



Clinical and Forensic

APPLICATIONS MANUAL



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CLINICAL



FORENSICS



UCT

Introduction and Application Hints

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)(CH₃CN); HPLC Grade
Chloroform (CHCl₃); HPLC Grade
Distilled or/ Deionized H₂O
(D.I. H₂O, 5 < pH < 7)

Ethyl Acetate (EtAc); HPLC Grade
Hexane; HPLC Grade
Isopropyl Alcohol (IPA); HPLC Grade
Methanol (CH₃OH): HPLC Grade
Dichloromethane (CH₂Cl₂): HPLC Grade

Solvent mixtures

Acetone / Hexane (1:99)
Acetonitrile / D.I. H₂O (20:80)
Ethyl Acetate / IPA (75:25)
Ethyl Acetate / Hexane (50:50), (75:25)

Methanol / D.I. H₂O (80:20)
Methanol / D.I. H₂O (70:30)
Methanol / D.I. H₂O (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 ₂ Cl ₂ / IPA/NH ₄ OH(78:20:2)	EtAc / IPA/NH ₄ OH (90:6:4)
Propoxyphene	CH ₂ Cl ₂ / IPA/NH ₄ OH(78:20:2)	EtAc / IPA/NH ₄ OH (90:6:4)
Cocaine / BE	CH ₂ Cl ₂ / IPA/NH ₄ OH(78:20:2)	EtAc / CH ₃ OH/NH ₄ OH(68:28:4)
Amphetamines	CH ₂ Cl ₂ / IPA/NH ₄ OH(78:20:2)	EtAc / IPA/NH ₄ OH (90:6:4)

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

Reagents

Acetic Acid, Glacial (CH₃COOH):17.4 M
Ammonium Hydroxide (NH₄OH): concentrated (14.8 M)
β-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 (H₃PO₄): concentrated (14.7 M)
Sodium Acetate Trihydrate (NaCH₃COO·3 H₂O): F.W. 136.08
Sodium Borate Decahydrate (Na₂B₄O₇·10 H₂O): F.W. 381.37
Sodium Hydroxide, (NaOH): F.W. 40.00
Sodium Phosphate Dibasic, Anhydrous (Na₂HPO₄): F.W. 141.96
Sodium Phosphate Monobasic, Monohydrate (NaH₂PO₄·H₂O): 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. H₂O add 28.6 mL glacial acetic acid. Dilute to 500 mL with D.I. H₂O. 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. H₂O. 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. H₂O; add 1.62 mL glacial acetic acid. Dilute to 500 mL with D.I. H₂O. 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. H₂O; Add 10.4 mL glacial acetic acid. Dilute to 500 mL with D.I. H₂O. 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. H₂O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months

7.4 M Ammonium Hydroxide:

To 50 mL D.I. H₂O add 50 mL concentrated NH₄OH. Mix. Storage: 25 °C in glass or fluoropolymer plastic. Stability: Storage condition dependent.

100 mM Hydrochloric Acid:

To 400 mL D.I. H₂O add 4.2 mL concentrated HCl. Dilute to 500 mL with D.I. H₂O. Mix. Storage: 25 °C in glass or plastic. Stability: 6 months

Methanol /Ammonium Hydroxide (98:2):

To 98 mL CH₃OH add 2 mL concentrated NH₄OH. 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. H₂O. Mix. Stability: 2 mos. at room temperature.

CH₂Cl₂ / IPA / NH₄OH (78:20:2):

To 20 mL IPA, add 2 mL concentrated NH₄OH. Mix. Add 78 mL CH₂Cl₂. 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 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 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 $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$ 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:

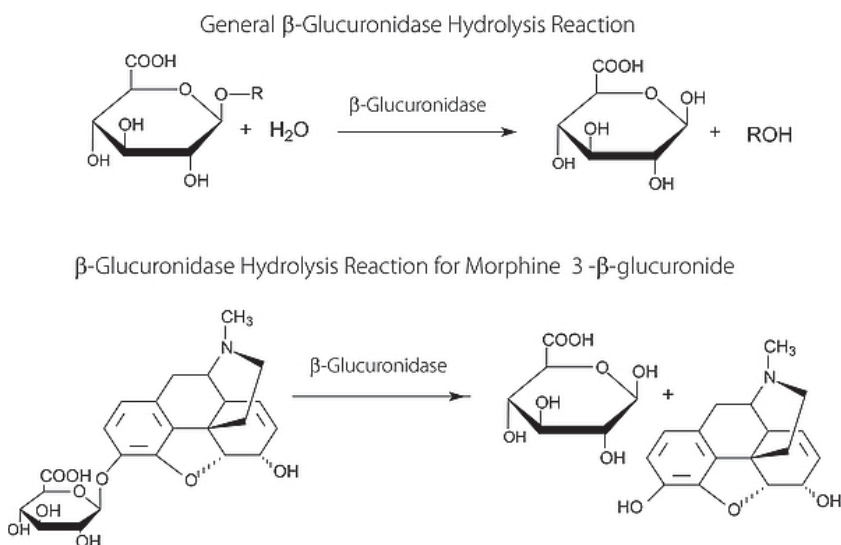
Dissolve 2.76 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 160 mL D.I. H_2O . Dilute to 200 mL with D.I. H_2O . Mix. Storage: 5°C in glass. Stability: 1 month. Inspect daily for contamination.

