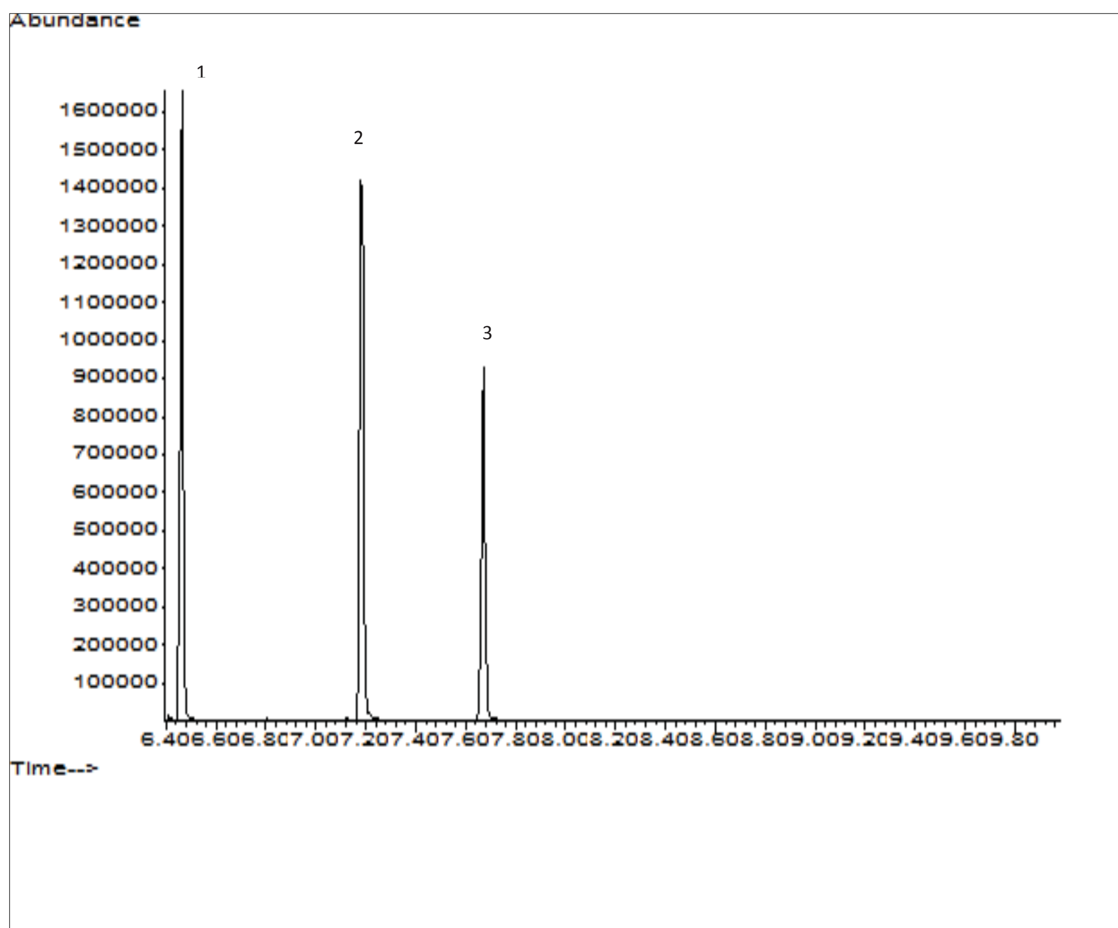
LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

Isocratic:

Time	%A	%B
0.00	25	75
20.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

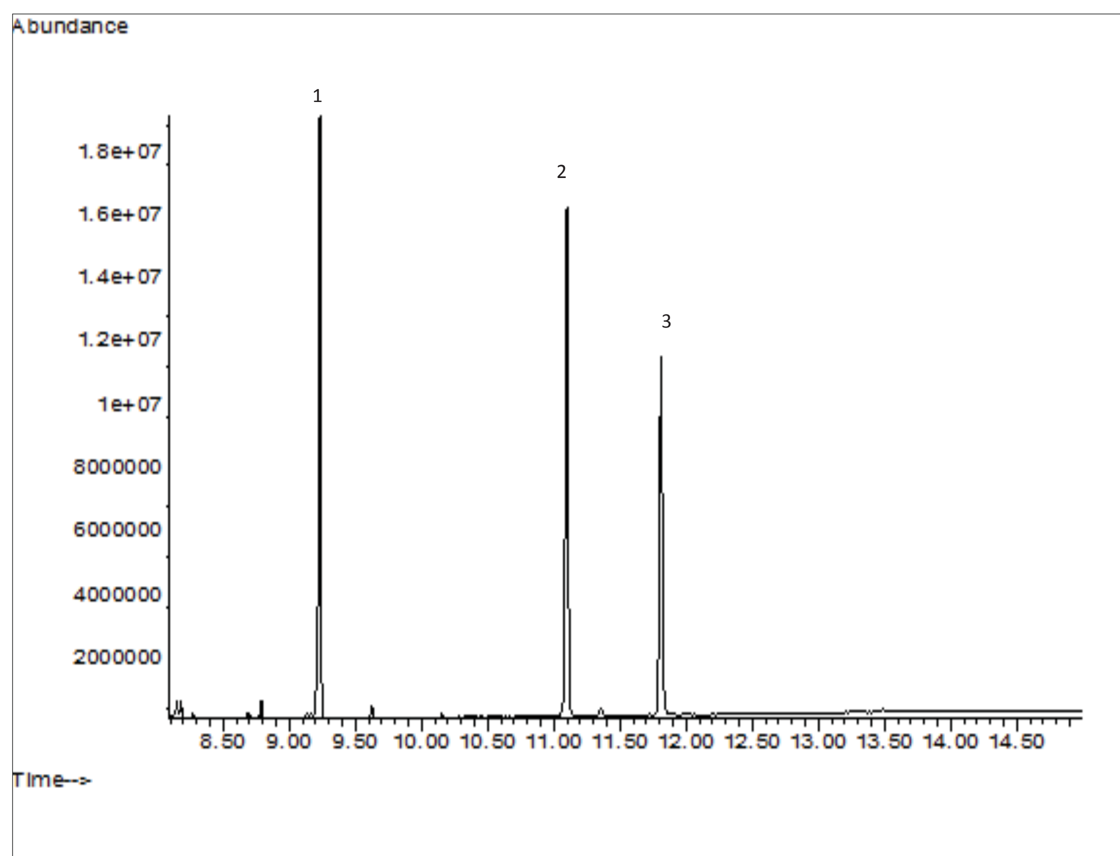
CHROMATOGRAM



TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
THC-D_3	389.25	374.3		
1. THC	386.30	371.3	303.15	6.463
THC-OH D_3	374.30	477.4		
2. THC-OH	371.3	474.4	459.3	7.178
THC-COOH D_3	374.3	491.4		
3. THC-COOH	371.2	488.4	473.3	7.670

CHROMATOGRAM



TBDMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
THC-D_3	374.2	431.3		
1. THC	371.2	428.3	372.2	9.209
THC-OH D_3	416.3	417.3		
2. THC-OH	413.3	369.2	414.3	11.075
THC-COOH D_3	416.3	518.3		
3. THC-COOH	413.3	515.3	572.4	11.783

PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30m X 0.25 mm, 0.25 µm

Injector: 2 µL Splitless, 250 °C

Oven temperature program: 100 °C for 1 min; 40 °C/min to 280 °C; 10 °C/min to 310 °C for 1.5 min

Carrier gas: Helium

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

SYNTHETIC CANNABINOIDS "SPICE" DRUGS IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® THC EXTRACTION COLUMN

Part #
CSTHC206 – CLEAN SCREEN® THC 200 mg 6 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SMSTFA-1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS
SLDA100ID21-3UM – Selectra® DA HPLC Column, 100 x 2.1 mm, 3 µm

1. **PREPARE SAMPLE:**

Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
Add 1 mL of blood, plasma/ serum, 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

Urine: **PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:**
To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase. Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool
Do not adjust pH~ sample is ready to be added to the extraction column.

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 of 100 mM phosphate buffer containing 20% Acetonitrile

Dry Column (5 minutes at >10inches Hg)

5. **ELUTE SPICE DRUGS:**
2 x 3 mL Ethyl Acetate containing 10% CH_3OH
Collect eluate at 1-2 mL /minute

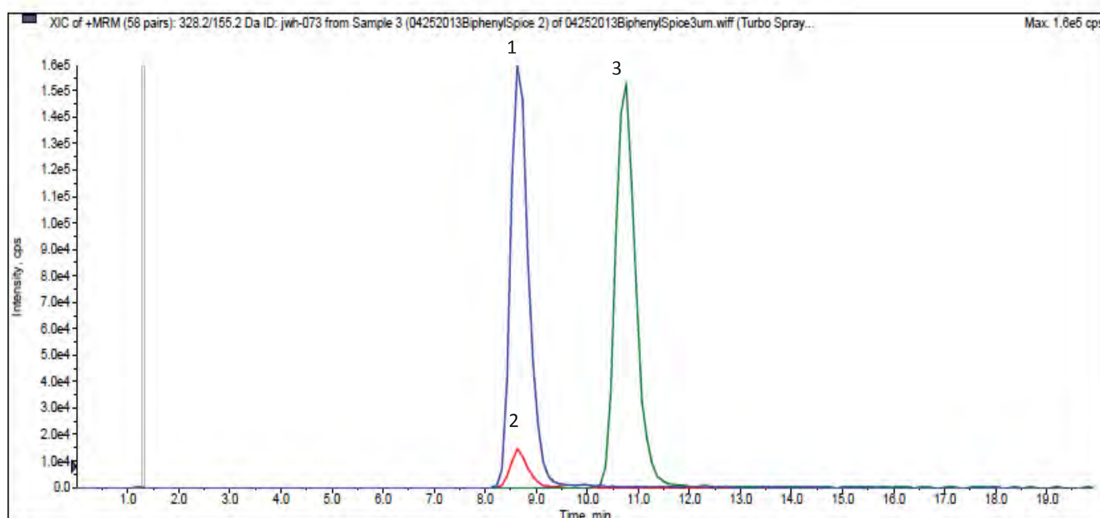
6. **DRY ELUATE:**
Evaporate to dryness at < 40 °C.

7. . **RECONSTITUTE / DERIVATIZE:**

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and
  50 µL MSTFA w/1% TMCS
  Overlay with N_2 and cap. Mix/vortex
  React 30 minutes at 70 °C; Cool and inject 1 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



PARAMETERS

Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. JWH015	328.2	155.1	8.65
2. JWH073	328.2	155.2	8.66
3. JWH018	342.2	155.1	10.74

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.7 mL/minute
Polarity: Positive
Reconstitute: 100 µL
Injection Volume: 3 µL
LC Column: Selectra® DA 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	20	80
20.00	STOP	

AM2201 METABOLITES IN URINE BY LC-MS/MS OR GC-MS
CLEAN SCREEN® THC EXTRACTION COLUMN

Part #
CSTHC206 – CLEAN SCREEN THC 200mg 5mL Tube
BETA-GLUC-10 - Slelectrazyme® Beta-glucuronidase
SMSTFA-1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS
SLC-1850ID21-3UM - Selectra® C18 HPLC Column, 50 x 2.1 mm, 3µm

1. **PREPARE SAMPLE:**

   Urine: **PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:**
   To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase. Optionally, add 1 mL of 1M acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 1-2 hours at 65°C.
   Allow sample to cool
   Do not adjust pH~ sample is ready to be added to the extraction column.

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 of 100 mM phosphate buffer containing 20% Acetonitrile

   NOTE: Aspirate at full vacuum or pressure

5. **ELUTE AM2201 ANALYTES:**
   2 x 3 mL Ethyl Acetate containing 10 % CH_3OH
   Collect eluate at 1-2 mL /minute

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

7. . **RECONSTITUTE / DERIVATIZE:**

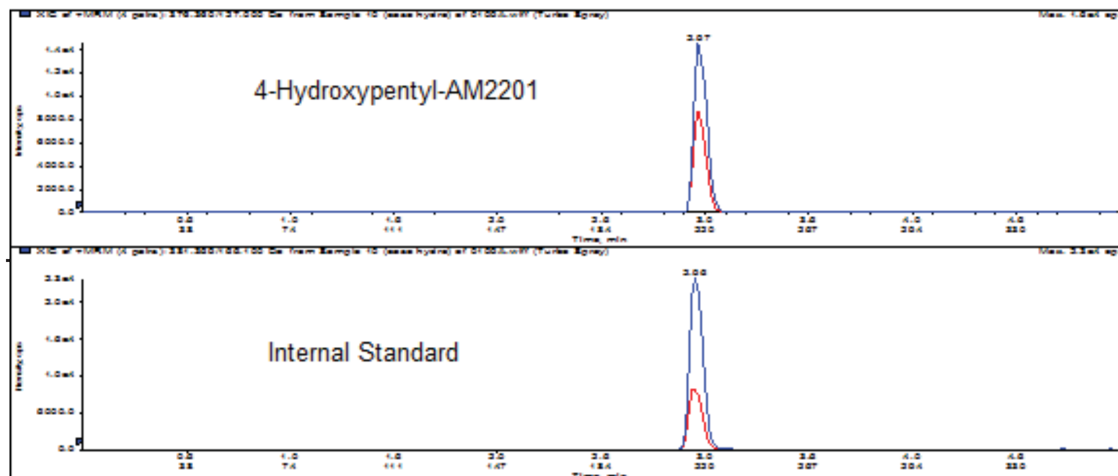
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL MSTFA w/ 1%TMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1 mL

NOTES:

This method is used for the extraction of AM2201 and metabolites in urine. LC/MS/MS instrumentation is used for analysis with a linear range of 1 ng/mL to 100 ng/mL with average recoveries found to be greater than 90%.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



PARAMETERS

Analyte	MRM Transitions		
	Precursor Ion	Product Ion 1	Product Ion 2
AM-2201	360.2	155.1	127.1
AM 2201 2-OH INDOLE	376.3	127.1	154.9
AM 2201 4-OH INDOLE 1	376.3	127.1	154.9
AM 2201 4-OH INDOLE D_5	381.3	126.1	155.1
AM 2201 5-OH INDOLE	377.1	154.9	56.9
AM 2201 N4 OH PENTYL	377.1	155.0	57.0

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Acetonitrile
Flow Rate: 0.5 mL/minute
Polarity: Positive
Reconstitute: 100 µL
Injection Volume: 10 µL
LC Column: Selectra® C18 HPLC Column 50 x 2.1 mm 3 µm
Instrument: API 3200 QTrap MS/MS Compound MRM Transition

Gradient:

Time	%A	%B
0	65	35
0.5	65	35
3.5	10	10
4.0	65	35
5.0	65	35

Opiates

CPS analitica for Chemistry

EXTRACTION OF OPIATES FROM BLOOD/PLASMA/SERUM/URINE OR TISSUE USING CLEAN SCREEN XCEL® I COLUMN

Part#
CSXCE106 – CLEAN SCREEN XCEL® I 130 mg, 6 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SPIA-0-1– SELECTRA-SIL® PIA (propionic anhydride)
SPYR-0-50– SELECTRA-SIL® Pyridine
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. PREPARE SAMPLE

Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
Add 1-2 mL of blood, plasma/ serum, 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.
Centrifuge for 10 minutes at 2000 rpm and discard pellet

Urine: PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:
To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool
Do not adjust pH~ sample is ready to be added to the extraction column.

2. APPLY SAMPLE

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. WASH

1 x 3 mL D.I. H_2O
1 x 3 mL 98% Methanol: 2% Acetic Acid

Dry column thoroughly under full vacuum or positive pressure for a minimum of 5-10 minutes.

4. ELUTION

1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. DRY ELUTE

Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10 µL

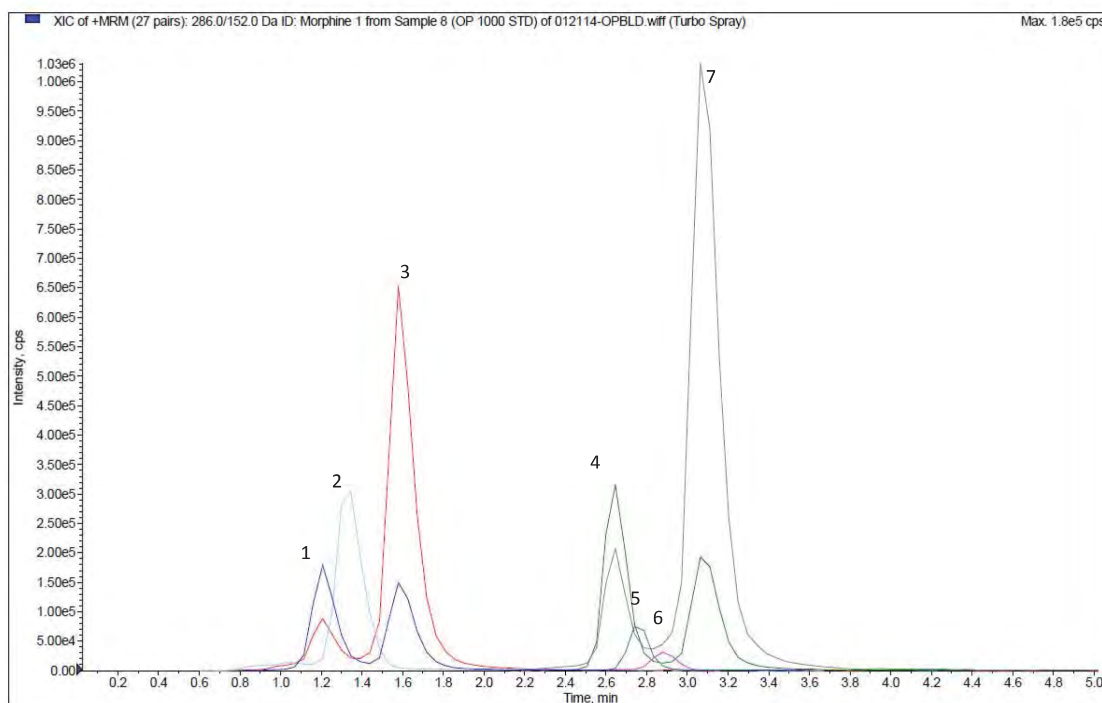
- **GC-MS**: **Derivatize with propionic anhydride: pyridine**
  Add 200 µL of a 1:1 solution of proprionic anhydride: pyridine
  Make this solution fresh daily.
  Mix/vortex.
  React for 60 minutes at 60 °C in a heater block.
  Remove from heat source to cool.
  Evaporate to dryness at < 40 °C.
  Reconstitute the residue with 50 µL of Ethyl Acetate / Methanol (70:30)

Alternate Derivatization

1. **DERIVATIZE with TMS**
   Add 50 µL Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
   Overlay with N_2 and cap. Mix/vortex.
   React 30 minutes at 70 °C. Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA solution

__Note__: *Hydroxylamine can be added to sample within method if oxime derivative is preferred.*
Following hydrolysis, add 200 µL 10% Hydroxylamine solution.
Heat to 60 °C for 30 min in a heating block.
Allow sample to cool then adjust pH back to 5 with 1.0 N NaOH.
Centrifuge for 10 minutes at 2000 rpm and discard pellet
Sample is now ready to be added to the extraction column

INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Morphine	286.0	152.0	1.21
2. Oxymorphone	302.0	227.0	1.30
3. Hydromorphone	286.0	185.0	1.60
4. Codeine	300.0	152.0	2.65
5. 6-MAM	328.0	165.1	2.75
6. Oxycodone	316.0	240.0	2.85
7. Hydrocodone	300.0	199.0	3.10

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

Injection Volume: 10 µL

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

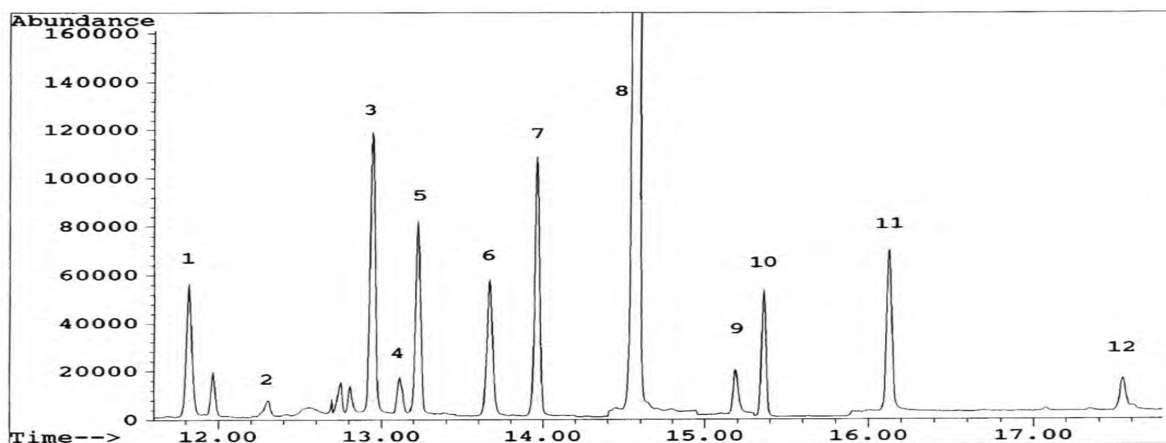
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	85	15
7.00	40	60
7.01	20	80
8.00	85	15
9.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



PROPYL DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion
1. Hydrocodone	299.0	242.0	214.0
2. Thebaine	311.2	296.2	312.2
3. Codeine	355.0	282.0	229.0
4. Oxycodone	371.0	314.0	298.0
5. Heroin	327.2	369.2	268.2
6. Hydromorphone	285.0	341.0	228.0
7. 6-Mam	327.2	268.0	383.2
8. Morphine	341.0	268.0	397.0
Morphine-D_3	344.3	271.3	400.3
9. Oxymorphone	357.0	300.0	413.0
10. Naloxone	327.1	383.2	328.2
11. Nalorphine	367.2	350.2	294.2
12. Norcodeine	223.1	224.1	236.1

BSTFA-OXIME DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Morphine TMS	**429.0**	**414.0**	**401.0**
Morphine-D_3 TMS	**432.0**	**417.0**	**404.0**
Morphine-D_6 TMS	**435.0**	**420.0**	**404.0**
Normorphine TMS	**487.0**	**472.0**	**414.0**
Diacetylmorphine	**369.0**	**327.0**	**268.0**
Oxymorphone Oxime TMS	**532.0**	**517.0**	**287.0**
Oxymorphone Oxime-D_3 TMS	**535.0**	**520.0**	**290.0**
Hydromorphone Oxime TMS	**444.0**	**429.0**	**355.0**
Hydromorphone Oxime-D_3 TMS	**447.0**	**432.0**	**358.0**
Codeine TMS	**371.0**	**356.0**	**343.0**
Codeine-D_3 TMS	**374.0**	**359.0**	**346.0**
Codeine-D_6 TMS	**377.0**	**349.0**	**316.0**
Dihydrocodeine TMS	**373.0**	**315.0**	**358.0**
Norcodeine TMS	**429.0**	**414.0**	**356.0**
6-MAM TMS	**399.0**	**400.0**	**340.0**
Oxycodone Oxime TMS	**474.0**	**459.0**	**417.0**
Oxycodone Oxime-D_3 TMS	**477.0**	**462.0**	**420.0**
Oxycodone Oxime-D_6 TMS	**480.0**	**465.0**	**420.0**
Hydrocodone Oxime TMS	**386.0**	**297.0**	**371.0**
Hydrocodone Oxime-D_3 TMS	**389.0**	**300.0**	**374.0**
Hydrocodone Oxime-D_6 TMS	**392.0**	**303.0**	**377.0**
Meperidine-D_4	**251.0**	**222.0**	**250.0**
Meperidine	**247.0**	**218.0**	**246.0**
Normeperidine-D_4 TMS	**308.0**	**280.0**	**309.0**
Normeperidine TMS	**305.0**	**276.0**	**304.0**
Tramadol TMS	**335.0**	**245.0**	**290.0**
O-Desmethyltramadol TMS	**393.0**	**378.0**	**303.0**
N-Desmethyltramadol TMS	**393.0**	**378.0**	**116.0**
Pentazocine TMS	**357.0**	**342.0**	**289.0**

PARAMETERS

GC/MS: Hewlett Packard 5971A/ 5890 GCMS System with 7673 ALS

GC capillary column: Rtx-5 30 m x 0.25 mm, 0.25 µm

Injector: 2 µL Splitless, 250 °C

Oven temperature program: 100 ºC (1 minute) to 250 ºC (25 ºC/minute): hold (2 minutes) to 290 ºC (10 ºC/minute): hold (0.5 minutes) to 325 ºC (25 ºC/minute): hold (3.1 minutes).

Carrier gas: Helium (1.2 mL/minute)

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

References: Hackett,J.; Telepchak, M.J.; Coyer, M.J. Automation of solid-phase extraction for urinary opiate analysis. American Laboratory. 2008.

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

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SPIA-0-1– SELECTRA-SIL® PIA (propionic anhydride)
SPYR-0-50– SELECTRA-SIL® Pyridine
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**

   Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
   Add 1-2 mL of blood, plasma/ serum, 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.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet

   Urine: PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:
   To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 1-2 hours at 65 °C.
   Allow sample to cool
   Do not adjust pH~ sample is ready to be added to the extraction column.

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 acetate buffer (pH 4.5).
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at full vacuum or pressure).

5. **ELUTE OPIATES:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

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**: **Derivatize with proprionic anhydride: pyridine**
  Add 200 µL of a 1:1 solution of proprionic anhydride: pyridine
  Make this solution fresh daily.
  Mix/vortex.
  React for 60 minutes at 60 °C in a heater block.
  Remove from heat source to cool.
  Evaporate to dryness at < 40 °C.
  Reconstitute the residue with 50 µL of Ethyl Acetate / Methanol (70:30)

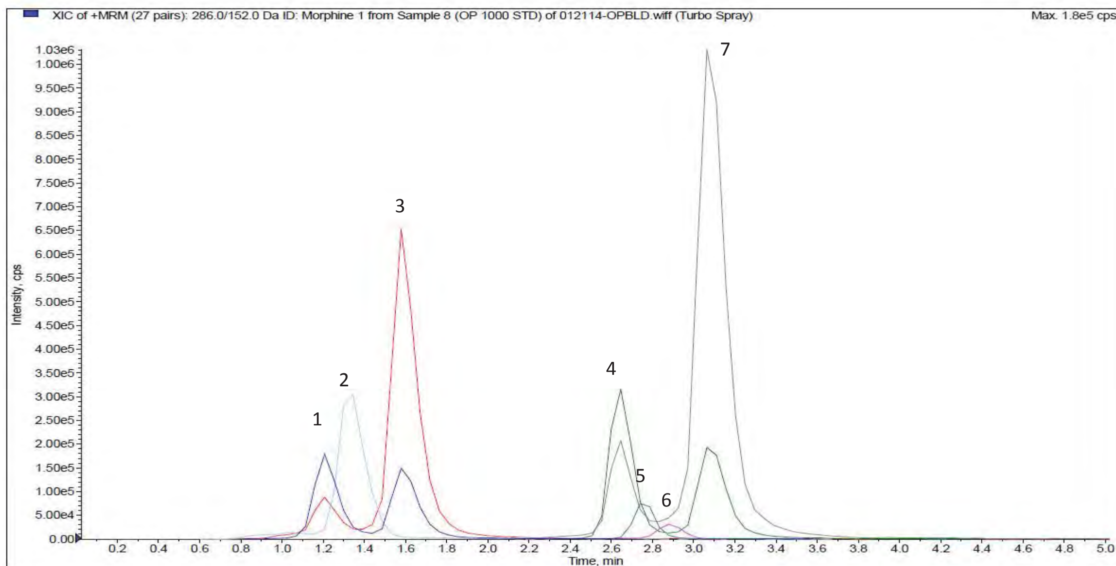
Alternate Derivatization

1. **DERIVATIZE with TMS**
   Add 50 µL Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
   Overlay with N_2 and cap. Mix/vortex.
   React 30 minutes at 70 °C. Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA solution

Note: *Hydroxylamine can be added to sample within method if oxime derivative is preferred.*
Following hydrolysis, add 200 µL 10% Hydroxylamine solution.
Heat to 60 °C for 30 min in a heating block.
Allow sample to cool then adjust pH back to 5 with 1.0 N NaOH.
Centrifuge for 10 minutes at 2000 rpm and discard pellet
Sample is now ready to be added to the extraction column

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Morphine	286.0	152.0	1.21
2. Oxymorphone	302.0	227.0	1.30
3. Hydromorphone	286.0	185.0	1.60
4. Codeine	300.0	152.0	2.65
5. 6-MAM	328.0	165.1	2.75
6. Oxycodone	316.0	240.0	2.85
7. Hydrocodone	300.0	199.0	3.10

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

Injection Volume: 10 µL

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

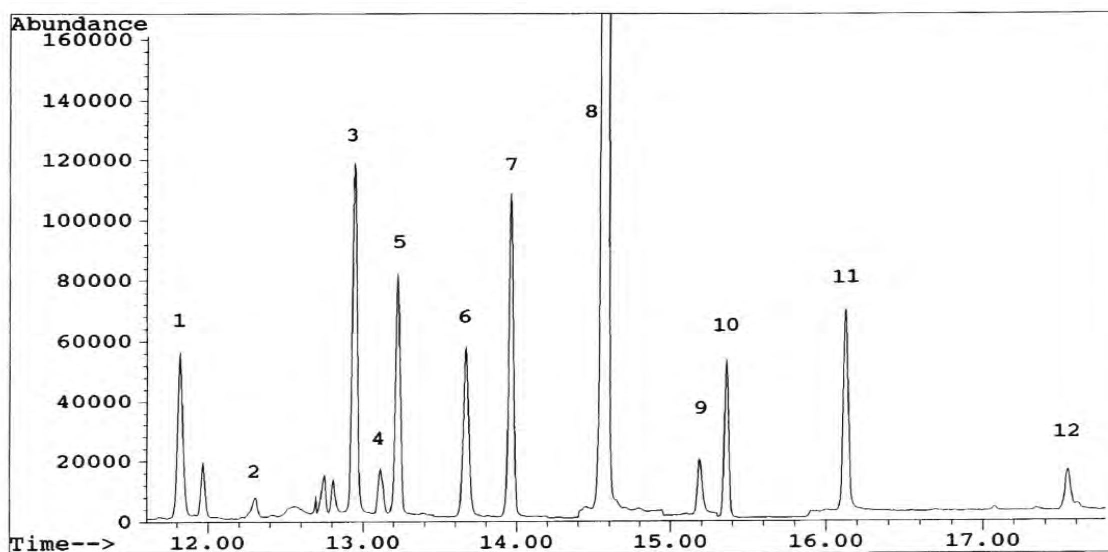
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	85	15
7.00	40	60
7.01	20	80
8.00	85	15
9.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



PROPYL DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion
1. Hydrocodone	299.0	242.0	214.0
2. Thebaine	311.2	296.2	312.2
3. Codeine	355.0	282.0	229.0
4. Oxycodone	371.0	314.0	298.0
5. Heroin	327.2	369.2	268.2
6. Hydromorphone	285.0	341.0	228.0
7. 6-Mam	327.2	268.0	383.2
8. Morphine	341.0	268.0	397.0
- Morphine-D_3	344.3	271.3	400.3
9. Oxymorphone	357.0	300.0	413.0
10. Naloxone	327.1	383.2	328.2
11. Nalorphine	367.2	350.2	294.2
12. Norcodeine	223.1	224.1	236.1

BSTFA-OXIME DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Morphine TMS	429.0	414.0	401.0
Morphine-D_3 TMS	432.0	417.0	404.0
Morphine-D_6 TMS	435.0	420.0	404.0
Normorphine TMS	487.0	472.0	414.0
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Oxymorphone Oxime TMS	532.0	517.0	287.0
Oxymorphone Oxime-D_3 TMS	535.0	520.0	290.0
Hydromorphone Oxime TMS	444.0	429.0	355.0
Hydromorphone Oxime-D_3 TMS	447.0	432.0	358.0
Codeine TMS	371.0	356.0	343.0
Codeine-D_3 TMS	374.0	359.0	346.0
Codeine-D_6 TMS	377.0	349.0	316.0
Dihydrocodeine TMS	373.0	315.0	358.0
Norcodeine TMS	429.0	414.0	356.0
6-MAM TMS	399.0	400.0	340.0
Oxycodone Oxime TMS	474.0	459.0	417.0
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Meperidine	247.0	218.0	246.0
Normeperidine-D_4 TMS	308.0	280.0	309.0
Normeperidine TMS	305.0	276.0	304.0
Tramadol TMS	335.0	245.0	290.0
O-Desmethyltramadol TMS	393.0	378.0	303.0
N-Desmethyltramadol TMS	393.0	378.0	116.0
Pentazocine TMS	357.0	342.0	289.0

PARAMETERS

GC/MS: Hewlett Packard 5971A/ 5890 GCMS System with 7673 ALS

GC capillary column: Rtx-5 30 m x 0.25 mm, 0.25 µm

Injector: 2 µL Splitless, 250 °C

Oven temperature program: 100 ºC (1 minute) to 250 ºC (25 ºC/minute): hold (2 minutes) to 290 ºC (10 ºC/minute): hold (0.5 minutes) to 325 ºC (25 ºC/minute): hold (3.1 minutes).

Carrier gas: Helium (1.2 mL/minute)

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

References: Hackett,J.; Telepchak, M.J.; Coyer, M.J. Automation of solid-phase extraction for urinary opiate analysis. American Laboratory. 2008.

OPIATES IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS STYRE SCREEN® DBX EXTRACTION COLUMN

Part #
SSDBX033 – STYRE SCREEN® DBX 30 mg, 3 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SPIA-0-1– SELECTRA-SIL® PIA (propionic anhydride)
SPYR-0-50– SELECTRA-SIL® Pyridine
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**

   Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
   Add 1-2 mL of blood, plasma/ serum, 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.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet

   Urine: PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:
   To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 1-2 hours at 65 °C.
   Allow sample to cool
   Do not adjust pH~ sample is ready to be added to the extraction column.

2. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute.

3. **WASH COLUMN:**
   1 x 1 mL D.I. H_2O.
   1 x 1 mL 100 mM acetate buffer (pH 4.5).
   1 x 1 mL CH_3OH.
   Dry column (5 minutes at full vacuum or pressure).

4. **ELUTE OPIATES:**
   2 x 0.5 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.
   NOTE: Prepare elution solvent daily. Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

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

6. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL
   - **GC-MS**: **Derivatize with propionic anhydride: pyridine**
     Add 200 µL of a 1:1 solution of propionic anhydride: pyridine
     Make this solution fresh daily.
     Mix/vortex.
     React for 60 minutes at 60 °C in a heater block.
     Remove from heat source to cool.
     Evaporate to dryness at < 40 °C.
     Reconstitute the residue with 50 µL of Ethyl Acetate / Methanol (70:30)

Alternate Derivatization

1. **DERIVATIZE with TMS**

   Add 50 µL Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS

   Overlay with N_2 and cap. Mix/vortex.

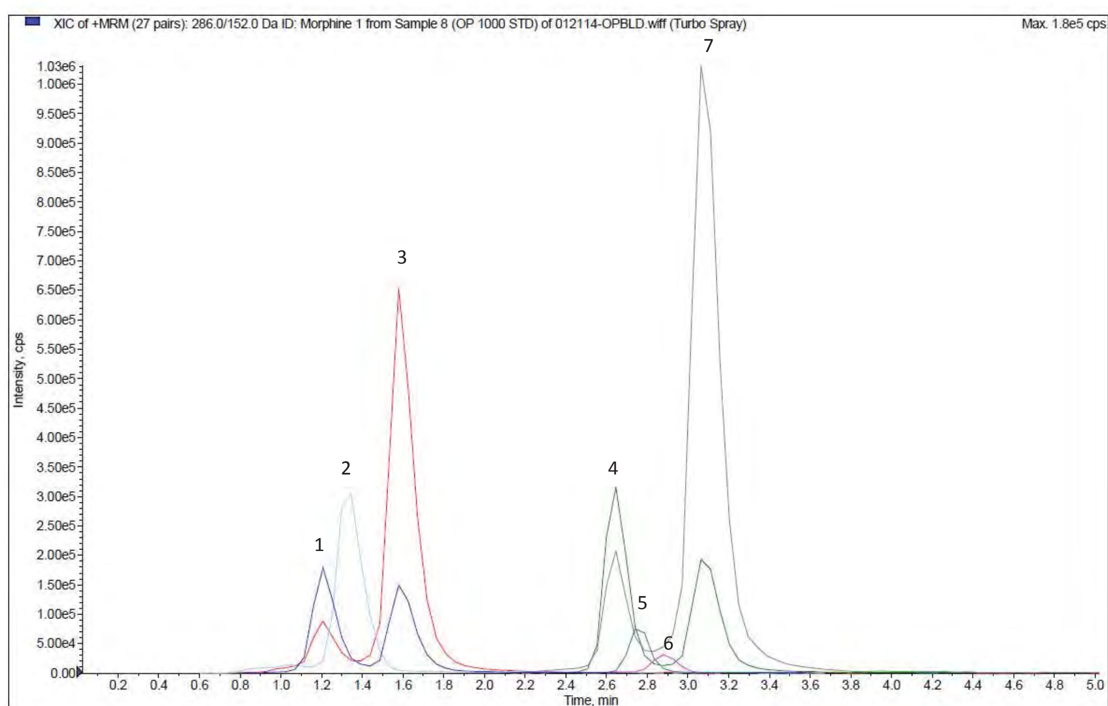
   React 30 minutes at 70 °C. Remove from heat source to cool.

   NOTE: Do not evaporate BSTFA solution

__Note__: *Hydroxylamine can be added to sample within method if oxime derivative is preferred.*
Following hydrolysis, add 200 µL 10% Hydroxylamine solution.
Heat to 60 °C for 30 min in a heating block.
Allow sample to cool then adjust pH back to 5 with 1.0 M NaOH.
Centrifuge for 10 minutes at 2000 rpm and discard pellet
Sample is now ready to be added to the extraction column

INSTRUMENT CONDITIONS (LC-MS/MS):

__CHROMATOGRAM__



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Morphine	286.0	152.0	1.21
2. Oxymorphone	302.0	227.0	1.30
3. Hydromorphone	286.0	185.0	1.60
4. Codeine	300.0	152.0	2.65
5. 6-MAM	328.0	165.1	2.75
6. Oxycodone	316.0	240.0	2.85
7. Hydrocodone	300.0	199.0	3.10

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

Injection Volume: 10 µL

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

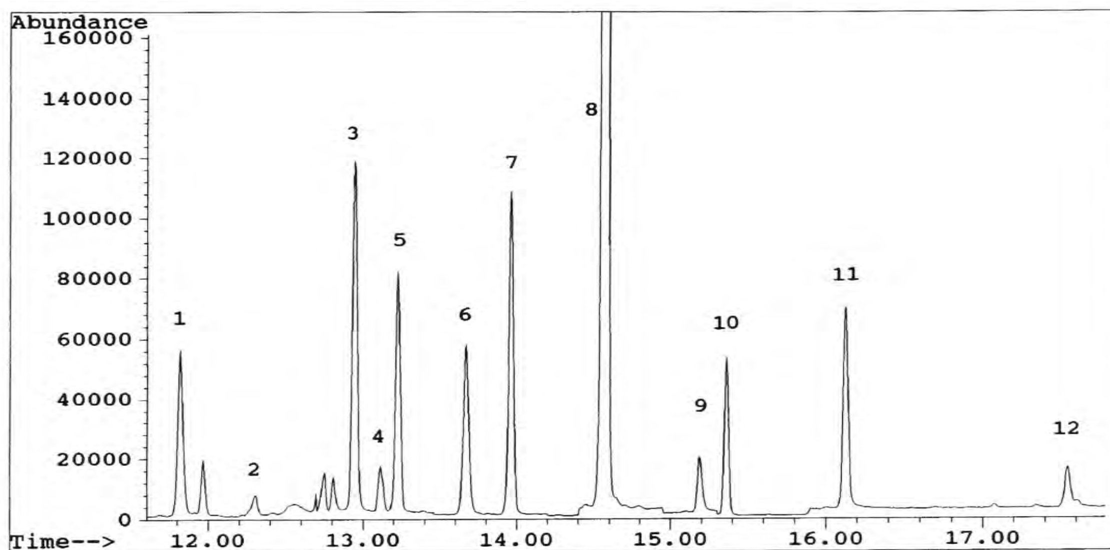
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	85	15
7.00	40	60
7.01	20	80
8.00	85	15
9.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



PROPYL DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion
1. Hydrocodone	299.0	242.0	214.0
2. Thebaine	311.2	296.2	312.2
3. Codeine	355.0	282.0	229.0
4. Oxycodone	371.0	314.0	298.0
5. Heroin	327.2	369.2	268.2
6. Hydromorphone	285.0	341.0	228.0
7. 6-Mam	327.2	268.0	383.2
8. Morphine	341.0	268.0	397.0
Morphine-D_3	344.3	271.3	400.3
9. Oxymorphone	357.0	300.0	413.0
10. Naloxone	327.1	383.2	328.2
11. Nalorphine	367.2	350.2	294.2
12. Norcodeine	223.1	224.1	236.1

BSTFA-OXIME DERIVATIVES

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Morphine TMS	**429.0**	**414.0**	**401.0**
Morphine-D_3 TMS	**432.0**	**417.0**	**404.0**
Morphine-D_6 TMS	**435.0**	**420.0**	**404.0**
Normorphine TMS	**487.0**	**472.0**	**414.0**
Diacetylmorphine	**369.0**	**327.0**	**268.0**
Oxymorphone Oxime TMS	**532.0**	**517.0**	**287.0**
Oxymorphone Oxime-D_3 TMS	**535.0**	**520.0**	**290.0**
Hydromorphone Oxime TMS	**444.0**	**429.0**	**355.0**
Hydromorphone Oxime-D_3 TMS	**447.0**	**432.0**	**358.0**
Codeine TMS	**371.0**	**356.0**	**343.0**
Codeine-D_3 TMS	**374.0**	**359.0**	**346.0**
Codeine-D_6 TMS	**377.0**	**349.0**	**316.0**
Dihydrocodeine TMS	**373.0**	**315.0**	**358.0**
Norcodeine TMS	**429.0**	**414.0**	**356.0**
6-MAM TMS	**399.0**	**400.0**	**340.0**
Oxycodone Oxime TMS	**474.0**	**459.0**	**417.0**
Oxycodone Oxime-D_3 TMS	**477.0**	**462.0**	**420.0**
Oxycodone Oxime-D_6 TMS	**480.0**	**465.0**	**420.0**
Hydrocodone Oxime TMS	**386.0**	**297.0**	**371.0**
Hydrocodone Oxime-D_3 TMS	**389.0**	**300.0**	**374.0**
Hydrocodone Oxime-D_6 TMS	**392.0**	**303.0**	**377.0**

Meperidine-D_4	251.0	222.0	250.0
Meperidine	247.0	218.0	246.0
Normeperidine-D_4 TMS	308.0	280.0	309.0
Normeperidine TMS	305.0	276.0	304.0
Tramadol TMS	335.0	245.0	290.0
O-Desmethyltramadol TMS	393.0	378.0	303.0
N-Desmethyltramadol TMS	393.0	378.0	116.0
Pentazocine TMS	357.0	342.0	289.0

PARAMETERS

GC/MS: Hewlett Packard 5971A/ 5890 GCMS System with 7673 ALS

GC capillary column: Rtx-5 30 m x 0.25 mm, 0.25 µm

Injector: 2 µL Splitless, 250 °C

Oven temperature program: 100 ºC (1 minute) to 250 ºC (25 ºC/minute): hold (2 minutes) to 290 ºC (10ºC/minute): hold (0.5 minutes) to 325ºC (25ºC/minute): hold (3.1 minutes).

Carrier gas: Helium (1.2mL/minute)

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

References: Hackett,J.; Telepchak, M.J.; Coyer, M.J. Automation of solid-phase extraction for urinary opiate analysis. American Laboratory. 2008.

CLINICAL

FORENSICS

UCT

Steroids

CPS analitica for Chemistry

ANABOLIC STEROIDS IN URINE BY LC-MS/MS OR GC-MS
CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #
ZSDAU020 – CLEAN SCREEN® DAU, 200 mg, 10mL Tube
BETA-GLUC-10 - Selectrazyme® Beta-glucuronidase
SMSTFA-1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS
SLDA50ID21-5UM - Selectra® DA HPLC Column, 50 x 2.1 mm, 5µm

1. **PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:**
   To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.
   Vortex and heat for 3 hours at 65°C.
   Allow sample to cool
   Adjust sample pH to 7.0 ± 0.5 with approximately 3-4mL of D.I. H_2O

2. **CONDITION CLEAN SCREEN® 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 10% (v/v) CH_3OH in D.I. H_2O
   Dry column (10 minutes at > 10 inches Hg).

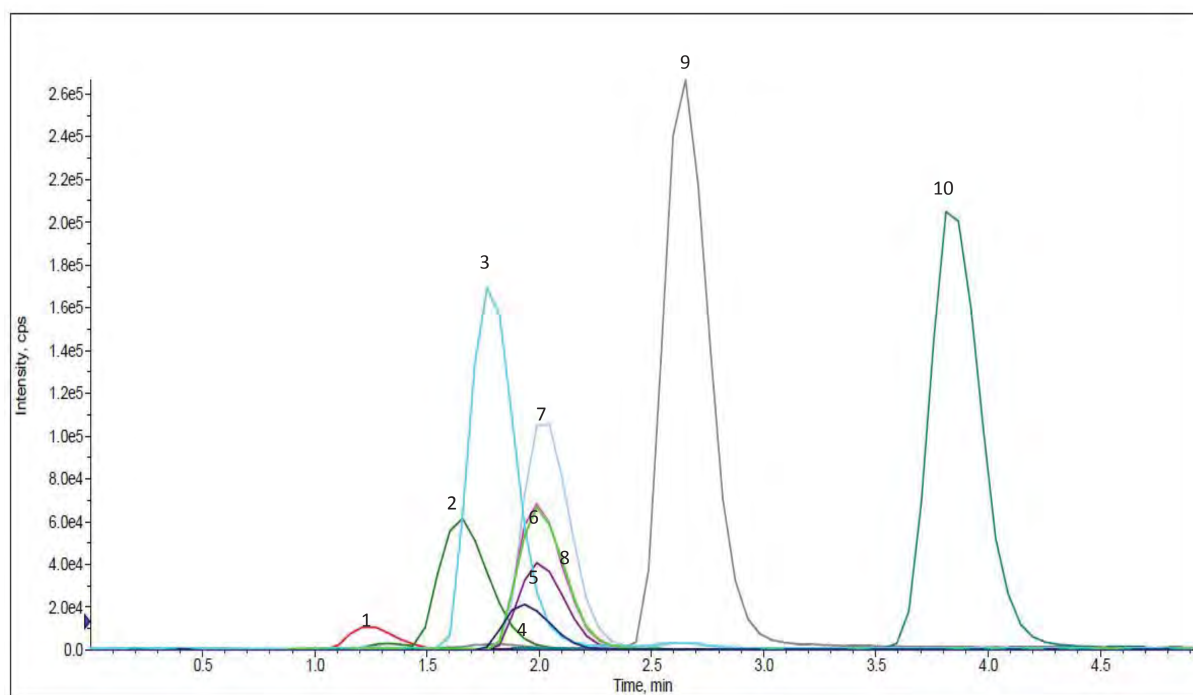
5. **ELUTE ANABOLIC STEROIDS (Choose a or b):**
   a. 1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2); Collect eluate at 1 to 2 mL/minute.
   NOTE: Prepare elution solvent daily. Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).
   b. 1 x 3 mL CH_2Cl_2/IPA (80:20).