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).

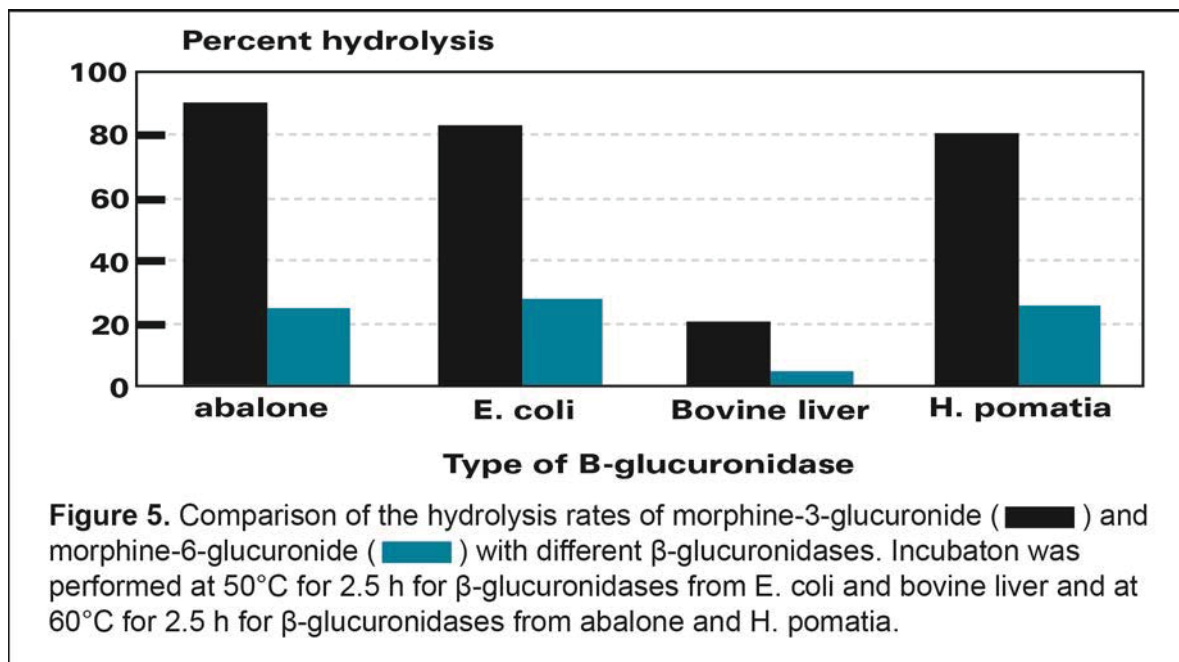


Benefits to Hydrolyzing with Selectrazyme® β -Glucuronidase

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



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.

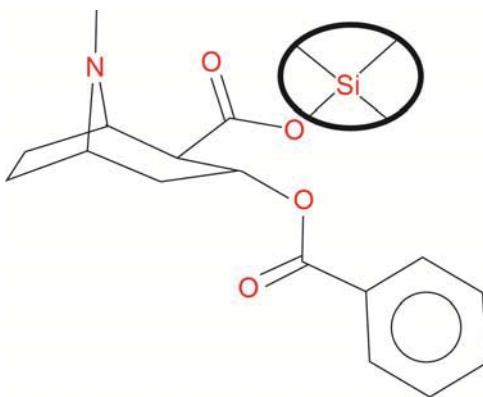


Figure 1. Trimethylsilyl derivative of benzoylecgonine. The underivatized compound has a carboxyl group and is too polar to pass through a GC column.

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 fluoroacyl 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 desiccator. 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 derivatizing agent of choice for are listed in Table 1.

Drug	Derivatized Functional Group	Derivative (BSTFA unless other specified)
amphetamine	-N ₂ H	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 ₂ H	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 ₂ H	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. H₂O 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. H₂O 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		



CLINICAL



FORENSICS



UCT

Screening Methods





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 CH₂Cl₂/ 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 CH₃OH solution has been used to prevent volatilization by the formation of the hydrochloric salt of the drugs. Add 1 drop of the solution prior to evaporating than continue to dryness.

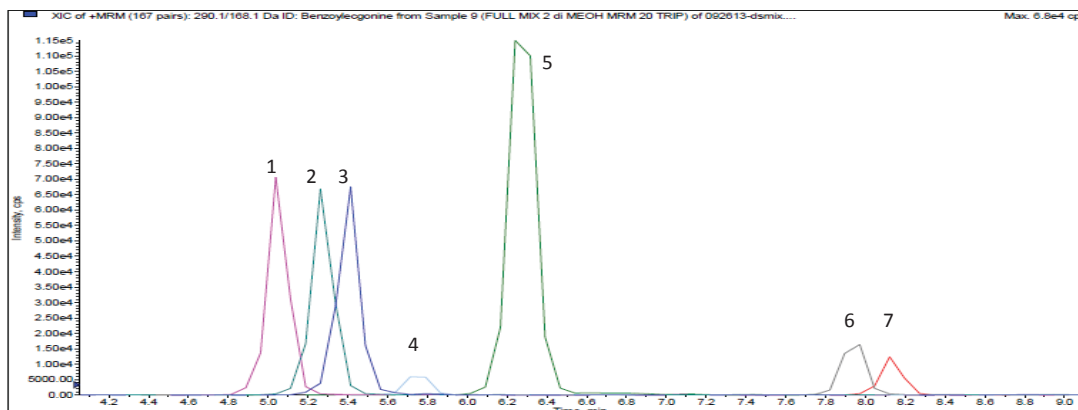
(4) The hexane wash step can be removed if user is looking to analyze for Parent THC

(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):

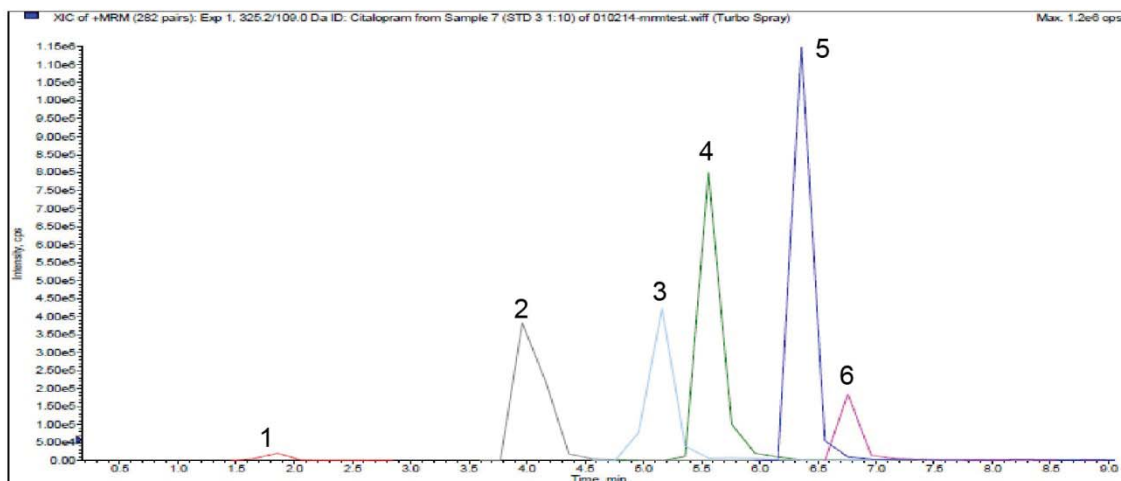
CHROMATOGRAMS

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. Benzoylcegonine	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. H₂O **Mobile Phase B:** 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 20 µL