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 50 µL of Ethyl Acetate and
     50 µL MSTFA (with 1%TMCS)
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1 -2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Cortisone	363.2	121.1	1.24
2. 11-Deoxycortisone	347.1	97.1	1.65
3. Boldenone	287.0	121.0	1.78
4. 17-OH Progesterone D_8	339.5	100.1	1.93
5. 17-OH Progesterone	331.3	97.1	1.99
6. Testosterone-D_3	292.0	97.1	2.00
7. Testosterone	289.3	97.1	2.02
8. Nandralone	275.0	109.0	2.00
9. Androstendione	287.3	97.1	2.64
10. Progesterone	315.3	97.1	3.84

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.35mL/minute

Polarity:Positive

Reconstitute: 100µl

Injection Volume: 10µl

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

LC Column: Selectra® DA HPLC Column 50 x 2.1mm 3µm

Isocratic:

Time	%A	%B
0.00	20	80
5.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion
Testosterone-TMS	432	301	209
19-Noretiocholanone-TMS	405	315	225
Oxymethalone	640	52	462
Dehydroepiandosterone-2TMS	432	327	297
10-Nortestosterone-2TMS	418	287	194
Oxymethalone Metabolite #1	640	52	462
Oxymethalone Metabolite #2	625	462	370
11-ß-Hydroxyandosterone	522	417	158
Methandienone	409	313	281
19-Norandosterone-2TMS	405	315	225
Alpha-Hydroxyetiocholanone	504	417	-
17-α-Epitestosterone-TMS	432	341	327
Stanazolol	472	381	342

Hair

CPS analitica
for Chemistry

BENZODIAZEPINES IN HAIR BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

CSDAU206 – CLEAN SCREEN® DAU 200 mg, 6 mL Tube

SMTBSTFA-1-1 – SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS

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

or

SLPFPP100ID21-5UM – Selectra® PFPP HPLC Column, 100 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**
   Add 10 -50 mg of decontaminated hair into a clean glass sample tube
   Add 3 mL of 0.1 M phosphate buffer and internal standards and mix
   Sonicate for 12 hours at room temperature
   Centrifuge for 10 minutes at 3000 rpm
   Transfer clear liquid to a clean glass sample tube

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 5% Acetonitrile in 100 mM phosphate buffer (pH 6.0.0).
   Dry column (5 minutes at full vacuum or pressure).

5. **ELUTE BENZODIAZEPINES:**
   1 x 3 mL Ethyl Acetate containing 2% NH_4OH
   collect eluate at 1 to 2 mL/minute.

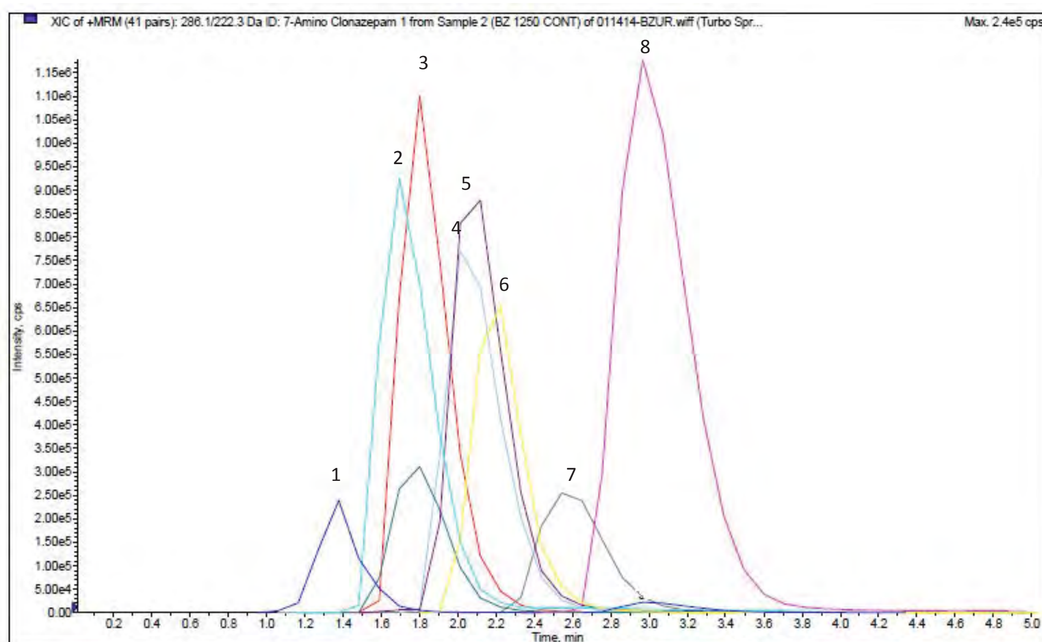
   NOTE: Prepare elution solvent daily.

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 50 µL of Acetonitrile and
     50 µL MTBSTFA w/ 1% TBDMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. 7-Amino Clonazepam	286.09	222.3	1.40
2. Oxazepam	287.09	241.3	1.70
3. Alpha- Hydroxy- Alprazolam	325.18	297.1	1.80
4. Clonazepam	316.13	270.2	2.10
5. Nordiazepam	271.09	140.1	2.10
6. Temazepam	301.12	255.2	2.20
7. Alprazolam	309.16	205.3	2.60
8. Diazepam	285.1	193.1	3.00

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.1 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

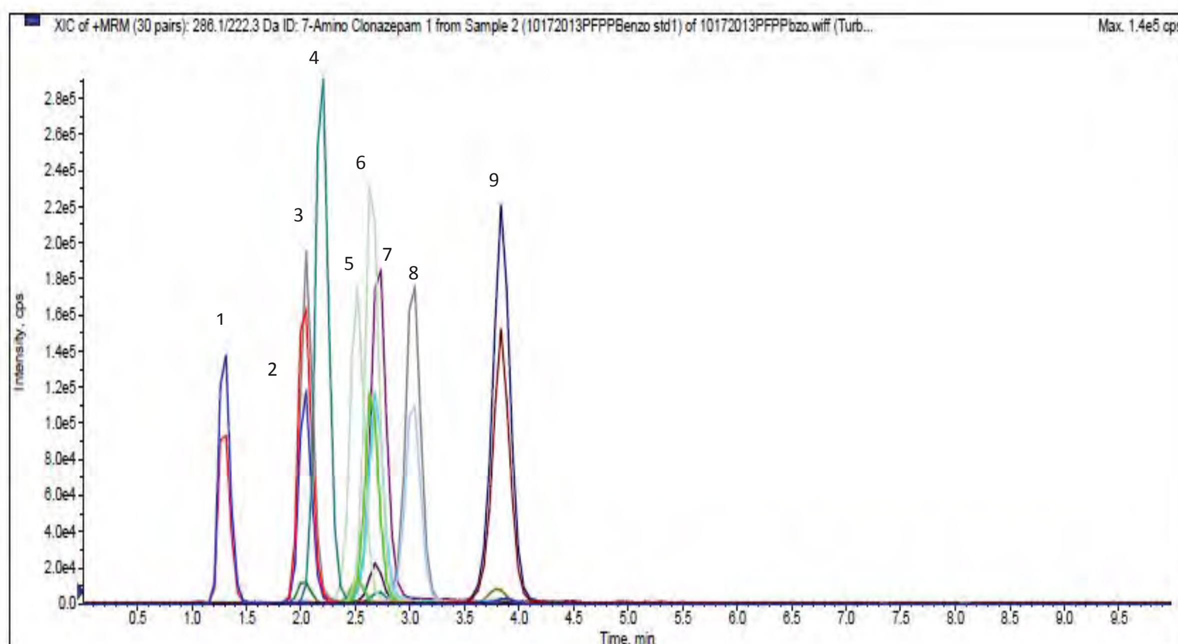
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Isocratic Flow:

Time	%A	%B
0.00	50	50
7.50	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. 7-Amino Clonazepam	**286.09**	**222.3**	**1.30**
2. Lorazepam	**321.06**	**303.3**	**2.04**
3. Alpha- Hydroxy- Alprazolam	**325.18**	**297.1**	**2.05**
4. Oxazepam	**287.09**	**241.3**	**2.19**
5. Clonazepam	**316.13**	**270.2**	**2.51**
6. Temazepam	**301.12**	**255.2**	**2.65**
7. Alprazolam	**309.16**	**205.3**	**2.71**
8. Nordiazepam	**271.09**	**140.1**	**3.03**
9. Diazepam	**285.1**	**193.1**	**3.84**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

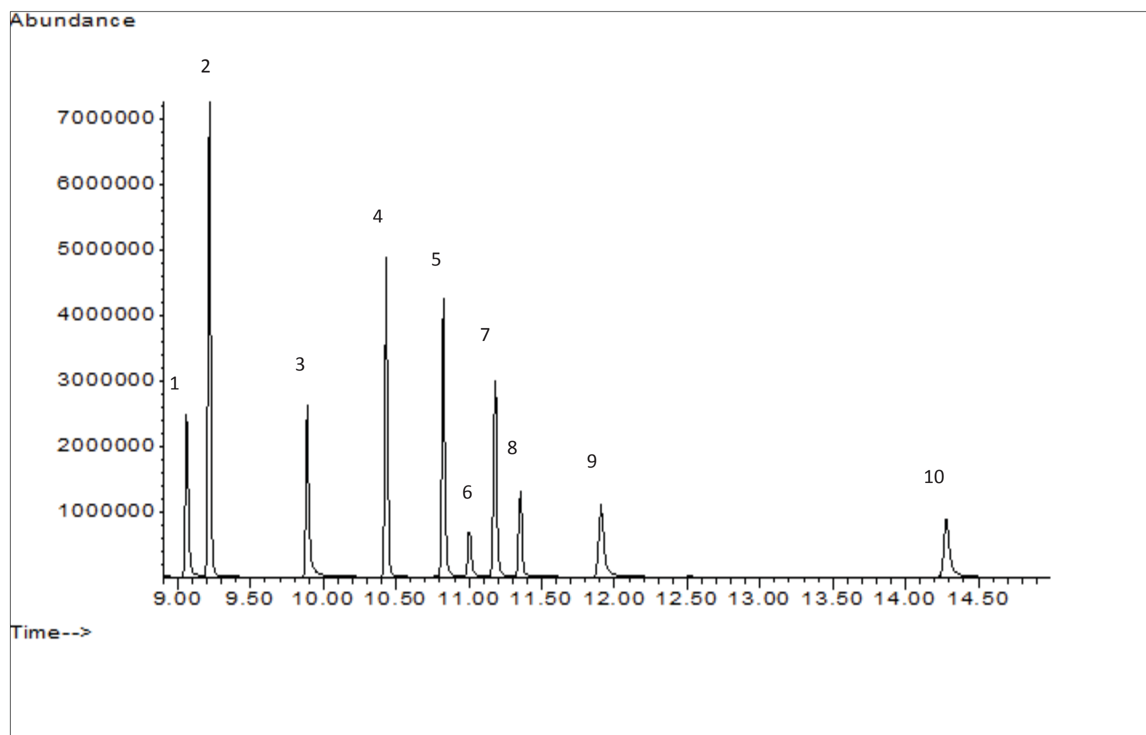
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic Flow:

Time	%A	%B
0.00	40	60
10.0	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



TBDMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Diazepam	256.0	283.0	221.0	9.06
2. Nordiazepam TBDMS	327.0	383.1	369.0	9.22
3. Midiazolam	310.0	325.0	297.0	9.89
4. Oxazepam 2TBDMS	457.1	513.2	383.1	10.43
5. Temazepam TBDMS	357.0	283.0	385.1	10.82
6. 7-Amino Clonazepam TBDMS	342.0	399.1	328.0	11.00
7. Lorazepam 2TBDMS	491.1	513.2	533.1	11.18
8. Clonazepam TBDMS	372.0	326.0	429.0	11.36
9. Alprazolam	279.0	204.0	308.0	11.91
10. Alpha-Hydroxy Alprazolam TBDMS	381.0	423.1	346.0	14.28

PARAMETERS

GC/MS: Agilent - 5975C XL / 6890N GC/MS System with 7683B ALS System

GC capillary column: Rxi-5sil MS 30 m x 0.25 mm, 0.25 µm

Injector: 1 µL Splitless 250 °C

Oven temperature program: 160 °C for 0.5 min; 15 °C/min to 310 °C for 4.50 minutes

Carrier gas: Helium

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

CARBOXY-THC IN HAIR BY LC-MS/MS OR GC-MS USING CLEAN SCREEN® THC EXTRACTION COLUMN

Part #

CSTHC206 – CLEAN SCREEN® THC 200 mg, 6 mL Tube

SMSTFA-1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS

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

1. **PREPARE SAMPLE:**
   Into a clean glass tube add approx 100 mg of decontaminated hair
   Add 1 mL of D.I. H_2O, add internal standard* and 100 µL of 10 M NaOH
   Digest at 70^{0}C for 12 hours
   Cool and adjust to pH 3

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 100 mm HCl
   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 CH_3CN /HCl (30:70)
   Dry column (10 minutes at full vacuum or pressure)

5. **ELUTE THC-COOH:**
   1 x 3 mL Hexanes/ Ethyl Acetate/ Acetic Acid (49:49:2)
   Collect eluate at 1 to 2 mL/minute

NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency

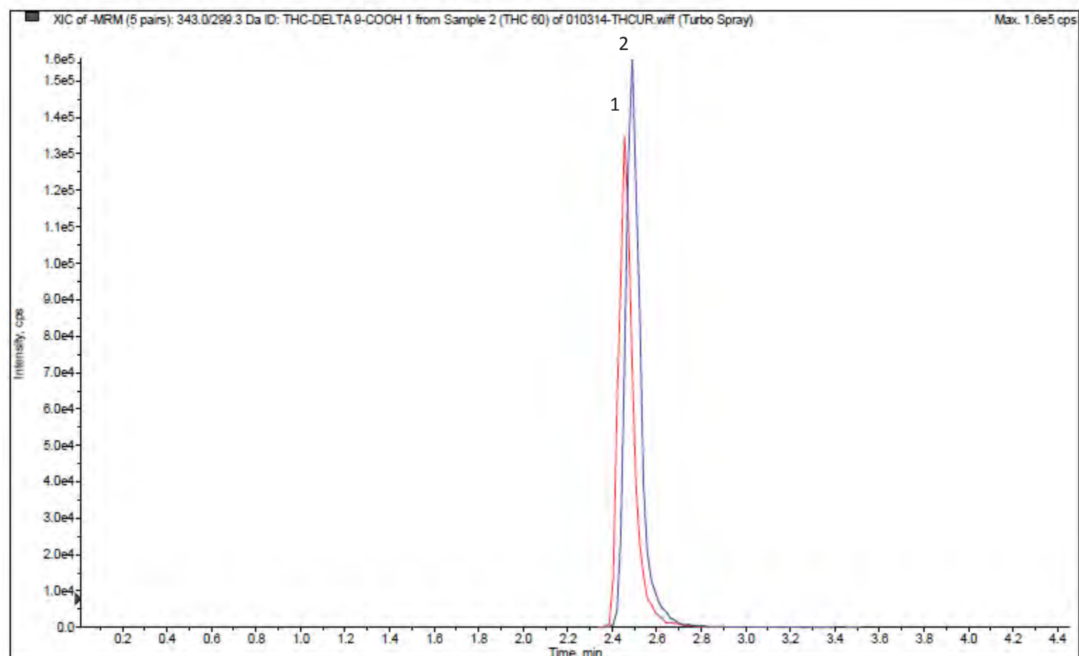
6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

7. **RECONSTITUTE:**

   Reconstitute sample in 100 µL of mobile phase
   Inject 20 µL.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. THC-DELTA 9-COOH D_9	352	308	2.44
2.THC-DELTA 9-COOH	343	299	2.49

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 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	60	40
2.00	30	70
2.50	10	90
2.51	60	40
4.00	STOP	

DETERMINATION OF GAMMA HYDROXYBUTYRATE (GHB) IN HAIR USING SOLID PHASE EXRACTION AND LC-MS/MS OR GC-MS CLEAN-UP® QAX EXTRACTION COLUMN

Part #
CUQAX156 – CLEAN-UP® QAX 500 mg, 6 mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

1. **PREPARE SAMPLE:**
   To a clean glass tube add 100 mg of decontaminated hair sample.
   Add 1 mL of CH_3OH and internal standard*, vortex mix
   Incubate at 40 °C for approx. 12 hours
   Centrifuge sample at 3000 rpm for 10 minutes
   Transfer organic phase to a clean glass tube
   Evaporate to dryness < 40 °C
   Dissolve residue in 3 mL of D.I. H_2O (pH 7)
   Vortex Mix

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 CH_3OH.
   Dry column (10 minutes at >10 inches Hg).

5. **ELUTE GHB:**
   2 x 3 mL 6% Glacial Acetic Acid/ 94% Methanol

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

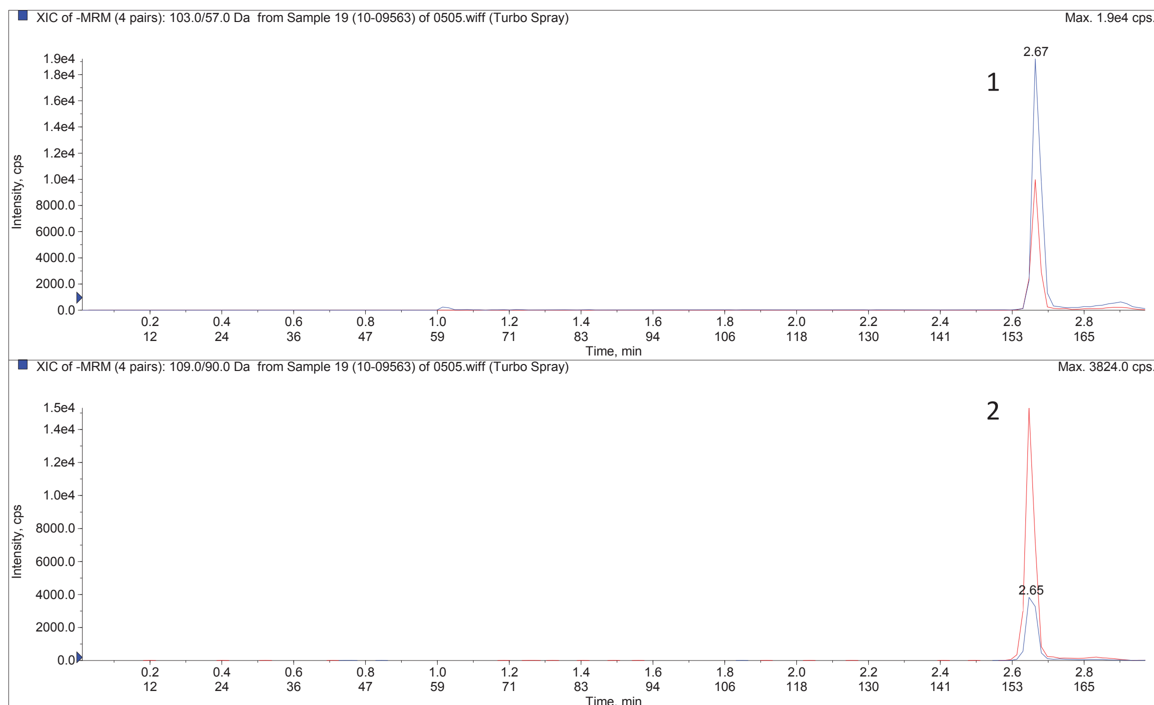
8. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL

   - **GC-MS:** **DERIVATIZE with TMS**
     Add 50 µL Ethyl Acetate and 50 µL BSTFA (with 1% TMCS)
     Overlay with N_2 and cap. Mix/vortex.
     React 30 minutes at 70 °C. Remove from heat source to cool.
     NOTE: Do not evaporate BSTFA solution

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. GHB	**103.02**	**84.9**	**2.67**
2. GHB-D_6	**109.13**	**90.0**	**2.65**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Flow Rate: 1.25 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

LC Column: Biphenyl HPLC Column 150 x 4.6 mm 5 µm

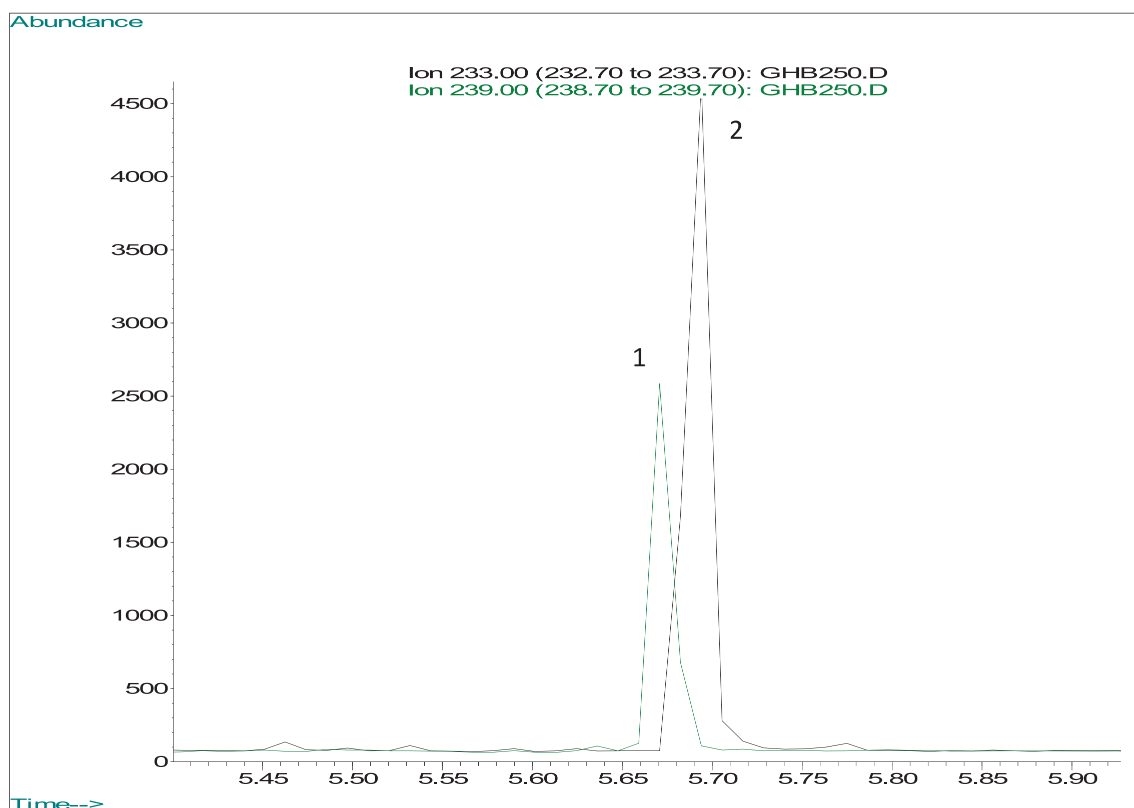
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Gradient:

Time	%A	%B
0.0	95	5
1.5	95	5
2.5	50	50
3.1	95	5
4.1	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



BSTFA-OXIME DERIVATIVES

Analyte	Quantify Ion	Qualifer Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. GHB-D_6	239	240		5.67
2. GHB	233	234	235	5.69

PARAMETERS

GC/MS: HP 5890 5972MSD GC/MS System with 7673 ALS System

GC capillary column: 30 m x0.25 mm (0.25 µm) RTX-5MS

Injector: 1 µL Splitless 250 °C

Oven temperature program: 70 °C for 1 min; 15 °C/min to 130 °C, then to 300 °C 50 °C/min. Hold for 0.1 min

Carrier gas: Helium

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

EtG/EtS IN HAIR BY LC-MS/MS USING 200 MG CLEAN SCREEN® ETG EXTRACTION COLUMN

Part #

CSETG203 – CLEAN SCREEN® ETG 300 mg, 3 mL Tube

1. **PREPARE SAMPLE:**

   Into a clean glass tube add approx. 50-100 mg of decontaminated hair.