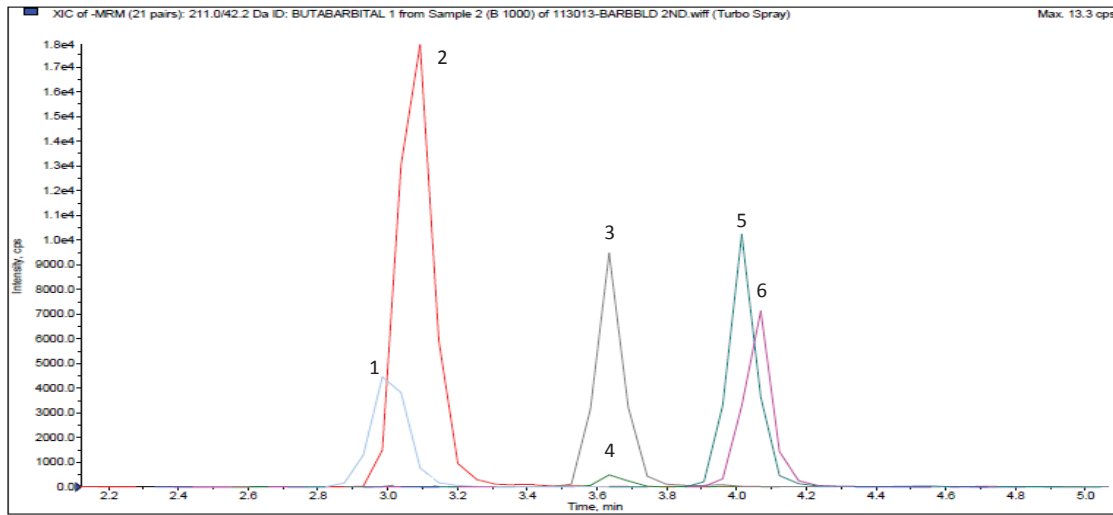
LC Column: Selectra[®] DA HPLC Column 50 x 2.1mm 5 µm

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. H₂O **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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

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 CH₃OH

Dry column (5 minutes at full vacuum or pressure)

8. ELUTE BASIC ANALYTES:

1 x 3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily

Add IPA/NH₄OH, mix, then add CH₂Cl₂ (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 CH₃OH solution has been used to prevent volatilization by the formation of the hydrochloric salt of the drugs. Add 1 drop of the solution prior to evaporating then continue to dryness.

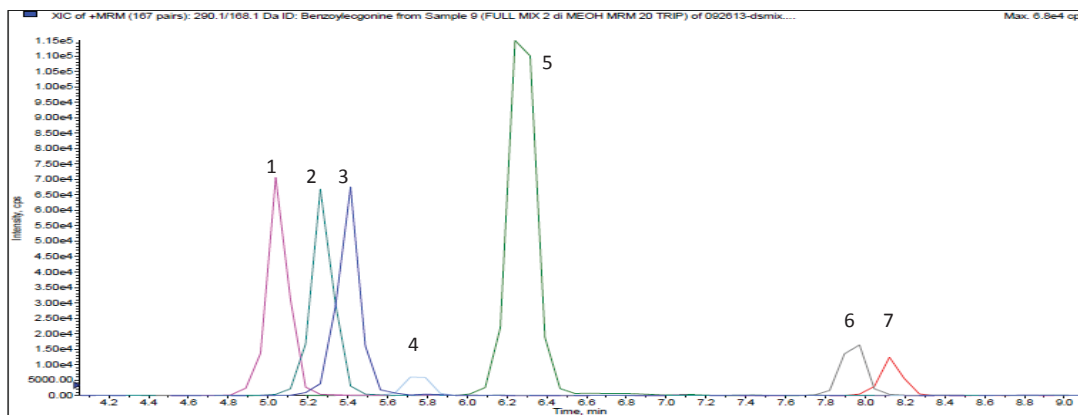
(4) The hexane wash step can be removed if user is looking to analyze for Parent THC

(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):

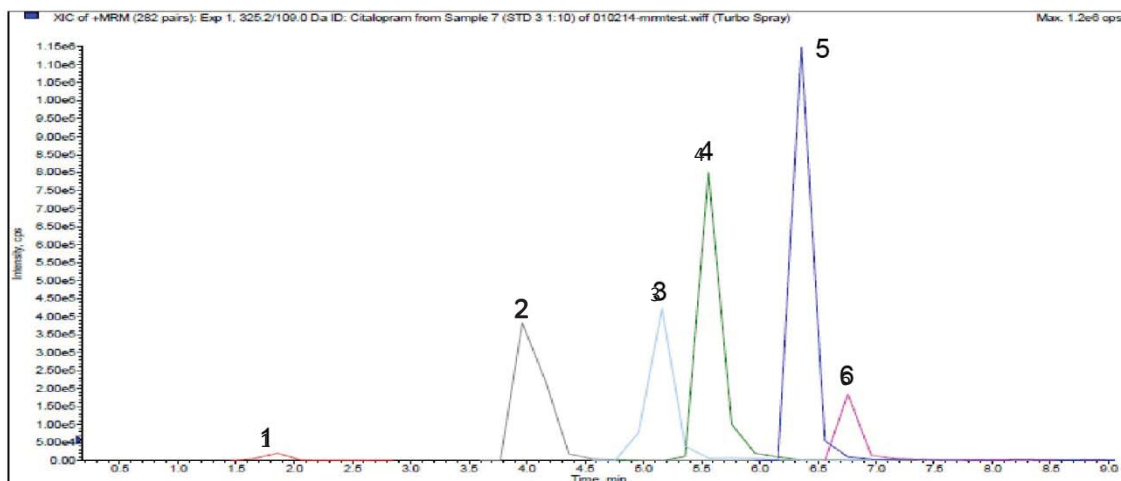
CHROMATOGRAMS

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. Benzoyllecgonine	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. H₂O **Mobile Phase B:** 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 20 µL

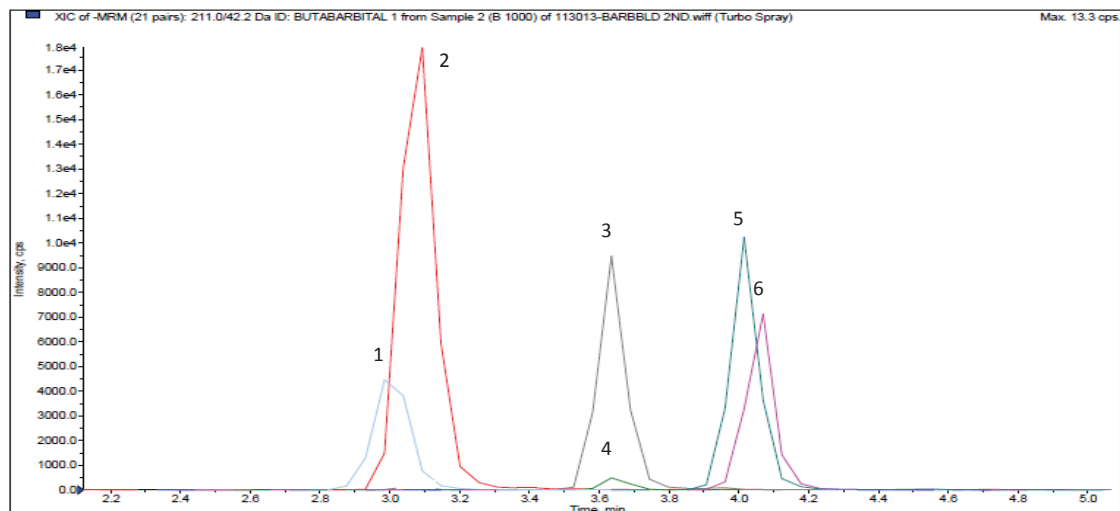
LC Column: Selectra[®] DA HPLC Column 50 x 2.1 mm 5 µm

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. H₂O **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	Clonazepam	Nordiazepam
Barbiturates	Cotinine	Phenytoin
Benzoic acid	Diazepam	Primidone
Caffeine	Glutethimide and metabolite	Salicylic acid
Carbamazepine	Ibuprofen	Theophylline
Carisoprodol	Meprobamate	Thiopental
Chlorpropamide	Methyl salicylate	

II. BASIC DRUG FRACTION (B)

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



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Acid & Neutral Drugs





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

SLDA501D21-5UM – Selectra[®] DA HPLC Column, 50 x 2.1 mm, 5 μ m

1. PREPARE SAMPLE:

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

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
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:

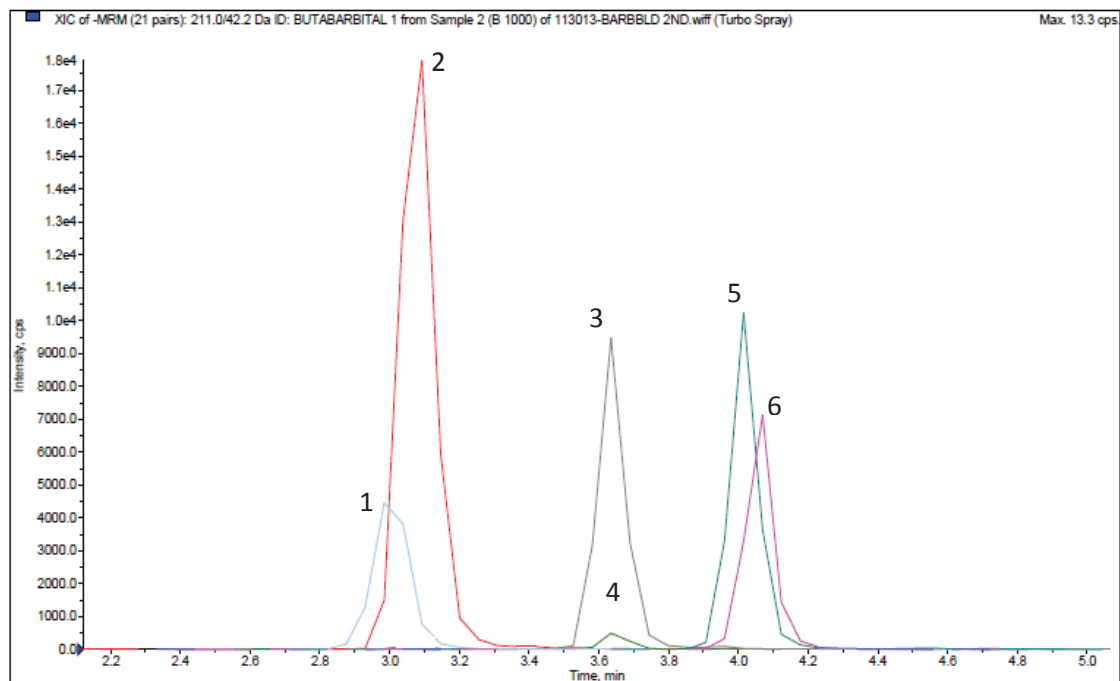
- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L.
- **GC-MS:** Dissolve residue in 100 μ L of Ethyl Acetate