   Add 1 mL of D.I. H_2O, add internal standards*

   Vortex mix

   Incubate at 40 ^{0}C for 12 hours

   Centrifuge as appropriate

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**

   1 x 3 mL CH_3OH containing 1% Formic Acid

   1 x 3 mL D.I. H_2O containing 1% Formic Acid

   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:

   Dry column (**10 minutes** at full vacuum or pressure)

5. **ELUTE EtG/EtS ANALYTES:**

   2 x 3 mL CH_3OH containing 1% Formic Acid

   Collect eluate at 1 to 2 mL/minute

6. **DRY ELUATE:**

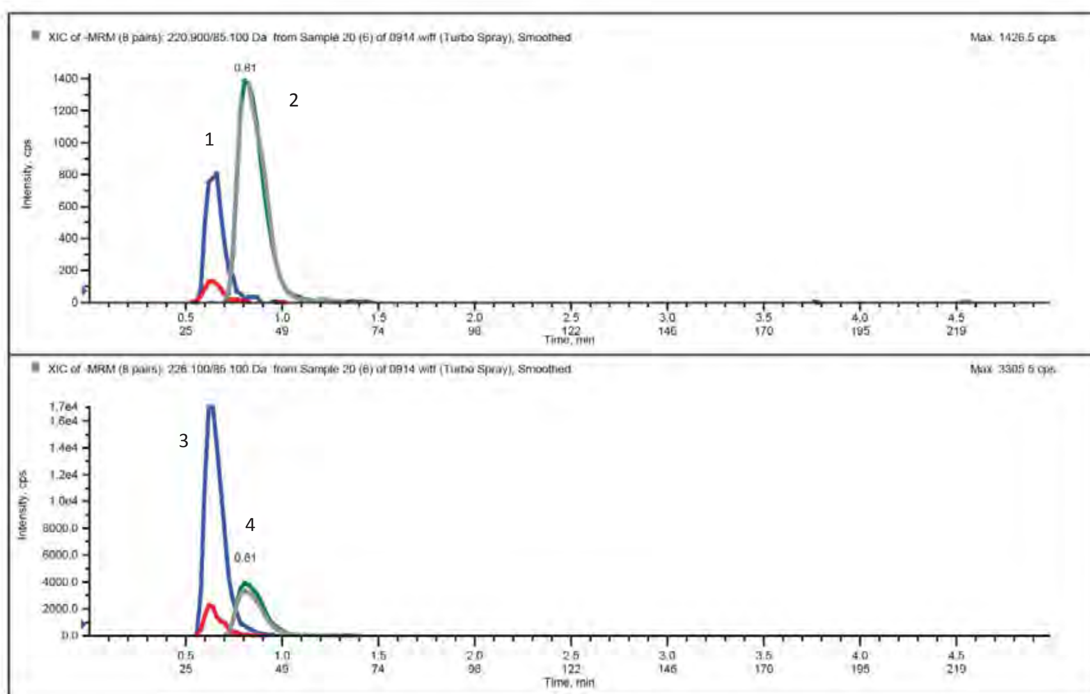
   Evaporate to dryness at < 40 °C

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 Acetonitrile

Flow Rate: 0.35 mL/minute

Polarity: Negative

Injection Volume: 20 µL

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

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

Isocratic:

Time	%A	%B
0.00	50	50
5.00	50	50

Oral Fluids

CPS analitica for Chemistry

AMPHETAMINES IN ORAL FLUID USING AN ORAL FLUID SAMPLING DEVICE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #
CSDAU – CLEAN SCREEN® DAU
PFAA-0-1 – SELECTRA-SIL® PFAA
SPFPOH-1 – SELECTRA-SIL® PFPOH
SHFAA-0-1 – SELECTRA-SIL® HFAA
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm
or
SLPFPP50ID21-5UM – Selectra® PFPP HPLC Column, 50 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**

   EMPLOY ORAL FLUID SAMPLING DEVICE ACCORDING TO MAKERS INSTRUCTIONS

   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard.*
   Add 1 mL of ORAL FLUID EXTRACT. Add 2 mL of 100 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.
   Mix/vortex.
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 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 Acetic Acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE SMA's:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

6. **DRY ELUATE:**
   Add 100uL of 1% HCl in Methanol to each test tube
   Evaporate to dryness at < 40 °C

7. **RECONSTITUTE / DERIVATIZE:**
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.

- **GC-MS**: Fluoroacylate with PFPA (PFAA)
  Add 50 µL PFPA. Over lay with N_2 and cap
  *Improved derivatization by addition of PFPOH
  React 20 minutes at 70 °C. Evaporate to dryness <40 °C
  Reconstitute with 100 µL Ethyl Acetate

Alternate Derivatization

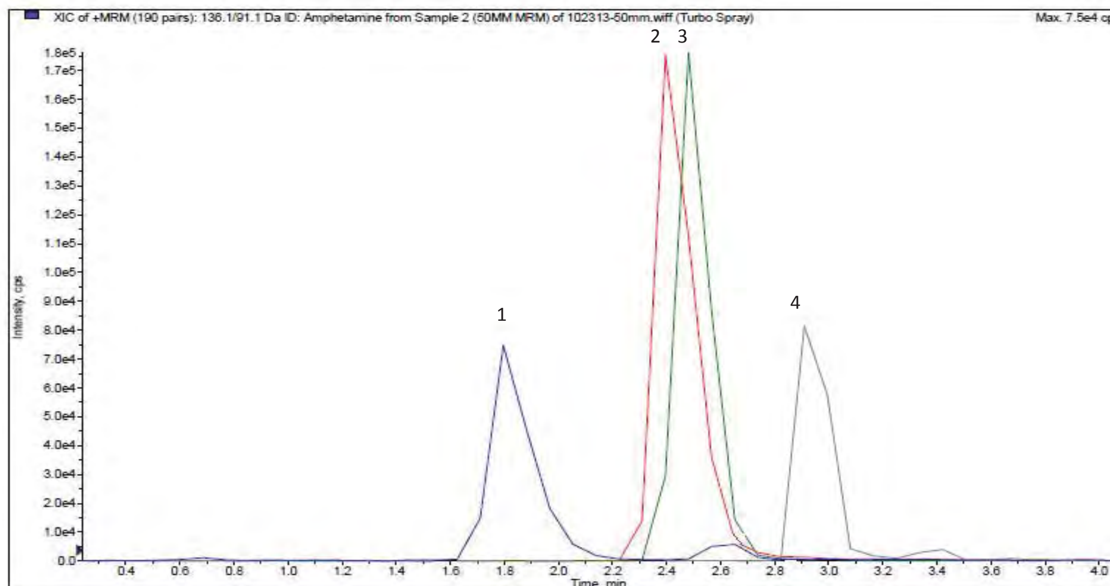
1. Fluoroacylate with HFPA (HFAA)
   Add 50 µL HFPA. Over lay with N_2 and cap
   Improved derivatization by addition of PFPOH
   React 20 minutes at 70 °C. Evaporate to dryness <40 °C
   Reconstitute with 100 µL Ethyl Acetate

2. Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate;
   React 45 minutes at 70 °C

Note: Sodium periodate can be added to sample during preparation if oxidation is preferred.
To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard(s). Add 1 mL of oral fluid extract and 1 mL 0.35 M sodium periodate.
Mix/vortex
Incubate at room temp.for 20 min.
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
Sample is now ready to be added to the extraction column

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	136.1	91.1	1.18
2. Methamphetamine	150.1	91.1	2.40
3. MDA	180.1	105.0	2.45
4. MDMA	194.1	105.1	2.95

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 20 µL

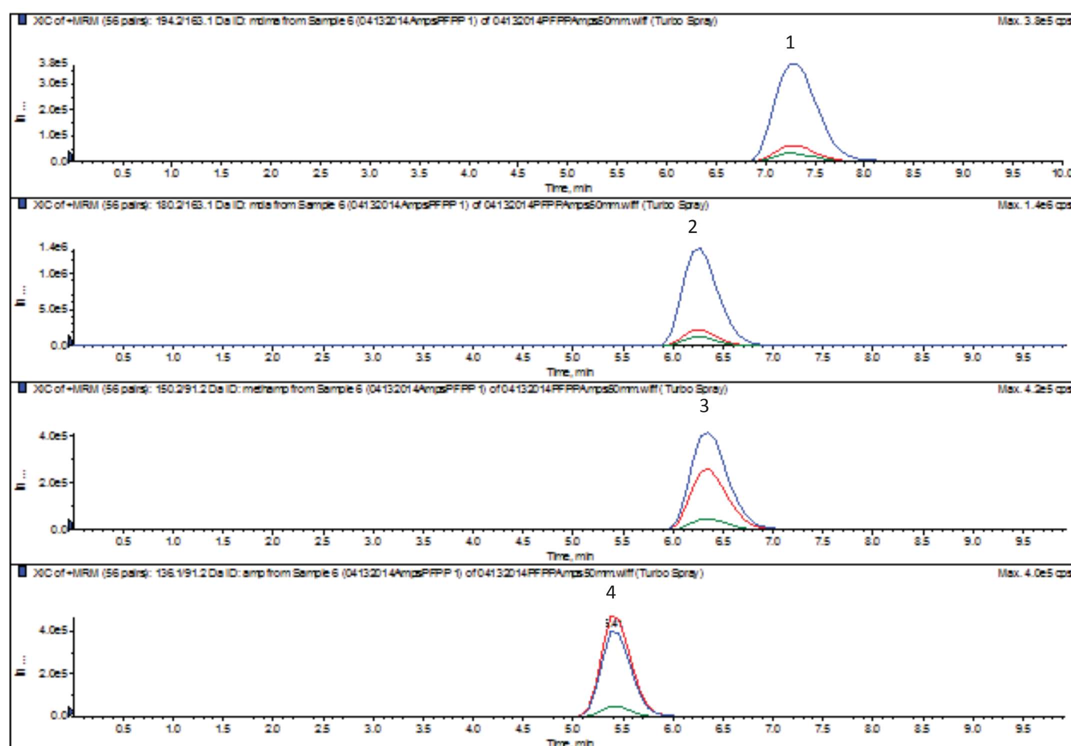
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Gradient:

Time	%A	%B
0.0	80	20
0.5	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	**136.1**	**91.2**	**5.41**
2. MDA	**180.2**	**163.1**	**6.24**
3. Methamphetamine	**150.1**	**91.2**	**6.35**
4. MDMA	**194.2**	**163.1**	**7.29**

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® PFPP HPLC Column 50 x 2.1 mm 5 µm

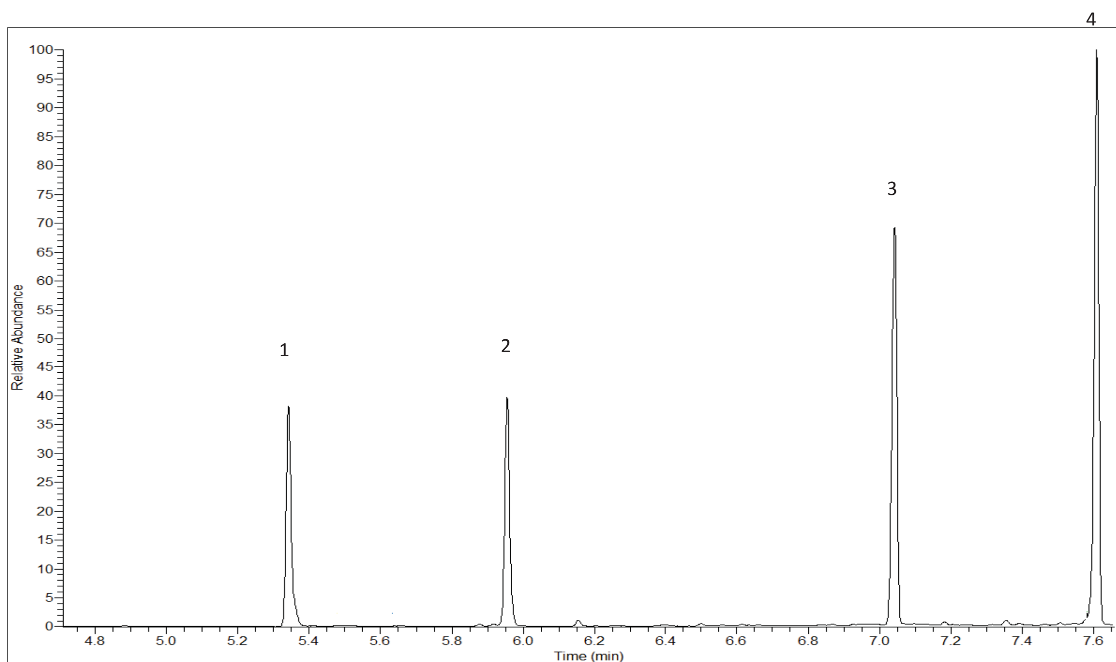
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	30	70
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

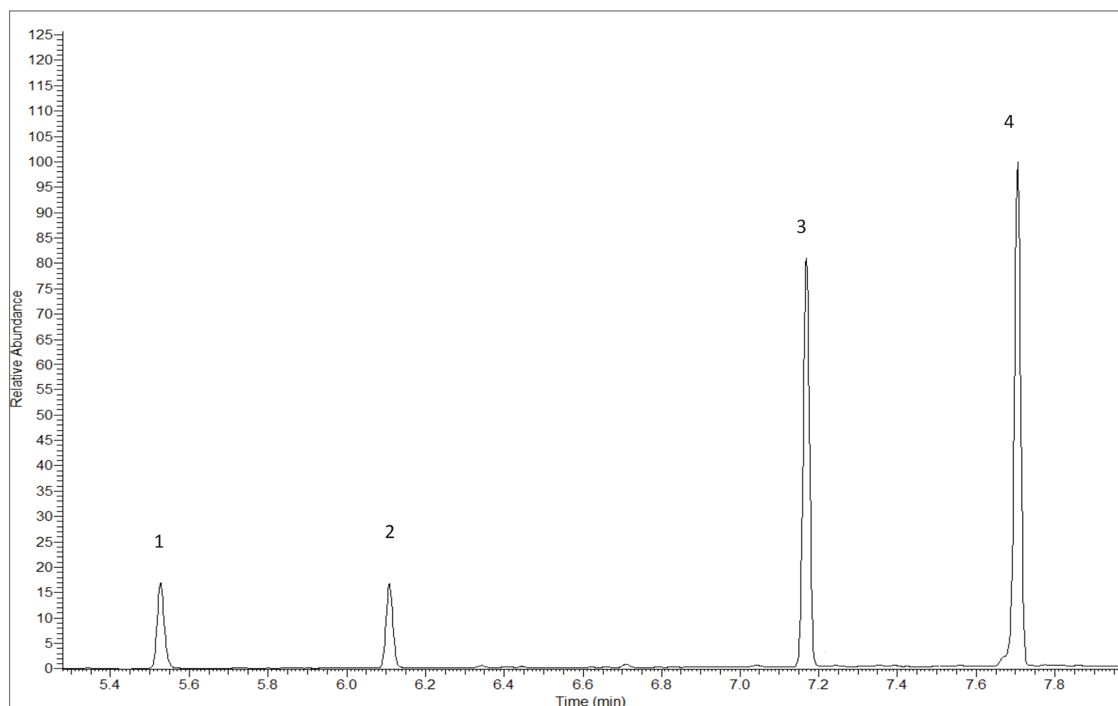
CHROMATOGRAM



Fluoroacylate with PFPA (PFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Amphetamine	190	118	91	5.34
2. Methamphetamine	204	160	118	5.95
3. MDA	162	325	135	7.61
4. MDMA	162	204	135	7.04

CHROMATOGRAM



Fluoroacylate with HFPA (HFAA) ions

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Amphetamine	240	91	118	5.53
2. Methamphetamine	254	210	118	6.11
3. MDA	375	162	135	7.17
4. MDMA	254	210	162	7.71

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: 70 °C (0.5) to 320 °C (25 °C/ minute): hold (2 minutes)

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

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

COCAINE AND BENZOYLECGONINE IN ORAL FLUID BY LC-MS/MS OR GC-MS 50 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube

SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

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

1. **PREPARE SAMPLE:**
   Add 100-500 µL of neat oral fluid sample to a clean test tube
   Add internal standard(s)* and let sit for 10 minutes at room temperature.
   Add 800 µL of 100mM phosphate buffer (pH 6.0)
   Mix/vortex for 10 seconds. Sample pH should be 6.0 ± 0.5
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 200 µL CH_3OH.
   1 x 200 µL D.I. H_2O.
   1 x 200 µL 100 mM HCl.
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**
   Do not exceed 1 mL/minute.

4. **WASH COLUMN:**
   1 x 500 µL D.I. H_2O.
   1 x 500 µL 100 mM HCl
   1 x 500 µL CH_3OH/D.I.H_2O (50:50)
   Dry column (5 minutes at full vacuum or pressure).

5. **ELUTE COCAINE/METABOLITES:**
   1 x 800 µL CH_2Cl_2/ IPA/ NH_4OH (70:26:4)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

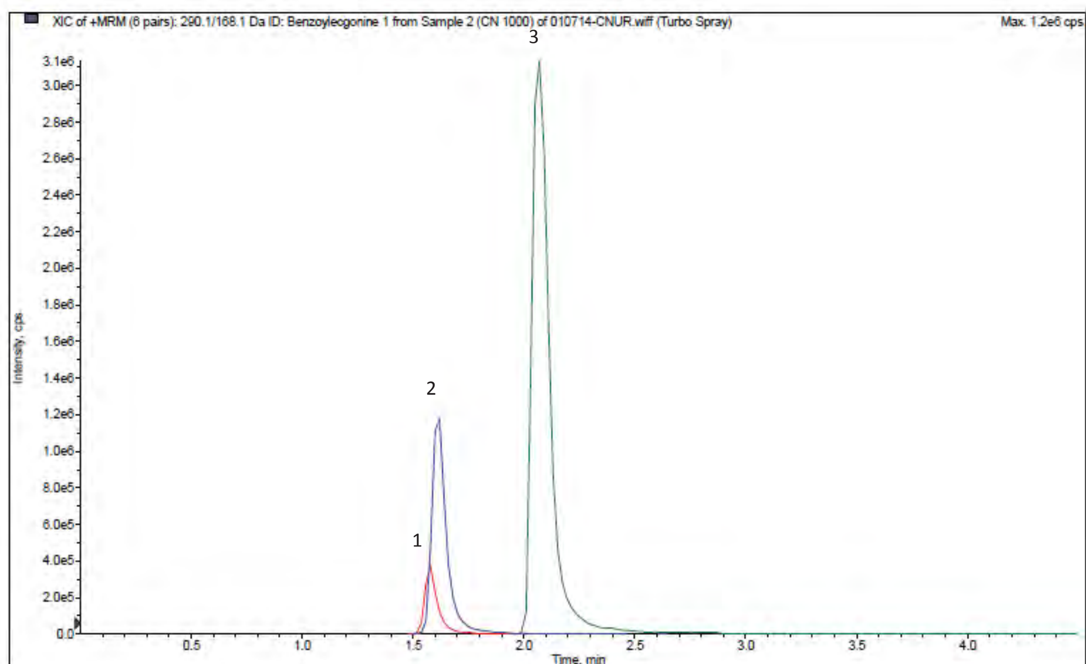
6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

7. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Benzolyecgonine D_8	**298.1**	**171.1**	**1.58**
2. Benzolyecgonine	**290.1**	**168.1**	**1.60**
3. Cocaine	**304.1**	**182.1**	**2.10**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

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

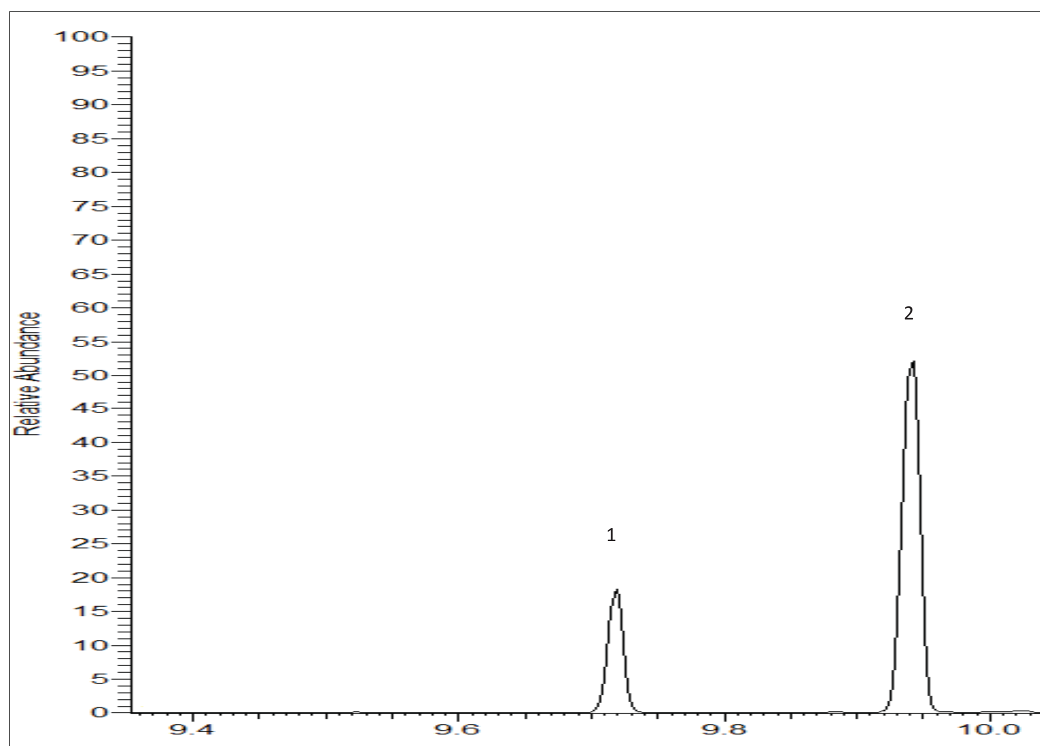
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	75	25
3.00	50	50
3.01	10	90
4.00	75	25
5.50	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1.Cocaine	**182**	**198**	**303**	**9.72**
Cocaine D_3	**185**	**201**	**306**	**-**
2.Benzolyecgonine TMS	**240**	**256**	**361**	**9.94**
Benzolyecgonine TMS D_3	**243**	**259**	**369**	**-**

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: 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 ºC

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

Part #

CSDAU – CLEAN SCREEN® DAU

SLDA100ID21-5UM – Selectra® 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® 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 1% acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE FENTANYL/NORFENTANYL:**
   1 x 3 mL Ethyl Acetate/ Acetonitrile / NH_4OH (78:20:2v/v)
   Collect eluate at 1 to 2 mL/minute

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

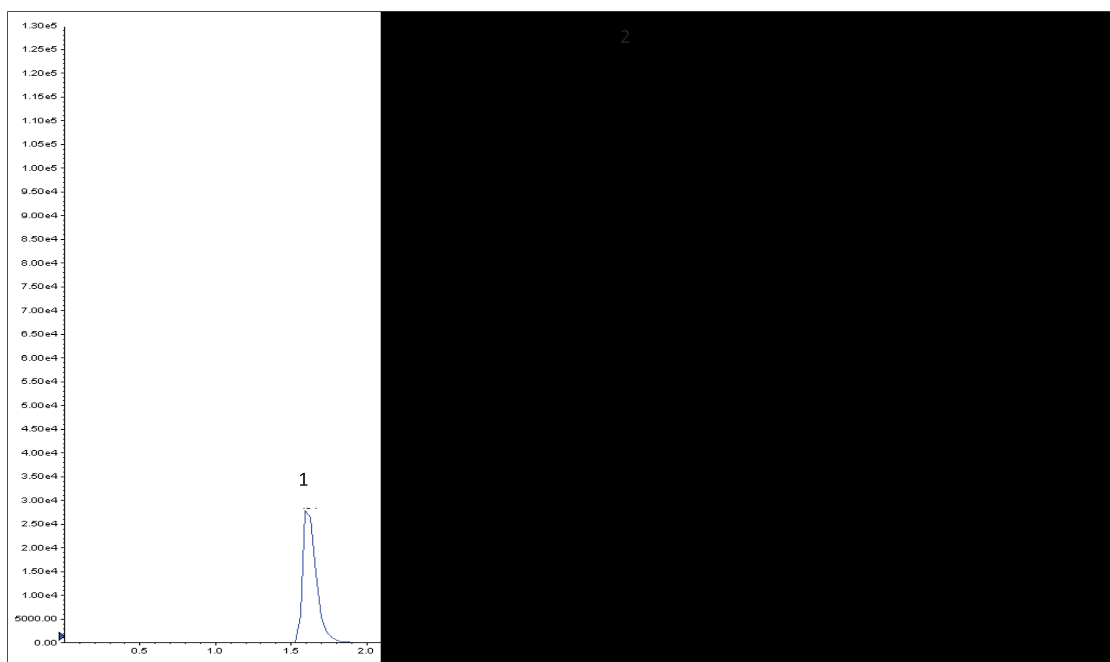
6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

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_5	238.3	84.1	-
2. Fentanyl	337.2	188.3	3.88
Fentanyl D_5	342.2	188.2	-