Alternate Derivatization

Add 25 μ L of 0.2 M TMPAH
Reaction occurs in injection port

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



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

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O **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

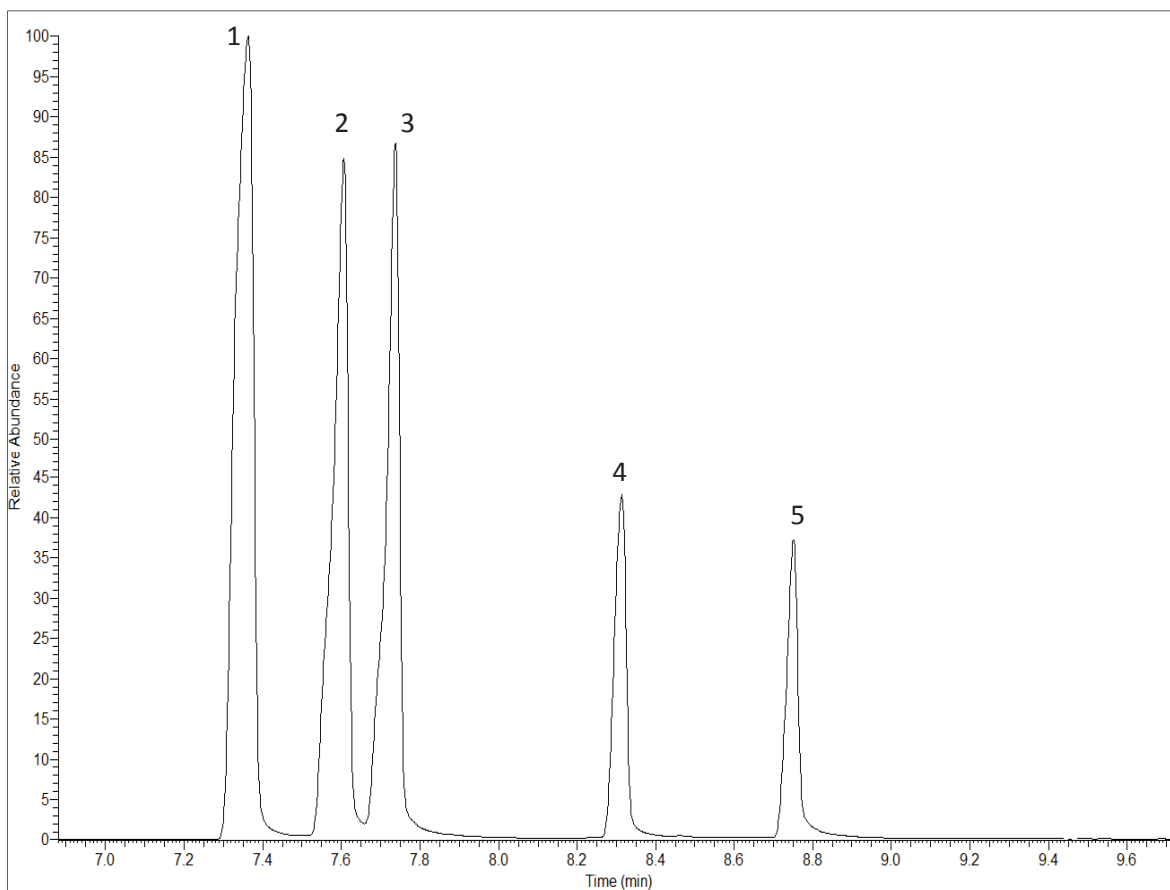
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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):