PARAMETERS

Mobile Phase A: 0.2% Formic Acid in D.I. H_2O

Mobile Phase B: 02% Formic Acid in Acetonitrile

Flow Rate: 0.6 mL/min

Polarity: Positive

Injection Volume: 10 µL

LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

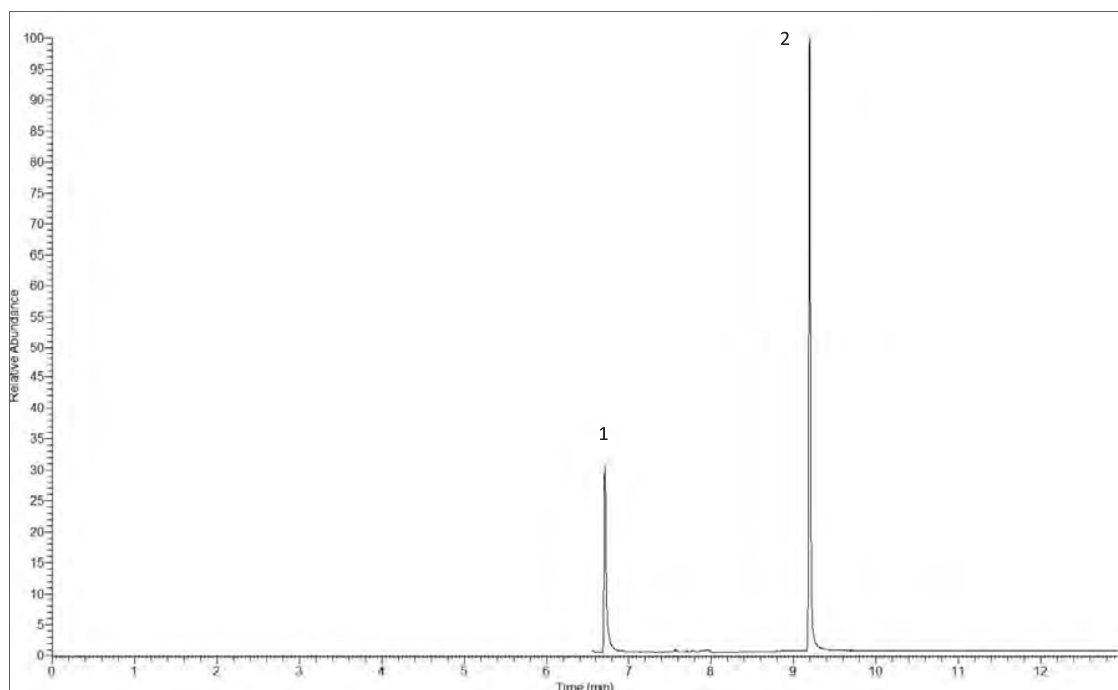
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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_5	98	164	180	6.69
2. Fentanyl	245	146	189	9.20
Fentanyl D_5	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 ºC, MS Source: 350 ºC, MS Quad: 150 ºC

THC AND THC-COOH FROM ORAL FLUID BY LC-MS/MS OR GC-MS STYRE-SCREEN® POLYMERIC QAX EXTRACTION COLUMN

Part #
SSQAX056 – Styre Screen® QAX 50 mg, 6 mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SMSTFA1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS
SLDA100ID21-5UM – Selectra® DA HPLC Column, 100 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**
   To 1 mL of oral fluid specimen (diluted in Quantisal buffer), add appropriate internal standards
   Mix/vortex for 30 seconds

3. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute.
   Dry column (2 minutes at full vacuum or pressure)

4. **WASH COLUMN:**
   1 x 750µL of 85:15:1 D.I. H_2O: ACN: NH_4OH
   Dry Column at full vacuum or pressure for 10 minutes

5. **ELUTE ANALYTES:**
   1 x 750 µL Hexane/: Ethyl Acetate/ Glacial Acetic Acid (90:10:2)
   Collect eluate at 1 to 2 mL/minute

NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency

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

7. **RECONSTITUTE / DERIVATIZE:**

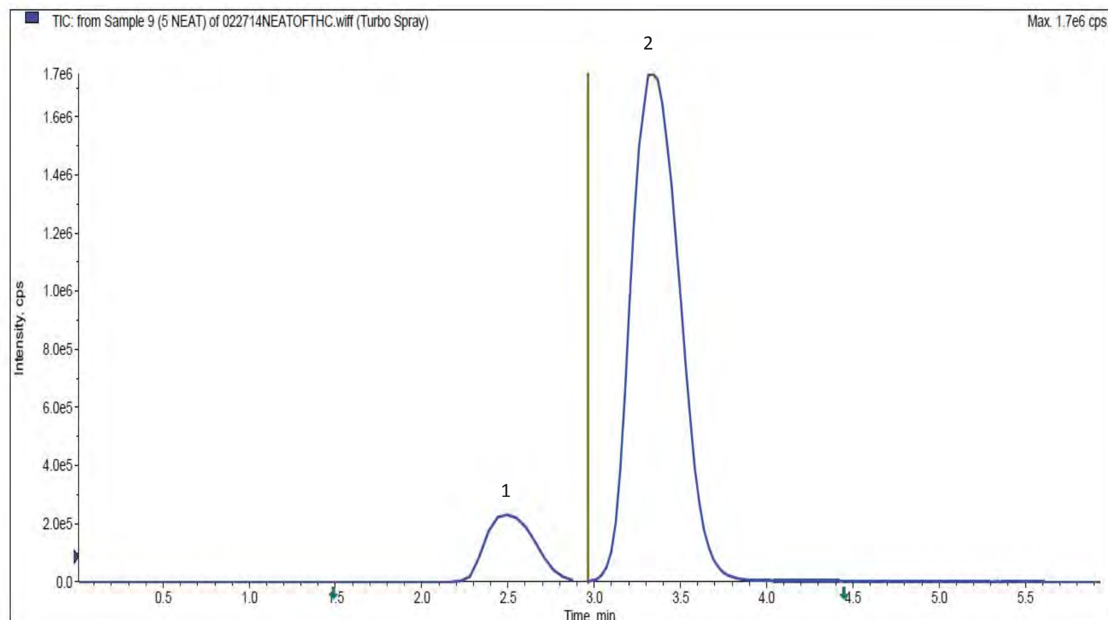
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70 °C; Cool and inject 1 µL

Alternate Derivatization

- Form TMS Derivatives by adding 50 µL MSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate; React 45 minutes at 70 °C

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
THC-DELTA 9-COOH D_9	352	254	2.50
1. THC-DELTA 9-COOH	343	299	2.52
DELTA 9-THC D_3	318	196	3.34
2. DELTA 9-THC	315	193	3.37

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.4 mL/minute

Polarity: Negative MRM (-): 2.995
Positive MRM (+): 3.018

Reconstitute: 100 µL

Injection Volume: 20 µL

LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

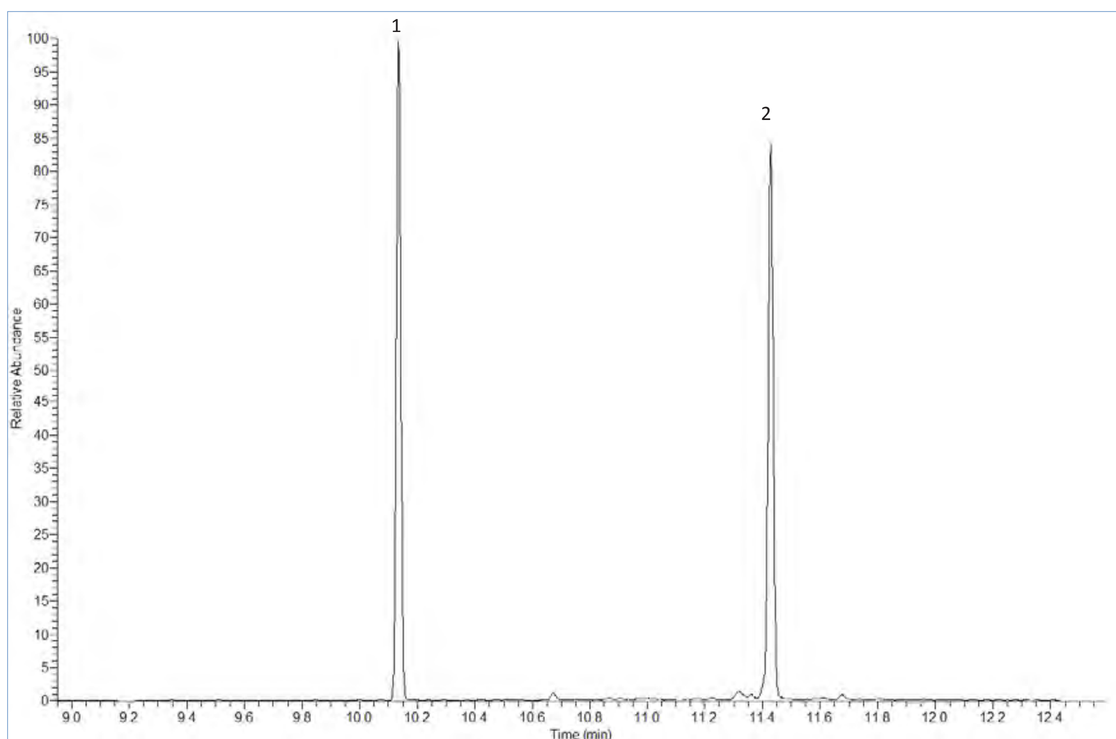
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Gradient:

Time	%A	%B
0.00	20	80
7.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



MSTFA/BSTFA TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
DELTA 9 THC-D_3	374	346	389	-
1. DELTA 9 THC	371	343	386	10.14
THC-COOH D_3	374	476	491	-
2. THC-COOH	371	473	488	11.43

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: 70 °C (0.5) to 320 °C (25 °C/ minute): hold (2 minutes)

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

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

THC AND THC-COOH FROM ORAL FLUIDS BY LC-MS/MS OR GC-MS USING CLEAN SCREEN XCEL® II COLUMN

Part #:
CSXCE2106 – CLEAN SCREEN XCEL® II 130 mg, 6 mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
SMSTFA-1-1– SELECTRA-SIL® MSTFA w/ 1% TMCS
SLDA100ID21-5UM – Selectra® DA HPLC Column, 100 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**
   To 1 mL of oral fluid specimen (diluted in Quantisal™ buffer), add appropriate internal standards
   Mix/vortex for 30 seconds

2. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute.
   Dry column (2 minutes at full vacuum or pressure)

3. **WASH COLUMN:**
   1 x 750 µL of 85:15:1 Di H_2O: Acetonitrile: NH_4OH
   Dry Column at full vacuum or pressure for 10 minutes

4. **ELUTE ANALYTES:**
   1 x 750 µL Hexane/ Ethyl Acetate/ Glacial Acetic Acid (90:10:2)
   Collect eluate at 1 to 2 mL/minute

NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency

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

6. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.

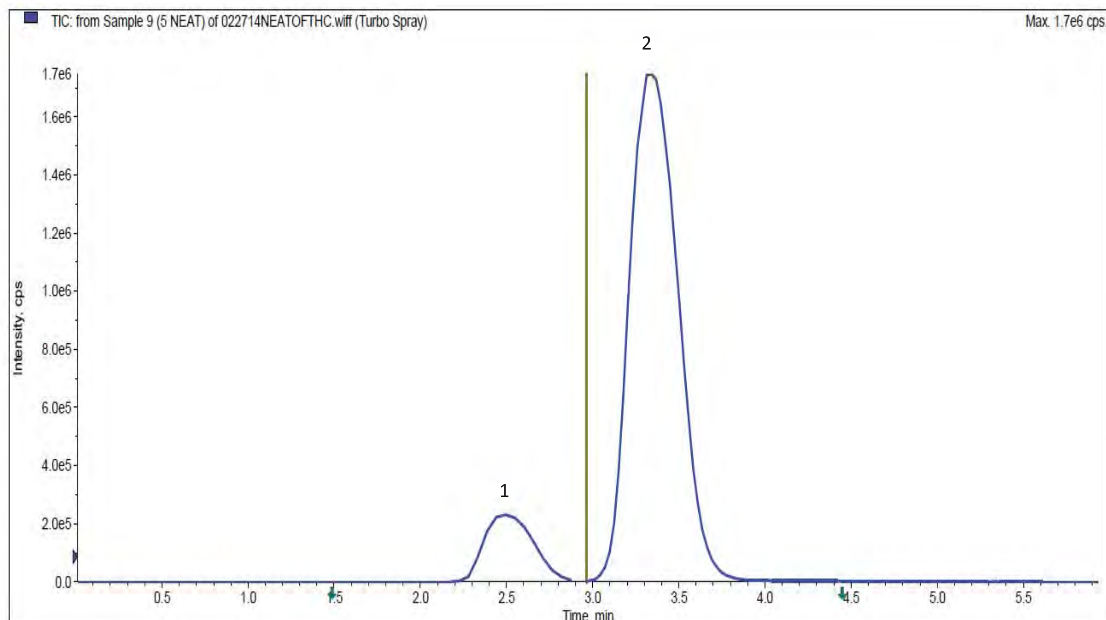
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70°C; Cool and inject 1 µL

Alternate Derivatization

1. Form TMS Derivatives by adding 50 µL MSTFA WITH 1% TMCS and 50 µL of Ethyl Acetate; React 45 minutes at 70 °C

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
THC-DELTA 9-COOH D_9	352	254	2.50
1. THC-DELTA 9-COOH	343	299	2.52
DELTA 9-THC D_3	318	196	3.34
2. DELTA 9-THC	315	193	3.37

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.4 mL/minute

Polarity: Negative MRM (-): 2.995
Positive MRM (+): 3.018

Reconstitute: 100 µL

Injection Volume: 20 µL

LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm

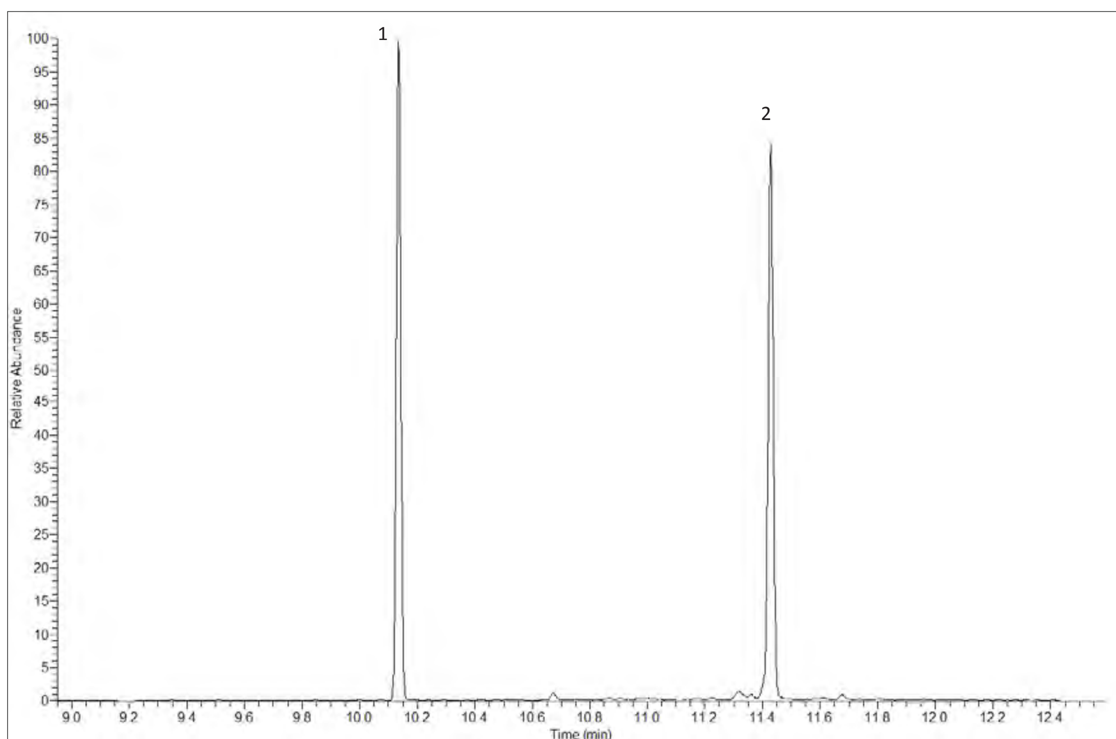
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Gradient:

Time	%A	%B
0.00	20	80
7.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



MSTFA/BSTFA TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
DELTA 9 THC-D_3	374	346	389	-
1. DELTA 9 THC	371	343	386	10.14
THC-COOH D_3	374	476	491	-
2. THC-COOH	371	473	488	11.43

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: 70 °C (0.5) to 320 °C (25 °C/ minute): hold (2 minutes)

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

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

Quantisal™ is a trademark of Immunalysis Corporation

THC-COOH FROM ORAL FLUIDS BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

ZSTHC020 - CLEAN SCREEN® THC 200 mg, 10 mL Tube

or

CSDAU206 - CLEAN SCREEN® THC 200 mg, 6 mL Tube

SMSTFA-1-1 – SELECTRA-SIL® MSTFA w/ 1% TMCS

SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

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

1. **PREPARE SAMPLE:**
   To 1 mL of oral fluid specimen 50ng/mL internal standard (THCA-D9) and let sit for ten minutes at room temperature
   Mix/vortex for 10 seconds
   Add 0.5 mL of Glacial Acetic Acid and vortex for 10 seconds

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 1 mL 0.1N HCl.
   NOTE: Aspirate at full vacuum or pressure

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

4. **WASH COLUMN:**
   1 x 2 mL D.I. H_2O
   1 x 2 mL 0.1 M HCl: Acetonitrile (70:30)
   Dry column (10 minutes at full vacuum or pressure)
   1 x 200 µL Hexane
   Aspirate at full vacuum or pressure (Additional step to remove any residual moisture)

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

NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease MSTFA derivatizing efficiency

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

7. **RECONSTITUTE / DERIVATIZE:**

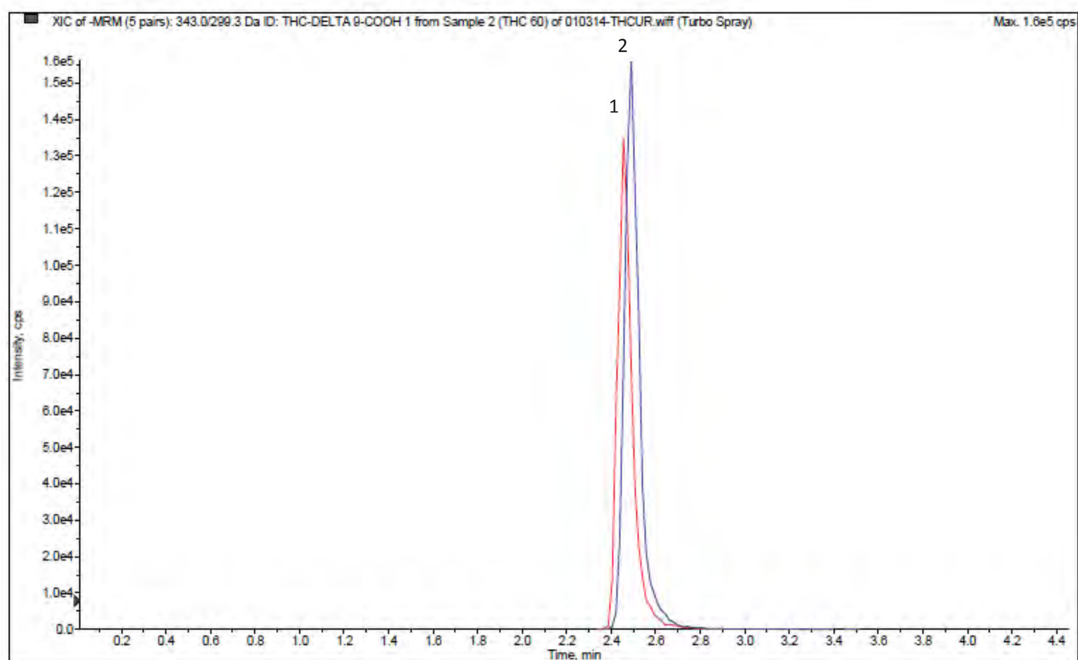
   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL MSTFA w/ 1% TMCS
     Overlay with N_2 and cap. Mix/vortex
     React 30 minutes at 70°C; Cool and inject 1 µL

Alternate Derivatization

1. Form TMS Derivatives by adding 50 µL BSTFA w/ 1% TMCS and 50 µL of Ethyl Acetate; React 45 minutes at 70 °C

Contributed by:
Janet Putnam, Assistant Laboratory Director/RP Advanced Toxicology Network, Memphis, TN

INSTRUMENT CONDITIONS (LC-MS/MS):



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. THC-DELTA 9-COOH D_9	**352**	**308**	**2.44**
2. THC-DELTA 9-COOH	**343**	**299**	**2.49**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

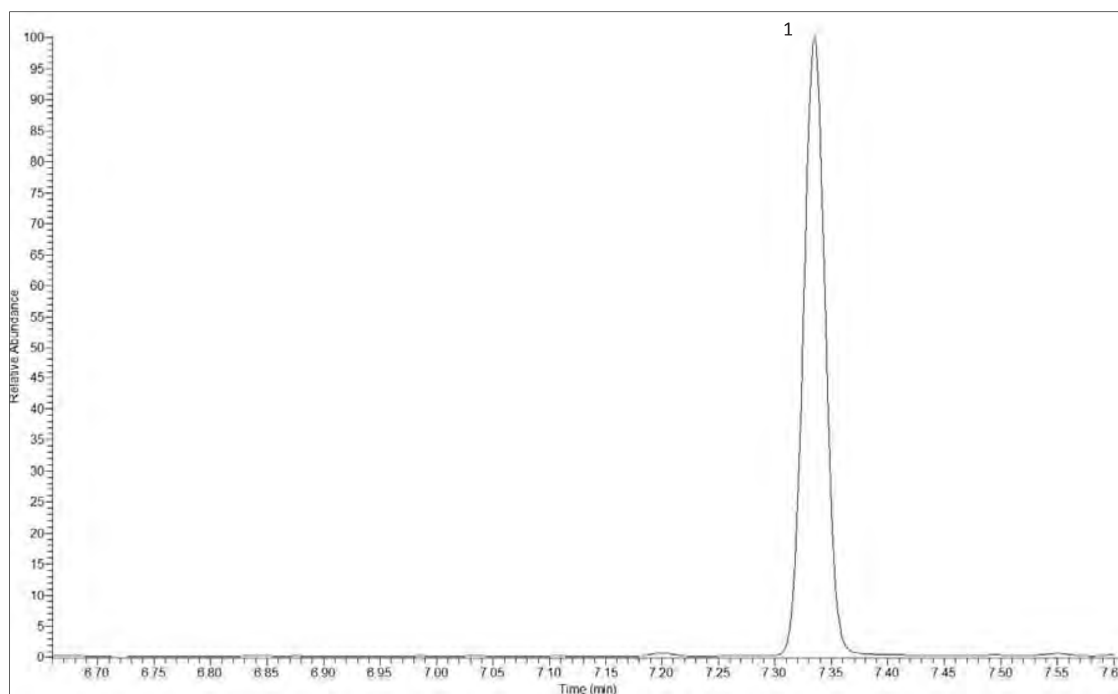
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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

Gradient:

Time	%A	%B
0.00	60	40
2.00	30	70
2.50	10	90
2.51	60	40
4.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):



<u>MSTFA/BSTFA TMS IONS</u>

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. THC-COOH	371	473	488	7.34
THC-COOH D_3	374	476	491	7.31

<u>PARAMETERS</u>

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: 170 °C (1) to 310 °C (30 °C/ minute): hold (5 minutes)

Carrier gas: Helium (1.2 mL/ minute)

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

UCT

FASt

CPS analitica for Chemistry

BASIC DRUGS IN URINE CLEAN SCREEN FASt® COLUMN

Part #:

CSFAS203 – CLEAN SCREEN FASt® 200 mg, 3 mL Tube

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

1. **PREPARE SAMPLE:**
   To 2 mL of urine add internal standard
   Adjust pH to 6

2. **LOAD SAMPLE and SAMPLE DILUTE RATIO:**
   Sample Dilution Ratio: Sample Volume* : Diluent** Volume

   NOTE: *If sample is hydrolyzed add appropriate aliquot volume after hydrolysis is complete.

Dilution Ratio	Urine	Diluent**
1:1	500 uL	500 uL
1:4	200 uL	800 uL
1:9	100 uL	900 uL

** Diluent is 50:50 (Methanol: D.I. H_2O)

Sample and diluents are added in an appropriately labeled tube.

Add appropriate volume internal standard(s). It is recommended to use an internal standard volume of no more than 200 uL.

3. **EXTRACTION and COLLECTION:**
   Set up extraction manifold with FASt cartridges and auto-sampler collection vials.

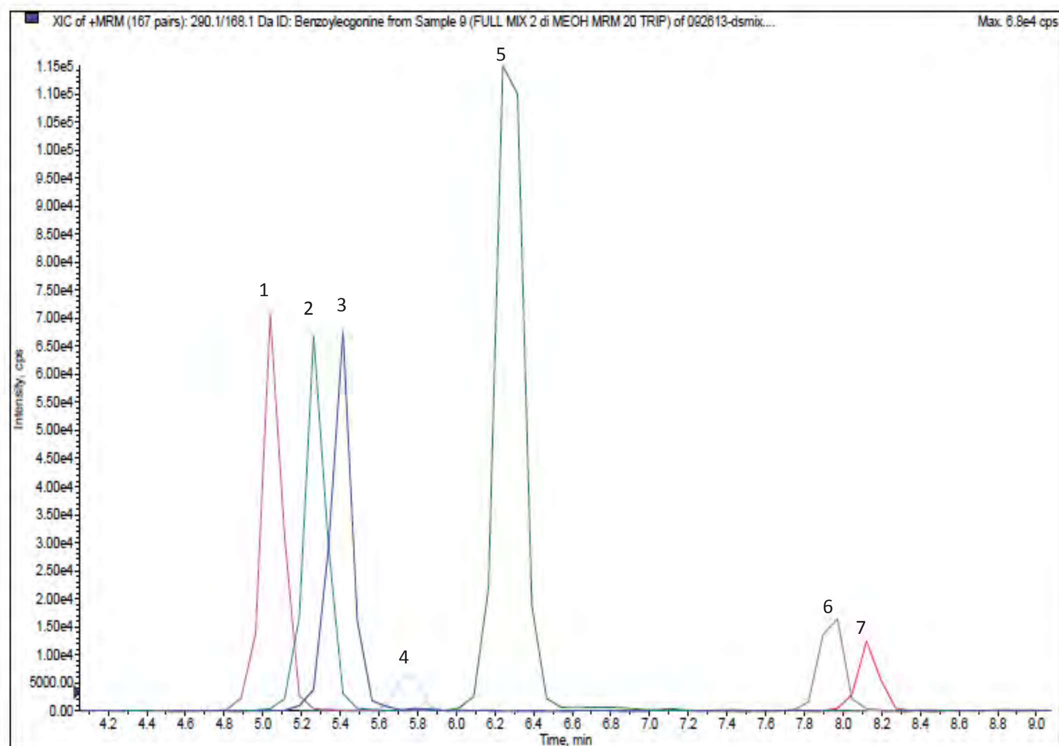
   Pour sample into FAST cartridge and elute sample directly into auto-sampler vials.

4. **ANALYSIS:**
   Cap vials and put directly onto LC/MS for analysis.

INSTRUMENT CONDITIONS (LC-MS/MS):

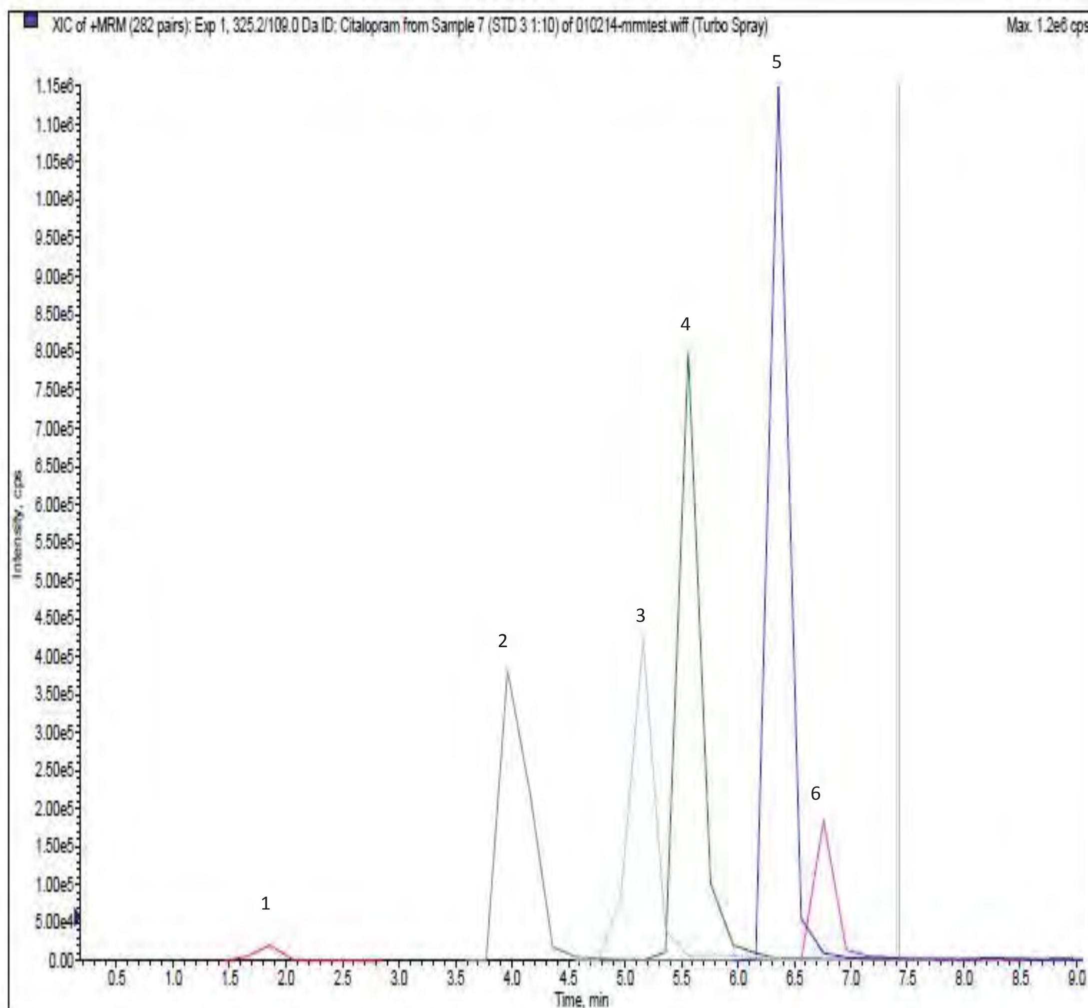
CHROMATOGRAMS

Basic Panel 1



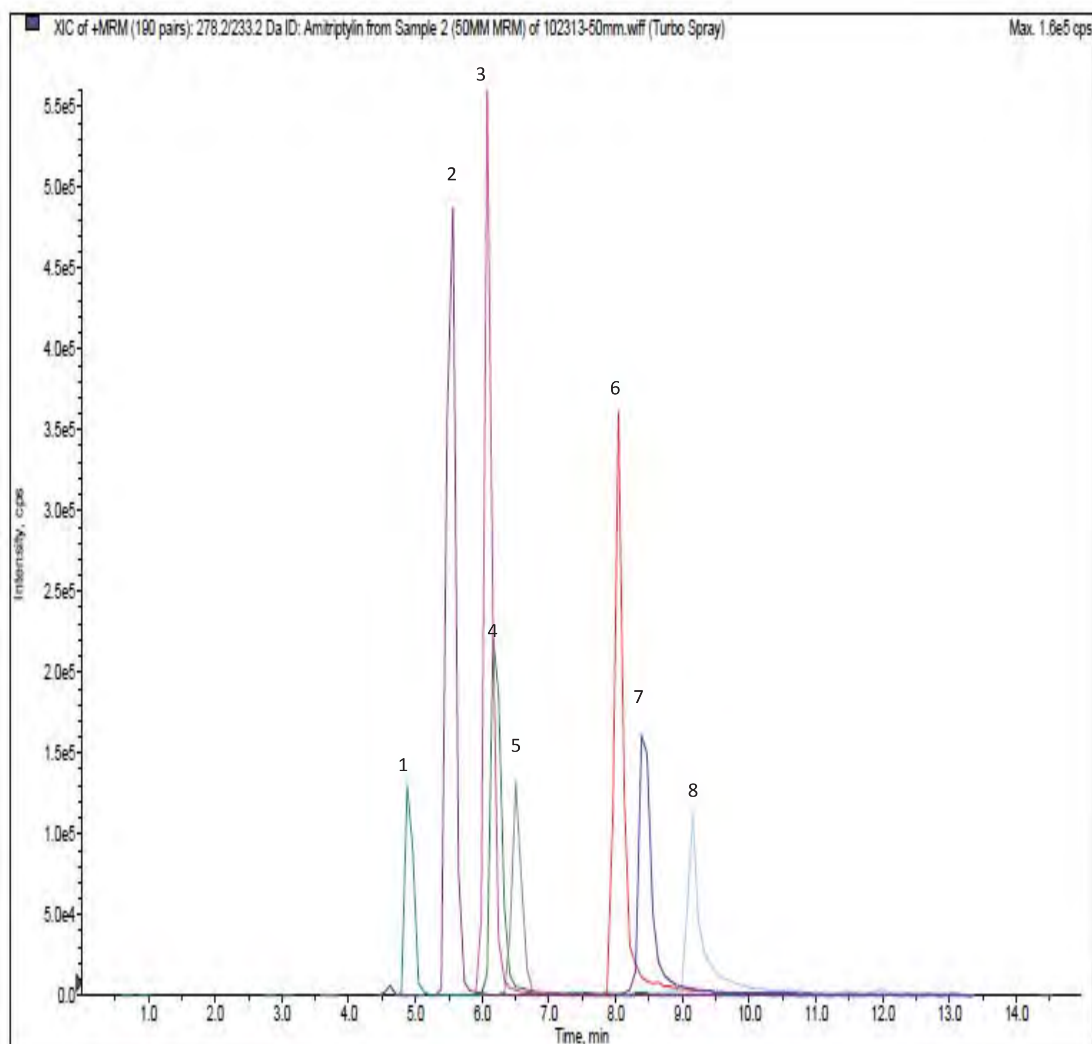
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylecgonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



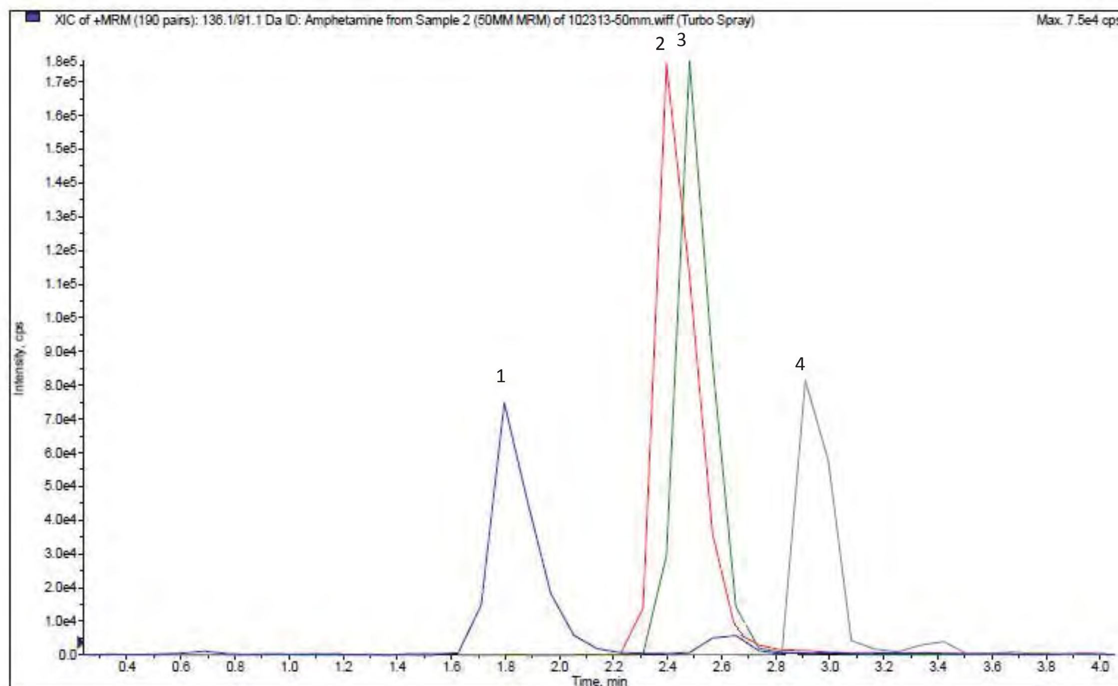
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxaine	278.2	260.2	4.90
2. Zolpidem	308.2	235.2	5.50
3. Trazadone	372.2	176.1	6.05
4. PCP	244.2	86.1	6.20
5. Quintiapine	384.2	253.1	6.50
6. Imipiramine	281.2	86.1	8.40
7. Amitriptyline	278.2	233.2	8.42
8. Sertraline	306.1	159	9.25

Amphetamine Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	**136.1**	**91.1**	**1.18**
2. Methamphetamine	**150.1**	**91.1**	**2.40**
3. MDA	**180.1**	**105.0**	**2.45**
4. MDMA	**194.1**	**105.1**	**2.95**

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 20 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	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

BENZODIAZEPINES IN URINE CLEAN SCREEN FASt® COLUMN

Part #

CSFAS203 – CLEAN SCREEN FASt ® 200 mg, 3 mL Tube

BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase

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

1. **PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:**

   To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.

   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.

   Vortex and heat for 1-2 hours at 65 °C.

   Allow sample to cool

   Do not adjust pH~ sample is ready to be added to the extraction column.

2. **LOAD SAMPLE and SAMPLE DILUTE RATIO:**

   Sample Dilution Ratio: Sample Volume* : Diluent** Volume

   NOTE: *If sample is hydrolyzed add appropriate aliquot volume after hydrolysis is complete.

Dilution Ratio	Urine	Diluent**
1:1	500 µL	500 µL
1:4	200 µL	800 µL
1:9	100 µL	900 µL

** Diluent is 50:50 (Acetonitrile: D.I. H_2O)

Sample and diluents are added in an appropriately labeled tube.

Add appropriate volume internal standard(s). It is recommended to use an internal standard volume of no more than 200 µL.

3. **EXTRACTION and COLLECTION:**

   Set up extraction manifold with FASt cartridges and auto-sampler collection vials.

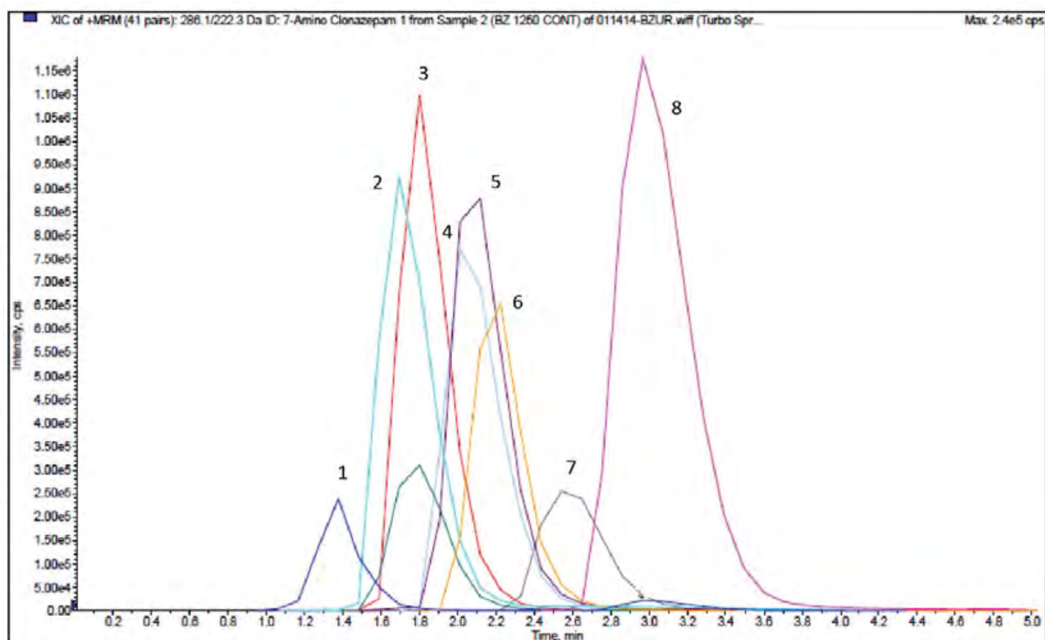
   Pour sample into FASt cartridge and elute sample directly into auto-sampler vials.

4. **ANALYSIS:**

   Cap vials and put directly onto LC/MS for analysis.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. 7-Amino Clonazepam	286.09	222.3	1.40
2. Oxazepam	287.09	241.3	1.70
3. Alpha- Hydroxy- Alprazolam	325.18	297.1	1.80
4. Clonazepam	316.13	270.2	2.10
5. Nordiazepam	271.09	140.1	2.10
6. Temazepam	301.12	255.2	2.20
7. Alprazolam	309.16	205.3	2.60
8. Diazepam	285.1	193.1	3.00

PARAMETERS

Mobile Phase A: .02% Formic Acid in D.I. H_2O

Mobile Phase B: .02% Formic Acid in Methanol

Flow Rate: 0.1 mL/minute

Polarity: Positive

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

Isocratic Flow:

Time	%A	%B
0.00	50	50
7.50	STOP	

OPIATES IN URINE CLEAN SCREEN FASt® COLUMN

Part #
CSFAS203 – CLEAN SCREEN FASt® 200 mg, 3 mL Tube
BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE FOR ENZYME HYDROLYSIS OF GLUCURONIDES:**

   To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.

   Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase.

   Vortex and heat for 1-2 hours at 65 °C.

   Allow sample to cool

   Do not adjust pH~ sample is ready to be added to the extraction column.

2. **LOAD SAMPLE and SAMPLE DILUTE RATIO:**

   Sample Dilution Ratio: Sample Volume* : Diluent** Volume

   NOTE: *If sample is hydrolyzed add appropriate aliquot volume after hydrolysis is complete.

Dilution Ratio	Urine	Diluent**
1:1	500 µL	500 µL
1:4	200 µL	800 µL
1:9	100 µL	900 µL

** Diluent is 50:50 (Methanol: D.I. H_2O)

Sample and diluents are added in an appropriately labeled tube.

Add appropriate volume internal standard(s). It is recommended to use an internal standard volume of no more than 200 µL.

3. **EXTRACTION and COLLECTION:**

   Set up extraction manifold with FASt cartridges and auto-sampler collection vials.

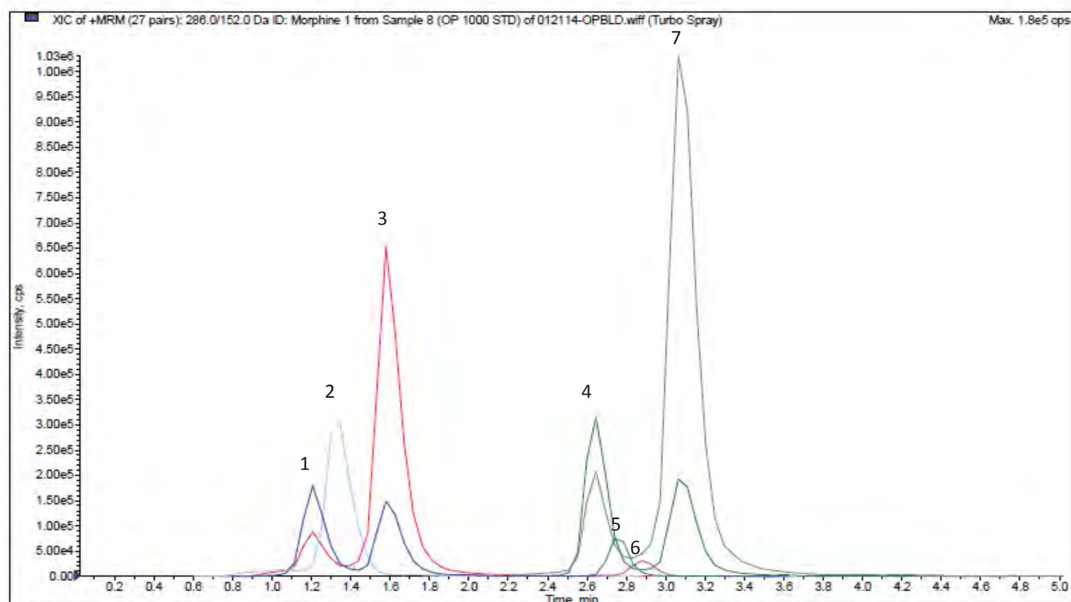
   Pour sample into FASt cartridge and elute sample directly into auto-sampler vials.

4. **ANALYSIS:**

   Cap vials and put directly onto LC/MS for analysis.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Morphine	286	152	1.21
2. Oxymorphone	302	227	1.30
3. Hydromorphone	286	185	1.60
4. Codeine	300	152	2.65
5. 6-MAM	328	165	2.75
6. Oxycodone	316	240	2.85
7. Hydrocodone	300	199	3.10

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

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	85	15
7.00	40	60
7.01	20	80
8.00	85	15
9.00	STOP	

THC-COOH IN URINE CLEAN SCREEN FASt ® THC COLUMN

Part #

CSFASTH203 – CLEAN SCREEN FASt® THC 200 mg, 3 mL Tube

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

1. PREPARE SAMPLE-BASE HYDROLYSIS OF GLUCURONIDES:

To 2 mL of urine add internal standard and 50 µL of 10 M NaOH

Mix/vortex

Hydrolyze for 15 minutes at 60-70 °C. Cool before proceeding

Adjust sample pH to 7.0 with 50 µL of 1:1 H_2O: Glacial Acetic Acid.

Add 200 µL pH 7.0 100 mM Phosphate Buffer

(pH should be ~7.0)

2. LOAD SAMPLE and SAMPLE DILUTE RATIO:

Sample Dilution Ratio: Sample Volume* : Diluent** Volume

NOTE: *If sample is hydrolyzed add appropriate aliquot volume after hydrolysis is complete.

Dilution Ratio	Urine	Diluent**
1:1	500 µL	500 µL
1:4	200 µL	800 µL
1:9	100 µL	900 µL

** Diluent is 50:50 (Acetonitrile: D.I. H_2O)

Sample and diluents are added in an appropriately labeled tube.

Add appropriate volume internal standard(s). It is recommended to use an internal standard volume of no more than 200 µL.

3. EXTRACTION and COLLECTION:

Set up extraction manifold with FASt cartridges and auto-sampler collection vials.

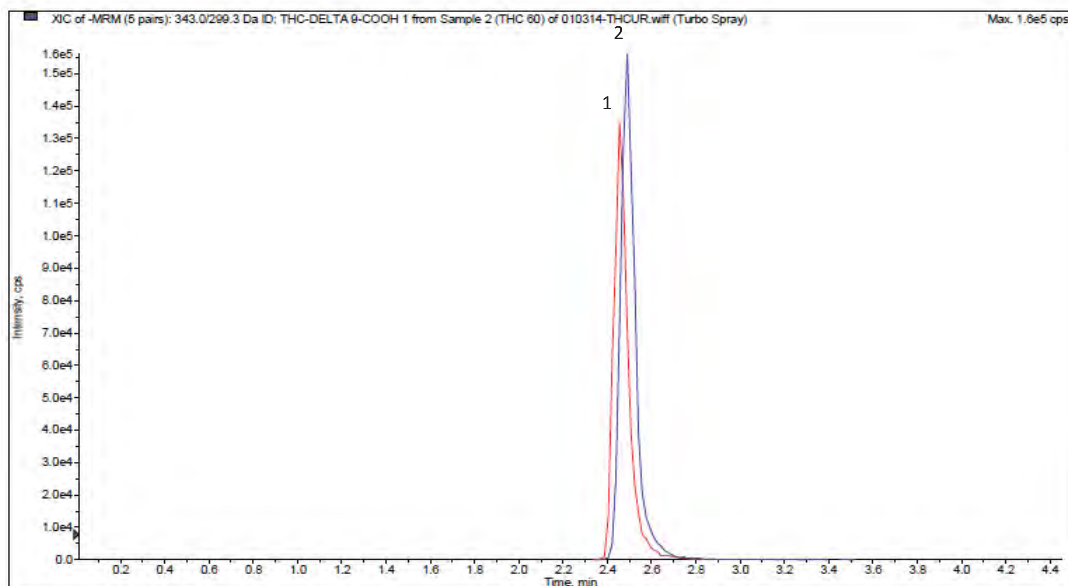
Pour sample into FAST cartridge and elute sample directly into auto-sampler vials.

4. ANALYSIS:

Cap vials and put directly onto LC/MS for analysis.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. THC-DELTA 9-COOH D_9	352	308	2.44
2. THC-DELTA 9-COOH	343	299	2.49

PARAMETERS

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative

Injection Volume: 20 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	60	40
2.00	30	70
2.50	10	90
2.51	60	40
4.00	STOP	

UCT

Veterinary and Racing

CPS analitica for Chemistry

3-HYDROXY LIDOCAINE, 4-HYDROXY GUANABENZ, 4-HYDROXY MEPIVICANE, 4-HYDROXY XYLAZINE, DETOMIDINE, AND O-DESMETHYL TRAMADOL IN EQUINE URINE BY LC/MS

Part #

XRDAH206 - XtrackT® DAU 200 mg, 6 mL Tube

SPHPH06001-10 - Select pH Buffer Pouches 100mM Phosphate pH 6.0

1. **PREPARE SAMPLE:**

   To 1 mlof 100 mM phosphate buffer (pH= 6) add 2 mL of Urine add Internal standards. Add 3 mL of 100 mM phosphate buffer Mix/vortex.

   Centrifuge as appropriate

2. **CONDITION XTRACKT® DAU EXTRACTION COLUMN:**

   1 x3mL CH_3OH
   1 x 3 mL D.I. H2O
   1 x 3 ml100 mM phosphate buffer (pH= 6)
   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. H2O
   1 x 3 mL Methanol containing 2% glacial acetic acid
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE:**

   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78: 20: 2 v/v)

   Collect the eluate at 1-2 mL minute (or gravity)

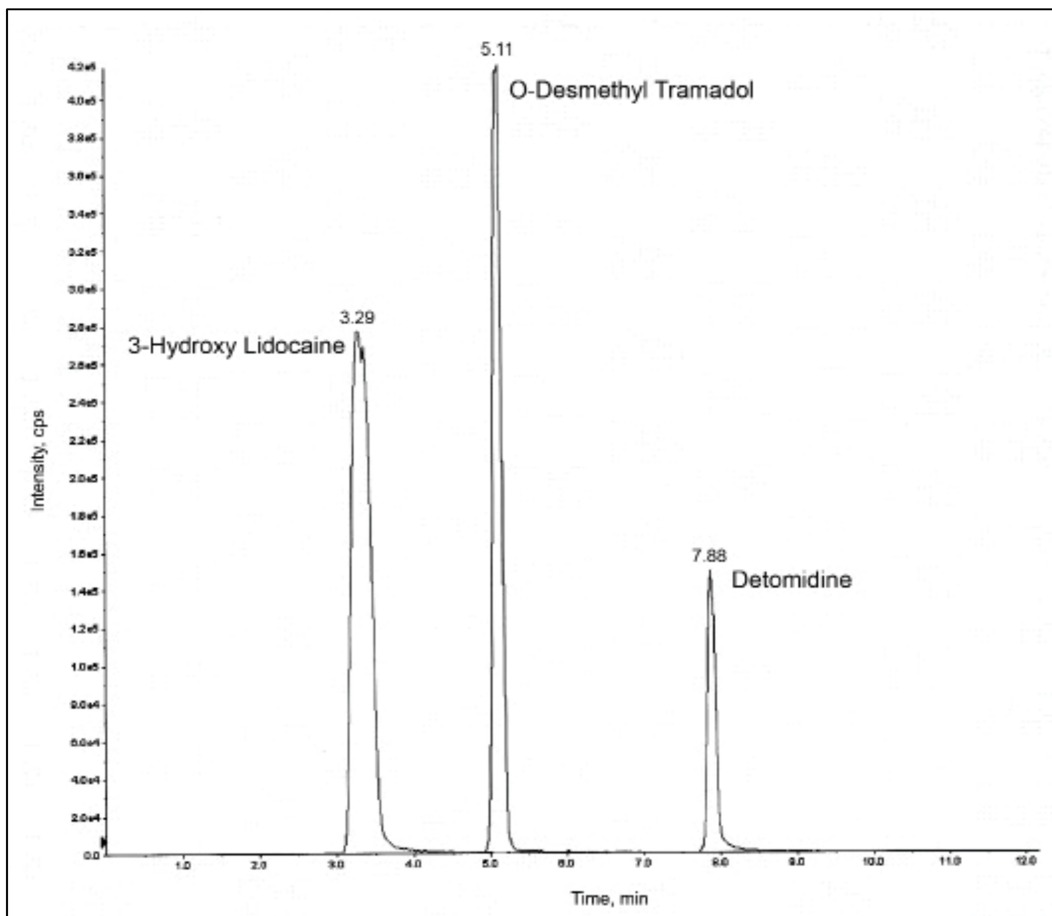
   NOTE: Prepare elution solvent daily

6. **DRY ELUATE:**

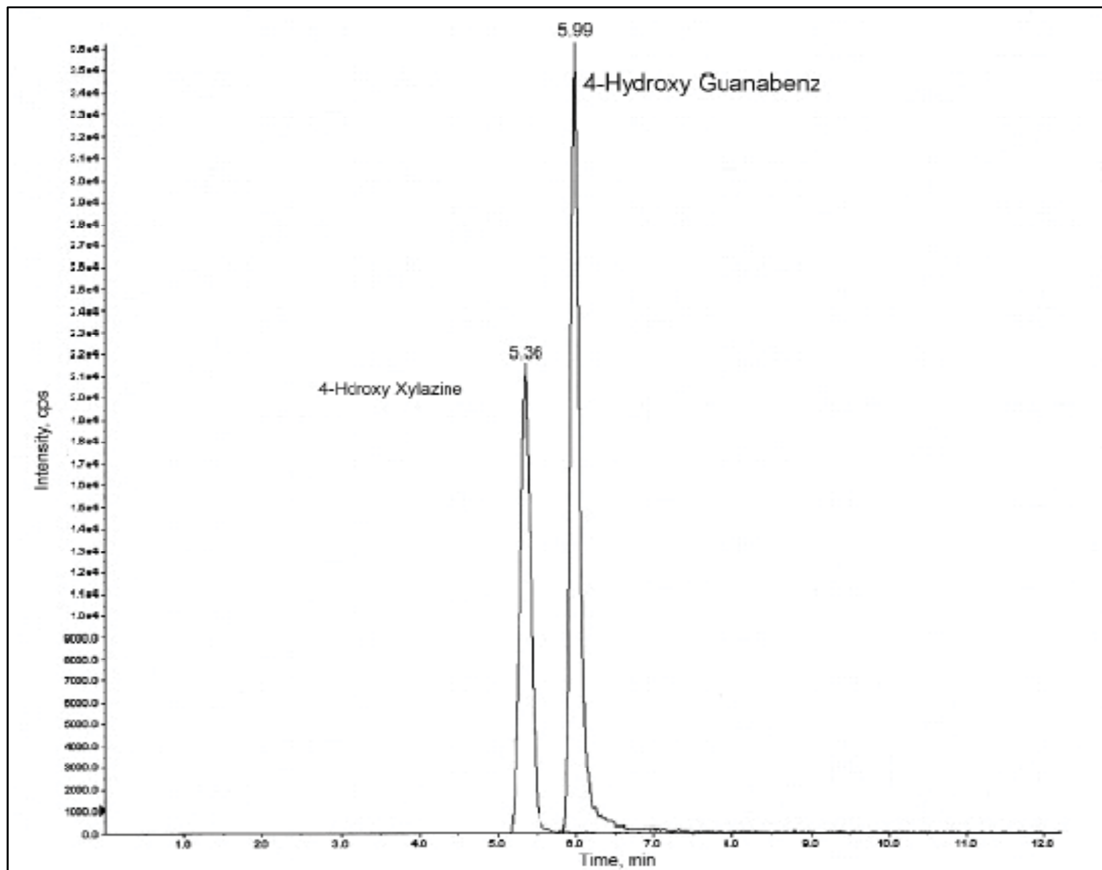
   Evaporate to dryness at< 40°C

7. **ANALYSIS:**

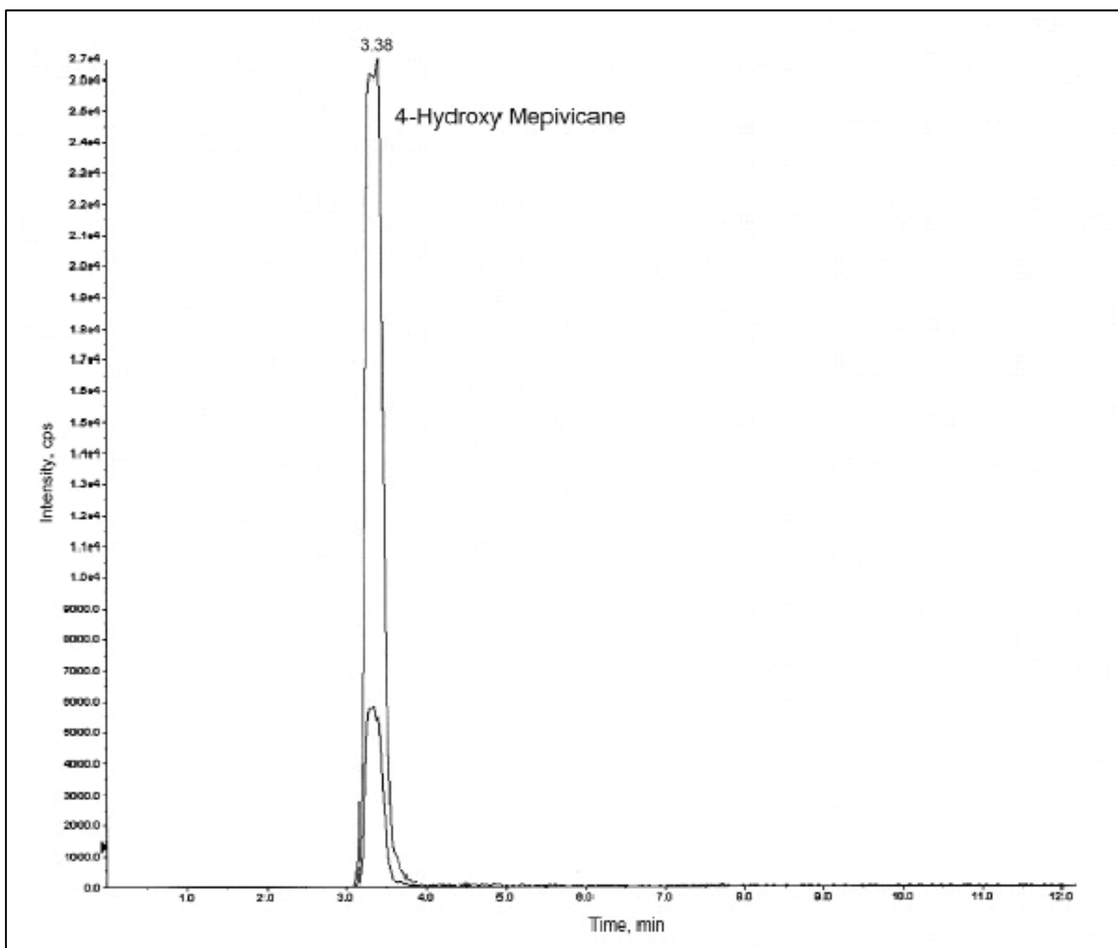
   Inject 10 µL sample



5.11
O-Desmethyl Tramadol
3.29
3-Hydroxy Lidocaine
7.88
Detomidine
Intensity, cps
Time, min



5.99
4-Hydroxy Guanabenz
5.36
4-Hdroxy Xylazine
Intensity, cps
Time, min



3.38
4-Hydroxy Mepivicane
Intensity, cps
Time, min

Compound	RT	Precursor Ion	Product Ion
3-Hydroxy Lidocaine	3.29	251.5	86.0
4-Hydroxy Mepivicane	3.38	264.1	98.1
O-Desmethyl Tramadol	5.11	250.4	58.0
4-Hydroxy Xylazine	5.36	237.9	137.0
4-Hydroxy Guanabenz	5.99	248.9	189.9
Detomidine	7.88	188.1	81.0

ABUSED DRUGS IN CANINE OR EQUINE URINE USING 500 mg XTRACKT® EXTRACTION COLUMN

Part #

XRDAH515 - XrackT® SPE Cartridge, 500 mg DAU, 15 mL Tube

BETA-GLUC-10 - Selectrazyme® Beta-glucuronidase

SLDA50ID21-5UM - Selectra® DA HPLC Column50 x 2.1 mm, 5µm

1. **PREPARE SAMPLE**

   A. PREPARE SAMPLE-ENZYMATIC HYDROLYSIS OF GLUCURONIDES

   To 5 mL of urine add internal standard(s) and 2 mL Selectrazyme®
   β-glucuronidase 5,000 F units/mL in 100 mM Acetate Buffer (pH 5.0).
   Optionally, add 1 mL of Acetate Buffer and 50 µL of concentrated
   β-glucuronidase.
   Mix/vortex. Hydrolyze at 65°C for 1-2 hours.
   Centrifuge for 10 min. at 2000 rpm, discard pellet.

   B. BASE HYDROLYSIS OF GLUCURONIDES

   To 2 mL of urine add internal standard(s) and 100 µL of 10 N NaOH.
   Mix/vortex. Hydrolyze at 60 °C for 20 minutes.
   Centrifuge for 10 min. at 2000 rpm, discard pellet.

   COMBINE HYDROLYSATES

   Combine both hydrolysis products with 5 mL of 100 mM phosphate buffer (pH 6.0).
   Adjust sample pH = 6.0 ± 0.5 with 0.5 M Phosphoric acid.

2. **CONDITION XtrackT® EXTRACTION COLUMN**

   1 x 5 mL CH_3OH
   1 x 5 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 100 mM phosphate buffer (pH 6.0).
   1 x 2 mL 1.0 M acetic acid.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 2 mL Hexane

5. **ELUTE ACIDIC AND NEUTRAL DRUGS**

   1 x 4 mL CH_2Cl_2; collect eluate at < 5 mL / minute.

6. **ELUTE STEROIDS**

   2 x 4 mL Ethyl Acetate; collect eluate at < 5 mL / minute.

7. **WASH COLUMN**

   1 x 5 mL Methanol; aspirate.

8. **ELUTE BASIC DRUGS**

   1 x 5 mL CH_2Cl_2 / IPA / NH_4OH (78:20:2).

NOTE: Prepare elution solvent fresh daily.

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

10. **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

Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and react at 70°C for 30 minutes; Cool and inject 1-2 µL

BARBITURATES IN EQUINE URINE FOR GC/MS CONFIRMATIONS

UCT Part Numbers:
XRDAH206 - 200 mg XtrackT® DAU Extraction Column in 6 mL cartridge, with CLEAN-THRU® Tips
or
XCDAH206 - 200 mg XtrackT® DAU Extraction Column in 6 mL cartridge without Tips
CLTTP050 - CLEAN-THRU® Tips
SBSTFA-0-1 – SELECTRA-SIL® BSTFA, 10 x 1gm vial pack
OPTIONAL:
STMPAH-0-1 – SELECTRA-SIL® TMPAH, 10 x 1gm vial pack

1. **PREPARE SAMPLE:**
   To 2 mL of urine add internal standard(s) and 1 mL of 100 mM phosphate buffer (pH= 5.0)
   Mix/vortex.
   Sample pH should be 5.0 ± 0.5
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate

2. **CONDITION XTRACKT® DAU EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM phosphate buffer (pH= 5.0)

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

6. **DRY ELUATE:**

   Evaporate to dryness at < 40 °C

   Reconstitute with 100 µL Ethyl Acetate

7. **QUANTITATIVE**

   Add 50 µL of both Ethyl Acetate and BSTFA

   (OPTIONAL DERIVATIZATION

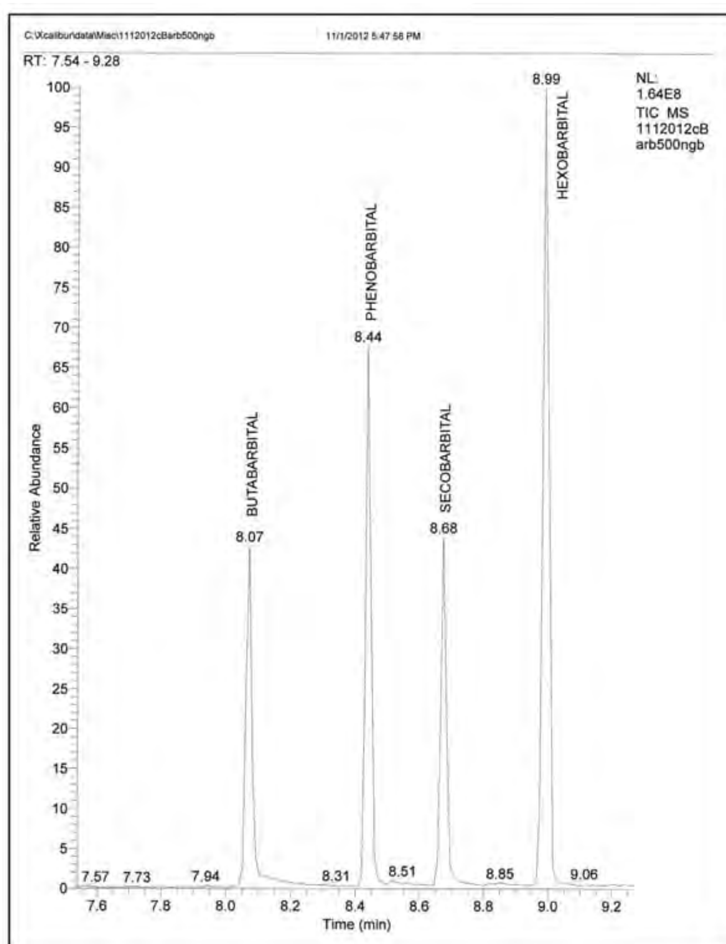
   Add 25-50 µL of 0.2 M TMPAH

   Reaction occurs in injection port)

   Inject 1 to 2 µL onto gas chromatograph

CHROMATOGRAM

Underivatized Barbituates



Mass Spec Table

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
Butabarbital	156	141	157
Phenobarbital	204	232	117
Secobarbital	168	167	195
Hexobarbital†	221	157	236

*Quantitation Ion

†Suggested internal standard for GC/MS

Other Barbituates that can be extracted using this protocol

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
Amobarbital	156	141	157
Butalbital	168	167	181
Pentobarbital	156	141	195
Thiopental	172	157	173

*Quantitation Ion

Mass Spec Ion Table for derivatized barbitutates

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
Butalbital	196	195	209
Butalbital-D_5†	201	214	
Amobarbital	169	184	185
Pentobarbital	169	184	112
Secobarbital	196	195	181
$^{13}C_4$ Secobarbital†	200	185	
Phenobarbital	232	146	175
Phenobarbital-D_5†	237	151	

*Quantitation Ion

†Suggested internal standard for GC/MS

BENZODIAZEPINES IN EQUINE OR CANINE URINE FOR GC/MS CONFIRMATIONS

Part #
XRDAH206 - XtrackT® DAU 200 mg, 6 mL Tube
SPHPHO6001-10 - Select pH Buffer Pouches 100mM Phosphate pH 6.0
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/1% TMCS
BETA-GLUC-10 - Selectrazyme® β - Glucuronidase

1. **PREPARE SAMPLE - ß-GLUCURONIDASE HYDROLYSIS:**

   To 2 mL of urine add internal standard(s) and 1 mL of ß-glucuronidase solution.
   ß-glucuronidase solution contains: 5,000 F units/mL Haliotis rufescens in 100 mM acetate buffer (pH=5.0).
   Mix/vortex.
   Hydrolyze for 3 hours at 65°C.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.

2. **CONDITION XTRACKT® DAU EXTRACTION COLUMN:**

   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM phosphate buffer (pH= 6.0)
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**

   Load at 1 mL/ minute

4. **WASH COLUMN:**

   1 x 2 mL D.I. H_2O
   1 x 2 mL 20% Acetonitrile in 100 mM phosphate buffer (pH= 6.0)
   Dry column (5 minutes at full vacuum or pressure)
   1 x 2 mL Hexane

5. **ELUTE BENZODIAZEPINES:**

   1 x 5 mL Ethyl Acetate containing 4% NH_4OH
   collect eluate at 1 to 2 mL/minute

6. **DRY ELUATE:**

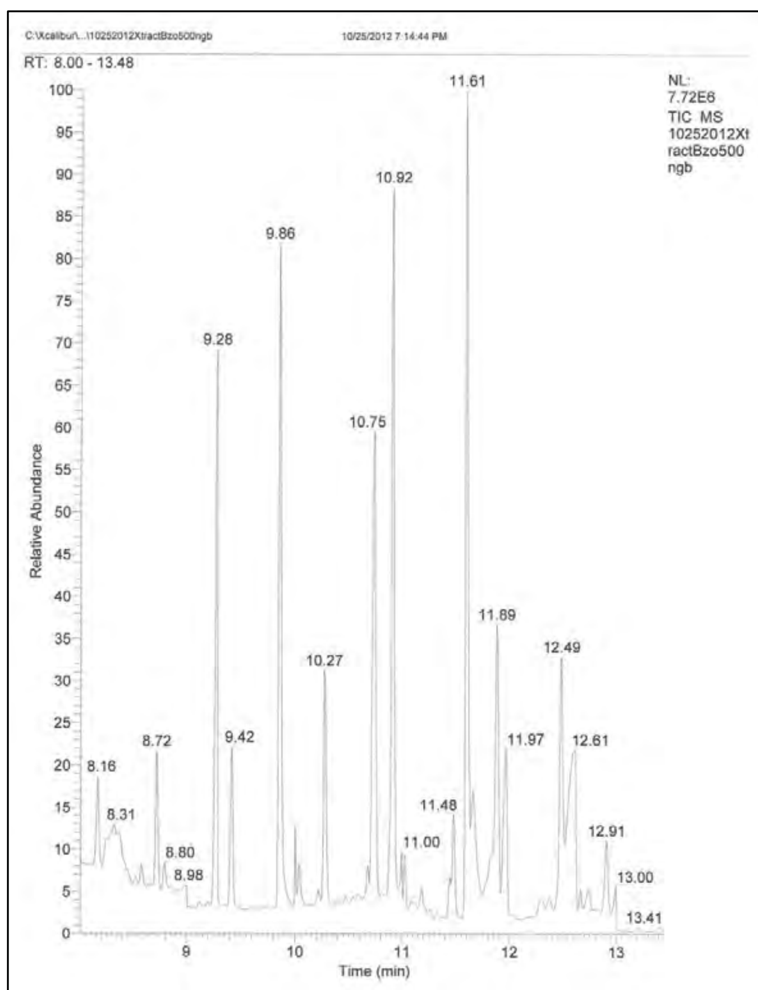
   Evaporate to dryness at < 40°C

7. **DERIVATIZE:**

   Add 50 µL Ethyl Acetate and 50 µL BSTFA w/1% TMCS
   Overlay with Nitrogen and cap. Mix/vortex
   React 20 minutes at 70°C. Remove from heat source to cool
   NOTE: Do not evaporate BSTFA solution

8. **ANALYZE:**

   Inject 1 to 2 µL onto gas chromatograph



Compound	Primary Ion*	Secondary Ion	Tertiary Ion
1. Diazepam	256	283	221
2. Nordazepam TBDMS	327	383	369
3. Midazepam	310	325	297
4. Oxazepam - 2TBDMS	457	513	383
Oxazepam - D_5 2TBDMS†	462	519	
5. Temazepam	357	283	385
6. 7-aminoclonazepam TBDMS	342	399	328
7. Lorazepam 2TBDMS	491	513	533
8. Clonazepam	372	326	429
9. Alprazolam	279	204	308
Alprazolam - D_5†	284	313	
10. Alphahydroxyl alprazolam TBDMS	381	423	346

*Quantitation Ion

†Suggested internal standard for GC/MS

BUPRENORPHINE AND NORBUPRENORPHINE IN EQUINE URINE FOR GC/MS CONFIRMATIONS

Part Numbers:
XRDAH206 - 200 mg XtrackT® DAU Extraction Column in 6 mL cartridge
SPHACE5001-10 - Select pH Buffer Pouches 100mM Acetate pH 5.0
SBSTFA-1-1 - BSTFA w/1% TMCS

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM Acetate buffer (pH= 5) add internal standard.
   Mix/ vortex and add 1 mL of Equine Urine.
   Add 2 mL of 100 mM Acetate buffer (pH= 5) and mix/ vortex
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge as appropriate.

   Enzyme Hydrolysis of Glucuronides.
   To 1 mL of 100 mM Acetate buffer add internal standard.
   Add 1 mL of Equine Urine. Mix/ vortex.
   Add 2 mL of 100 mM Acetate buffer (pH= 5).
   Hydrolyze with Helix Pomatia (5,000 units/mL), heat for 3 hours at 60°C Cool before proceeding.

2. **CONDITION XTRACKT® DAU EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM Acetate buffer (pH= 5.0)

NOTE: Aspirate at < 3 Inches Hg to prevent sorbent drying

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

4. **WASH COLUMN:**
   1 x 2 mL D.I. H_2O
   1 x 3 mL 100 mM acetate buffer (pH=5.0)
   1 x 3 mL Methanol
   Dry column (5-10) minutes at full vacuum or pressure

5. **ELUTE BUPRENORPHINE / NORBUPRENORPHINE:**
   1 x 3 mL $MeCl_2$ / IPA / NH_4OH (78/20/12). (Make elution solvent fresh)
   Collect eluate at 1 to 2 mL/minute
   NOTE: Before proceeding, insure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency

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

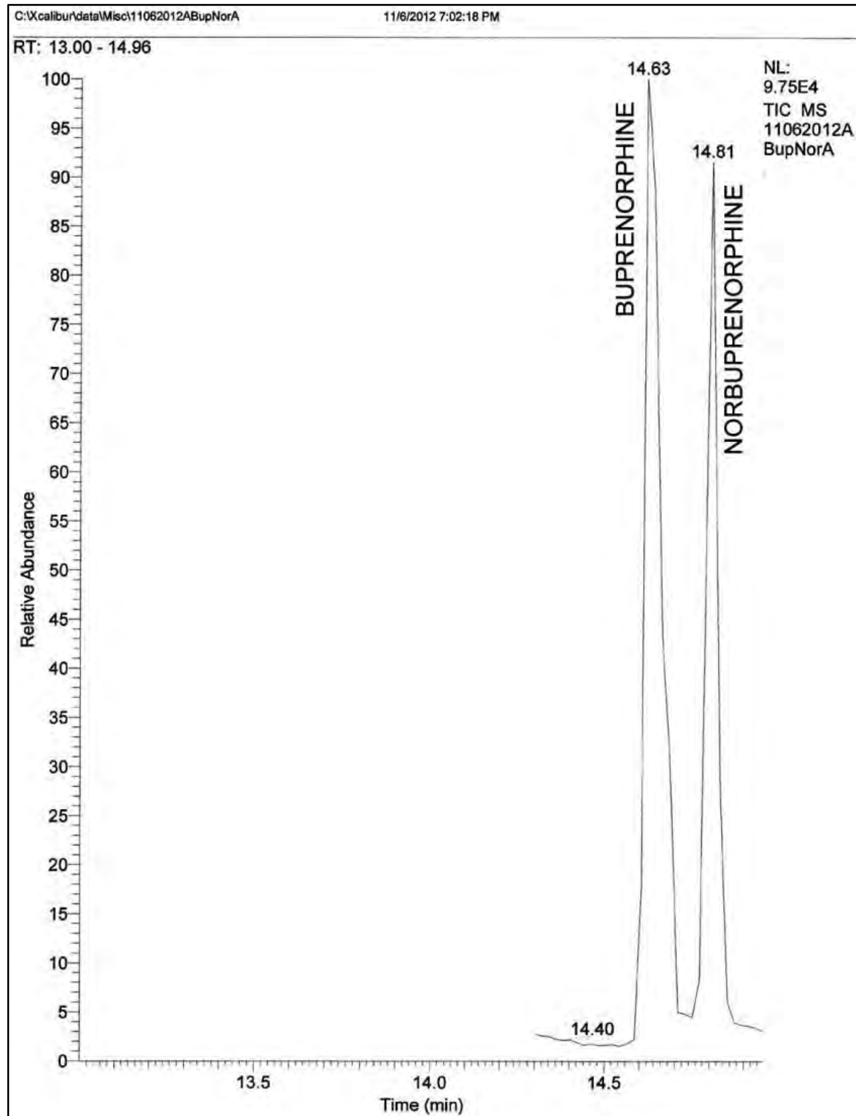
7. **DERIVATIZE:**
   Add 50 µL Ethyl Acetate and 50 µL BSTFA w/1 % TMCS

React 20 minutes at 70°C
Remove from heat source to cool
NOTE: Do not evaporate BSTFA

8. QUANTITATIVE:
Inject 1 to 2 µL onto gas chromatograph/mass spectrometer

CHROMATOGRAM

Buprenorphine and Norbuprenorphine



Mass Spec Table

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
Buprenorphine-TMS	452	467	487
Buprenorphine-D_4-TMS†	455	470	489
Norbuprenorphine-TMS	468	500	510
Norbuprenorphine-D_5-TMS†	503	525	542

*Quantitation Ion

†Internal Standard

CARISOPRODOL AND MEPROBAMATE IN EQUINE URINE FOR GC/MS CONFIRMATIONS

Part Numbers:
XRDAH206 - 200 mg XtrackT® DAU Extraction Column in 6 mL cartridge
SPHPHO6001-10 - Select pH Buffer Pouches 100mM Phosphate pH 6.0

1. **PREPARE SAMPLE:**
   To 2 mL of urine add internal standard(s) and 1 mL of 100 mM phosphate buffer (pH= 6)
   Mix/vortex
   Sample pH should be 6.0 ± 0.5
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate
   Centrifuge at 3000 RPM for 10 minutes

2. **CONDITION XTRACKT® DAU EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM phosphate buffer (pH= 6)

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

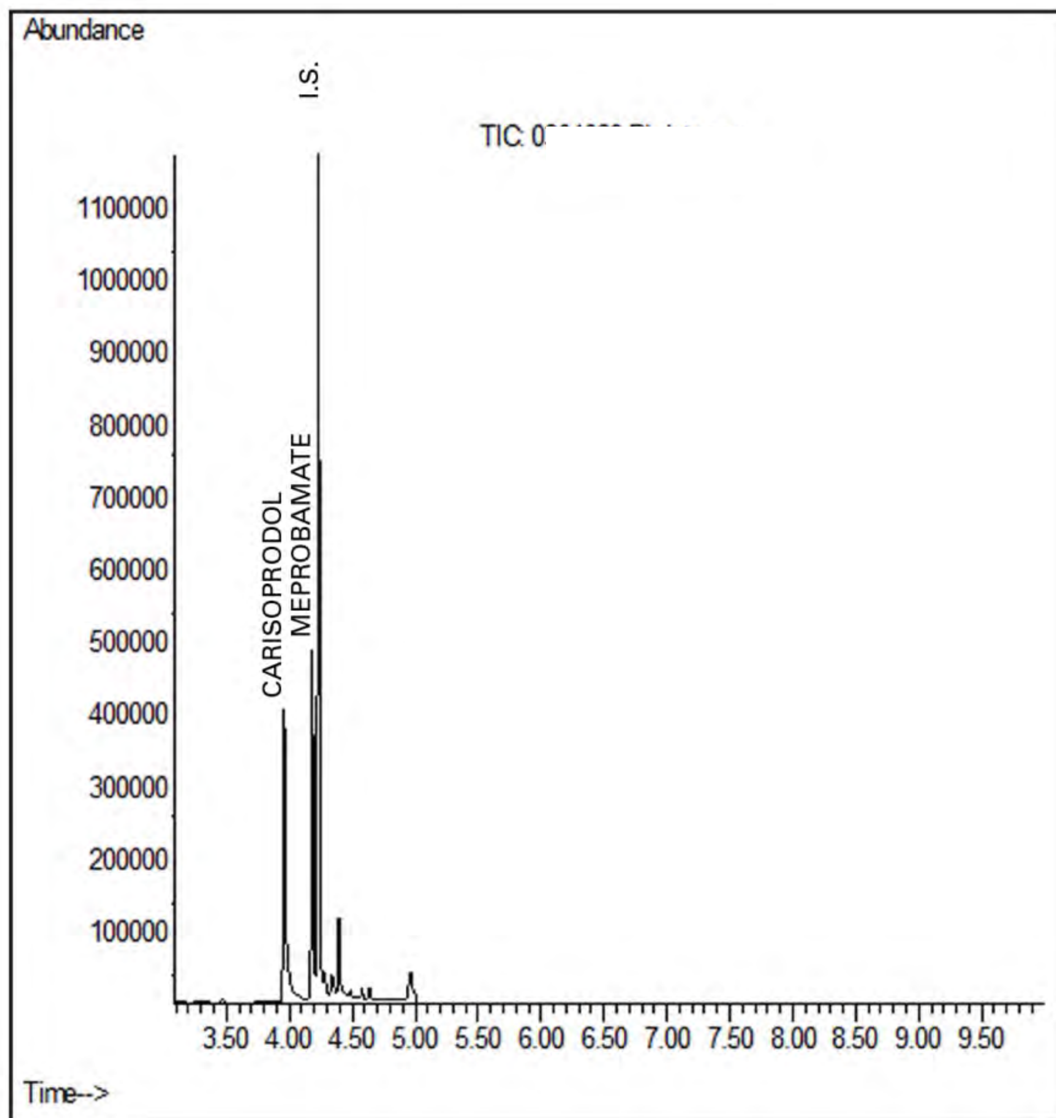
5. **ELUTE BARBITUATES:**
   1 x 3 mL Hexane/ Ethyl Acetate (50:50); Collect eluate at 1 to 2 mL / minute

6. **DRY ELUATE**:
   Evaporate to dryness at < 40°C
   Reconstitute with 100 µL Ethyl Acetate

7. **QUANTITATIVE:**
   Inject 1 to 2 µL onto gas chromatograph

CHROMATOGRAM

Carisoprodol, Meprobamate, and Hexobabital (Internal Standard)



Mass Spec Table

Compound	Primary Ion
Carisoprodol	221
Meprobamate	157
Hexobarbital	236

CLENBUTEROL AND SALBUTAMOL IN EQUINE URINE FOR GC/MS CONFIRMATIONS

Part #
XRDAH206 - XtrackT® DAU 200 mg, 6 mL Tube
SPHPHO6001-10 - Select pH Buffer Pouches 100mM Phosphate pH 6.0
SBSTFA-1-1 1 – SELECTRA-SIL BSTFA w/1% TMCS

1. **PREPARE SAMPLE:**

   To 1 mL of 100 mM phosphate buffer (pH= 6) add 1 mL of Urine
   Add Internal standards Add 3 mL of 100 mM phosphate buffer
   Mix/ vortex
   Centrifuge as appropriate

2. **CONDITION XTRACKT® DAU 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)
   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 CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE CLENBUTEROL / SALBUTAMOL:**

   1 x 3 mL CH_3OH containing 4% NH_4OH
   Collect the eluate at 1-2 mL minute (or gravity)

6. **DRY ELUATE:**

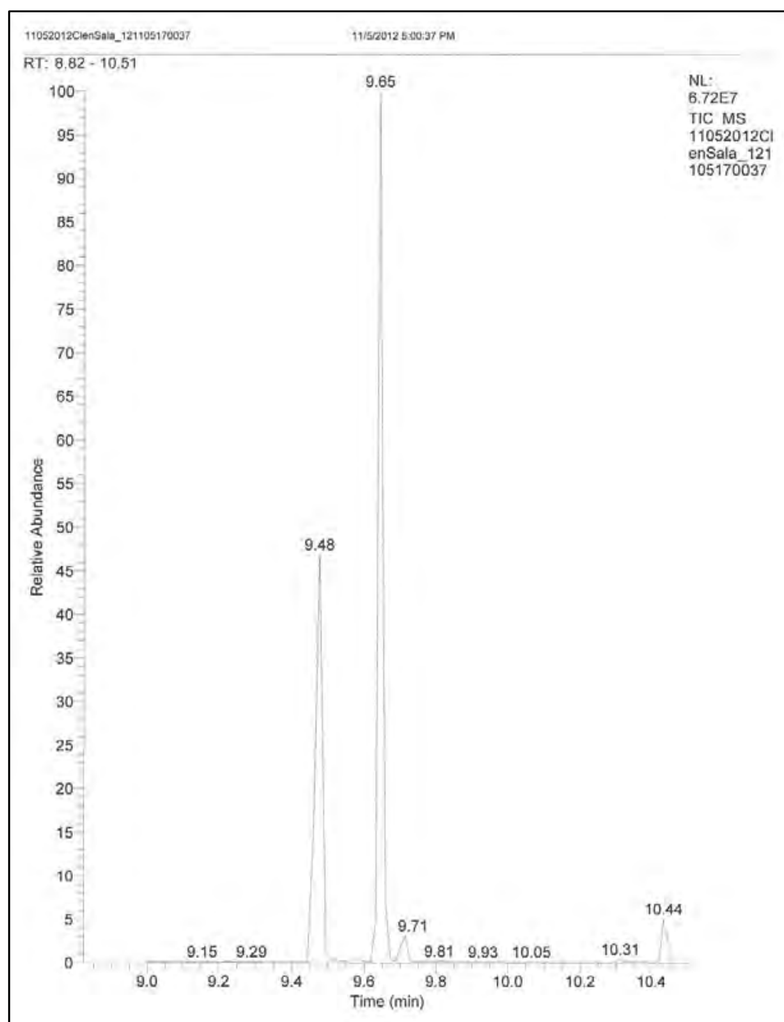
   Evaporate to dryness at < 40°C

7. **DERIVATIZE:**

   Add 50 µL Ethyl Acetate
   Add 50 µL BSTFA w/1% TMCS
   Heat at 70 °C for 30 minutes
   Cool to room temperature
   NOTE: Do not evaporate this solution

8. **ANALYSIS:**

   Inject 1 to 2 µL onto gas chromatograph



Compound	Primary Ion*	Secondary Ion	Tertiary Ion
Clenbuterol-TMS	86	262	243
Clenbuterol-D_3-TMS†	95	262	243
Salbutamol-TMS	369	86	207
Salbutamol-D_3-TMS†	372	86	210

*Quantitation Ion

†Suggested internal standard for GC/MS

THC, THC-OH, AND CARBOXY-THC IN EQUINE URINE FOR GC/MS CONFIRMATIONS

Part Numbers:
XRDAH206 - 200 mg XtrackT® DAU Extraction Column in 6 mL cartridge
SPHACE5010-10 - Select pH Buffer Pouches 1M Acetate pH 5.0
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/1% TMCS
BETA-GLUC-10 - SELECTRAZYME® β – Glucuronidase

1. **PREPARE SAMPLE - ENZYMATIC AND BASE HYDENZYMATIC AND BASE HYDROLYSIS OF GLUCURONIDES:**
   To 1 mL of urine add internal standard (s) and 50 µL of Beta Glucuronidase solution (*Haliotis rufescens*), add 2 mL of 1 M Acetate buffer pH= 5.
   Mix and incubate at 65 °C for 3 hours.
   Cool to room temperature

   Add 100 of 10 M NaOH. Mix/vortex
   Hydrolyze for 20 minutes at 60°C. Cool before proceeding
   Adjust sample pH to 3.0 with approx. 1.0 mL of glacial acetic acid. Check pH to insure that the pH value is ~ 3.0
   Centrifuge as appropriate

2. **CONDITION XTRACKT® DAU EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL Acetate buffer (pH= 3.0)

NOTE: Aspirate at full vacuum or pressure

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

4. **WASH COLUMN:**
   1 x 2 mL D.I. H_2O
   1 x 2 mL 100 mM HCl: Acetonitrile (95:5)
   Dry column (5-10 minutes at full vacuum or pressure)
   1 x 200 1 mL Hexane; Aspirate. (Additional step to remove any residual moisture)

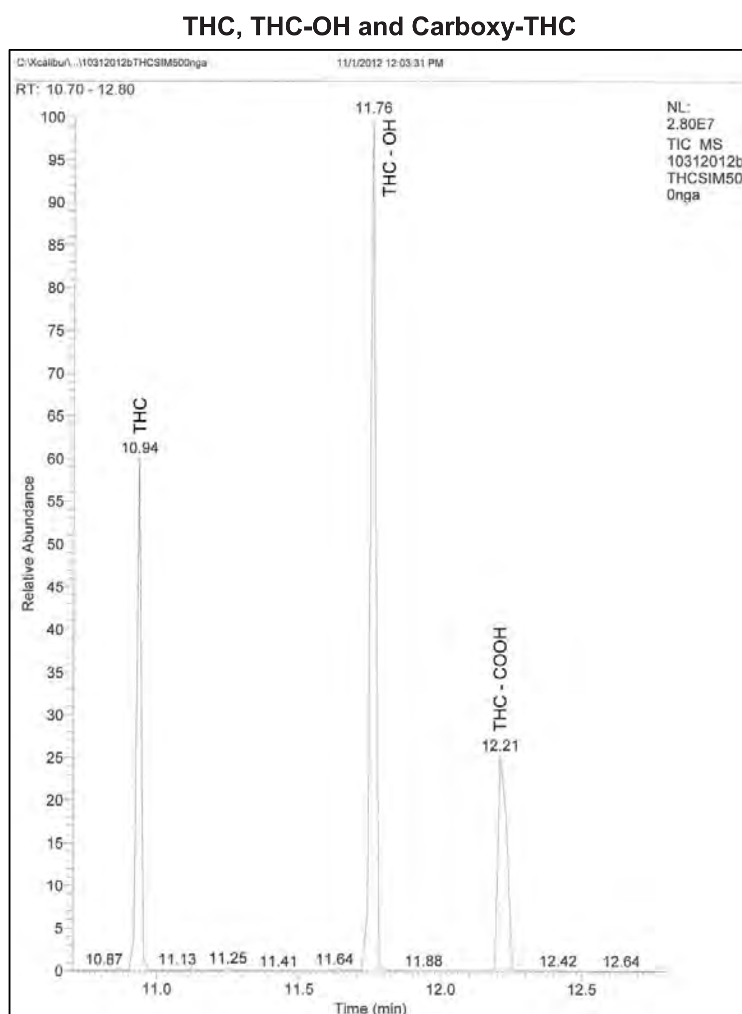
5. **ELUTE CANNABINOIDS:**
   1 x 3 mL Hexane/ Ethyl Acetate/ Glacial Acetic Acid (49:49:2)
   Collect eluate at 1 to 2 mL/minute
   NOTE: Before proceeding, ensure there are no water droplets at the bottom of the collection tube. This may increase drying time and decrease BSTFA derivatizing efficiency

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

7. **DERIVATIZE:**
Add 50 µL Ethyl Acetate and 50 µL BSTFA w/1% TMCS
Mix/vortex
React 20 minutes at 70°C
Remove from heat source to cool
NOTE: Do not evaporate BSTFA
Inject 1 to 2 µL onto gas chromatograph

CHROMATOGRAM

THC, THC-OH and Carboxy-THC



Mass Spec Table

Compound	Primary Ion*	Secondary Ion	Tertiary Ion
THC-TMS	371	343	366
THC-D_3-TMS†	374	346	889
THC-OH-TMS	371	459	474
THC-OH-D_3-TMS†	374	462	471
THC-COOH-TMS	371	473	488
THC-COOH-D_3-TMS†	374	476	491

*Quantitation Ion
†Suggested internal standard for GC/M

GLYCOPYRROLATE (ROBINUL) FROM EQUINE URINE BY LC-MSMS USING: 500 mg CLEAN-UP® CCX2 EXTRACTION COLUMN

Part #:

CUCCX25Z – CLEAN-UP® CCX2 500 mg, 10 mL Tube

1. SAMPLE PREPARATION

Buffer 5 mL of urine to pH 7.0 by adding 3 mL of 100 mM phosphate buffer (pH 7.0).

Add (12.5 ng) of mepenzolate (internal standard).

Add 5 mL of H_2O to the sample.

Vortex or shake thoroughly.

Centrifuge for 5 min at 800 rpm.

2. CONDITION CLEAN-UP® EXTRACTION COLUMN

1 x 3 mL CH_3OH.

1 x 3 mL D.I. H_2O.

1 x 1 mL 100 mM phosphate buffer (pH 7.0).

3. APPLY SAMPLE

Decant supernatant onto SPE column.

Load at 1 to 2 mL / min.

4. WASH COLUMN

5 mL of CH_3OH.

5 mL of D.I. H_2O.

Dry column (5 min > 10 inches Hg).

5. ELUTE GLYCOPYRROLATE

1 x 4 mL CH_3OH /0.5 M Ammonium Acetate buffer, pH 3.0 (95:5).

6. DRY ELUTE

Evaporate to dryness at 60 °C.

Reconstitute with 100 µL CH_3OH.

7. QUANTITATE

Inject 10 µL onto HPLC.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM

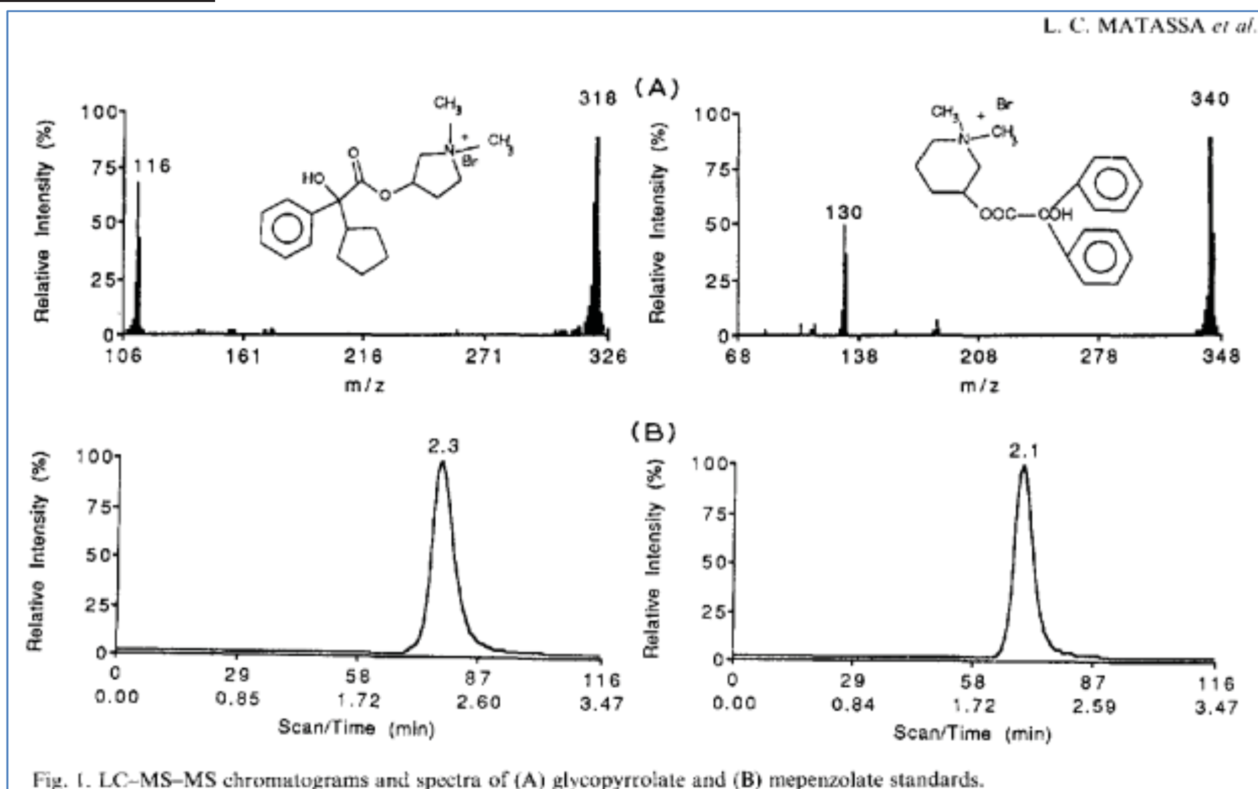


Fig. 1. LC–MS–MS chromatograms and spectra of (A) glycopyrrolate and (B) mepenzolate standards.

Analyte	MRM Transitions		Relative Retention Time (minutes)
	Q1	Q3	
1. Glycopyrrolate	318	116	2.3
2. Mepenzolate (IS)	340	130	2.1

PARAMETERS

Mobile Phase A: Methanol

Mobile Phase B: 50mM Ammonium Acetate pH 3.0

Flow Rate: 0.8mL/minute

Polarity: Positive

Injection Volume: 10µl

LC Column: Hamilton PRP-1, 150 mm x 4.1 mm I.D. 5µm

Instrument: Sciex Model API III with Applied Biosystems 140A solvent delivery system

Isocratic Flow:

Time	%A	%B
0.00	80	20
10.0	STOP	

Reference: Matassa, L.C.et al. Solid-phase extraction techniques for the determination glycopyrrolate from equine urine by liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry; Journal of Chromatography, 573 (1992) 43-48.

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