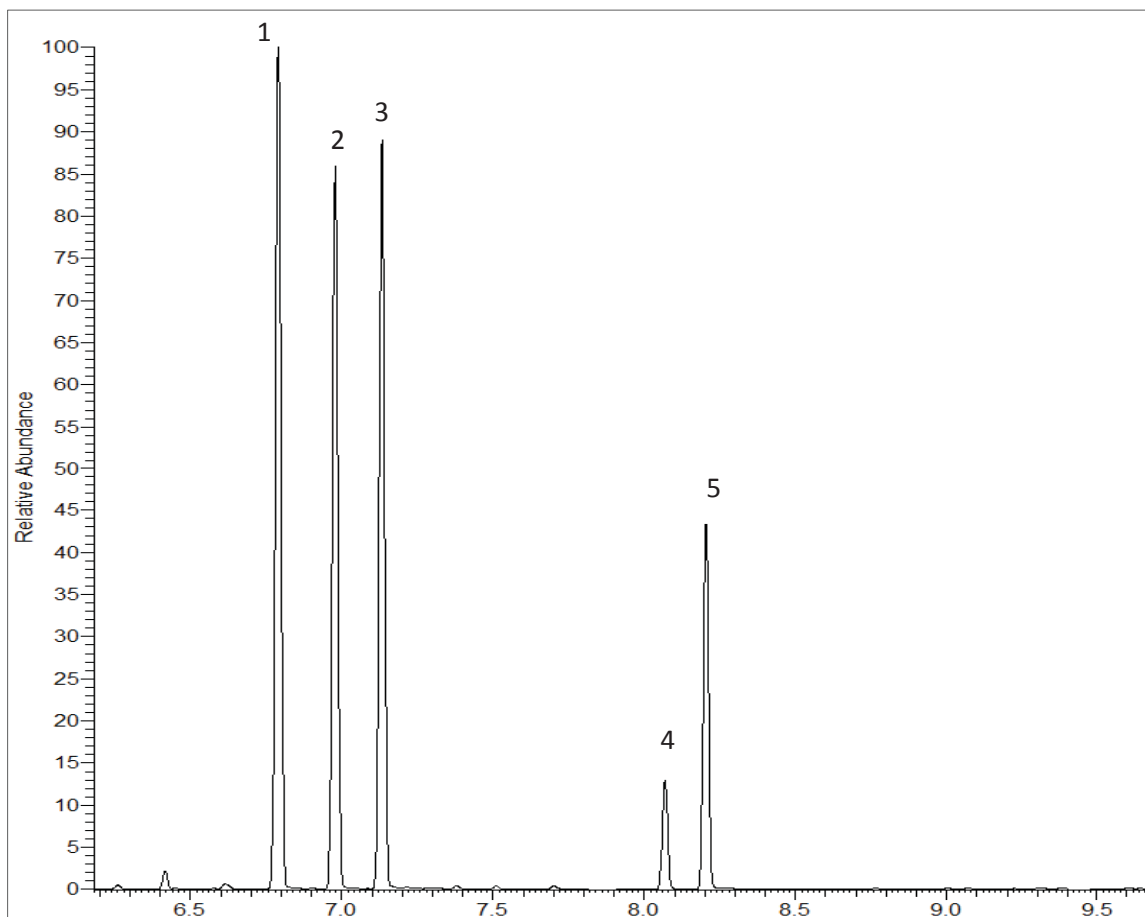
CHROMATOGRAM 1 (UNDERIVATIZED)



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 (0.25 μ m) TG-1MS

Injector: 1 μ L 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 °C, MS Source: 350 °C, 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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
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. RECONSTITUTE: sample in 1000 µL of 0.1 % Formic Acid (aq).**
Inject 20 µL.

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) Gold C₁₈ (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:

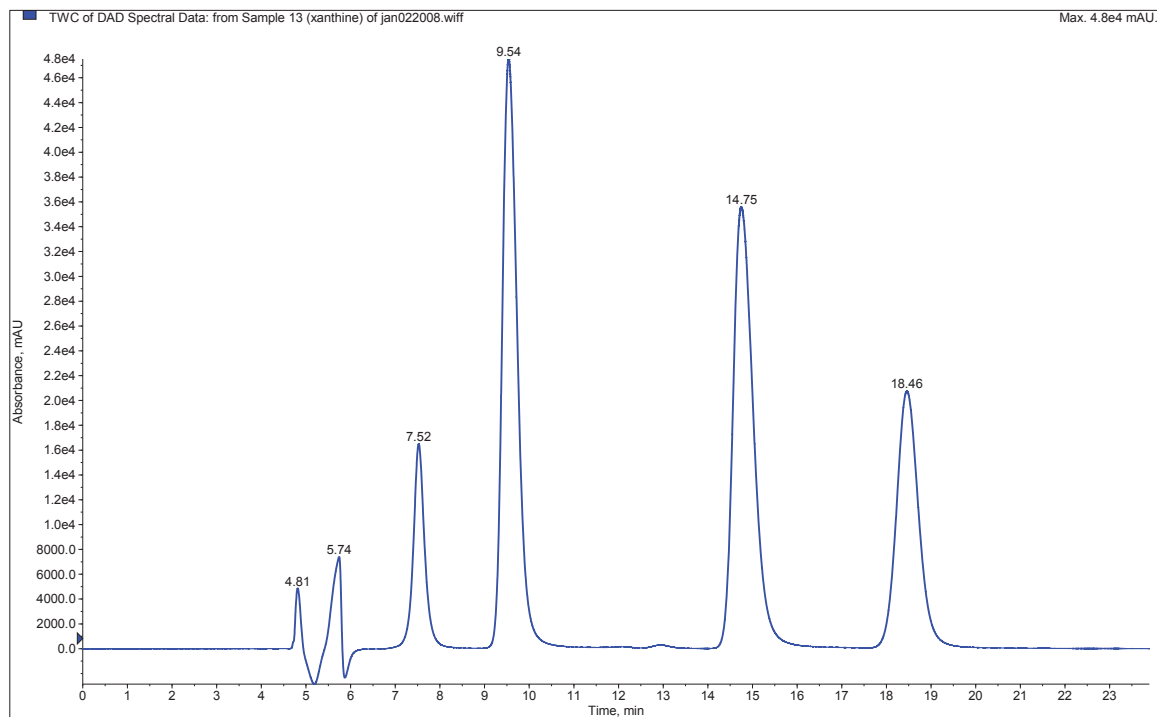
Compound

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 μ m
or

SLC-18100ID21-3UM – Selectra[®] C18 HPLC Column, 100 x 2.1 mm, 3 μ m

1. PREPARE SAMPLE:

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

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
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:

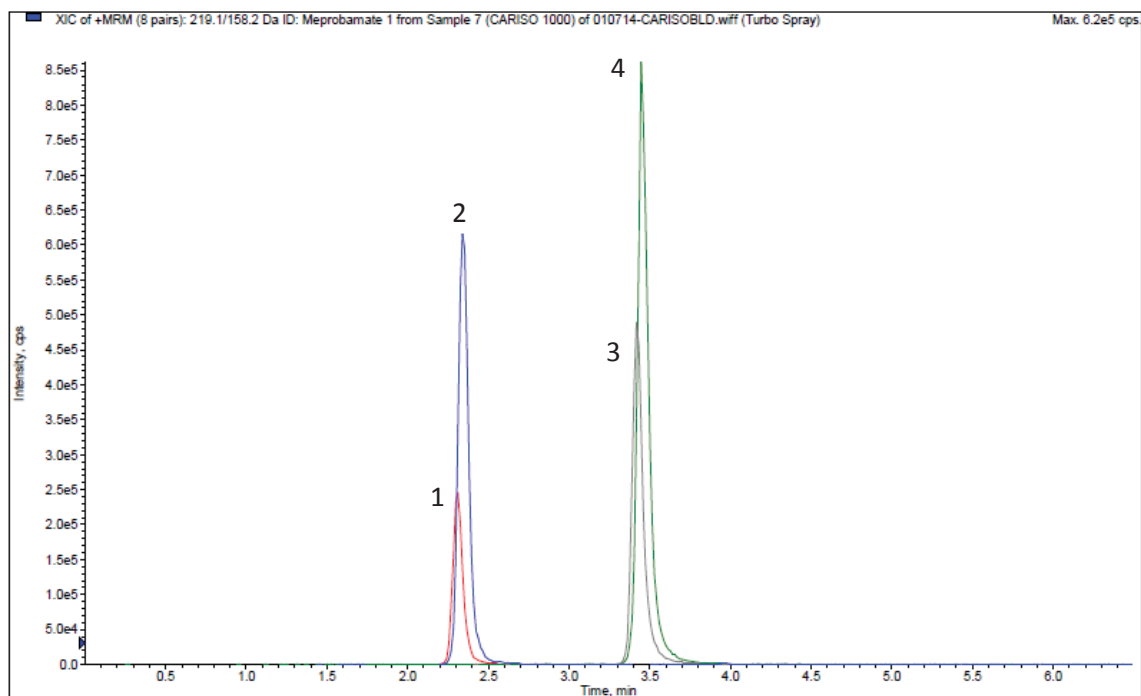
Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

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

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.MEPROBAMATE D 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. H₂O

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

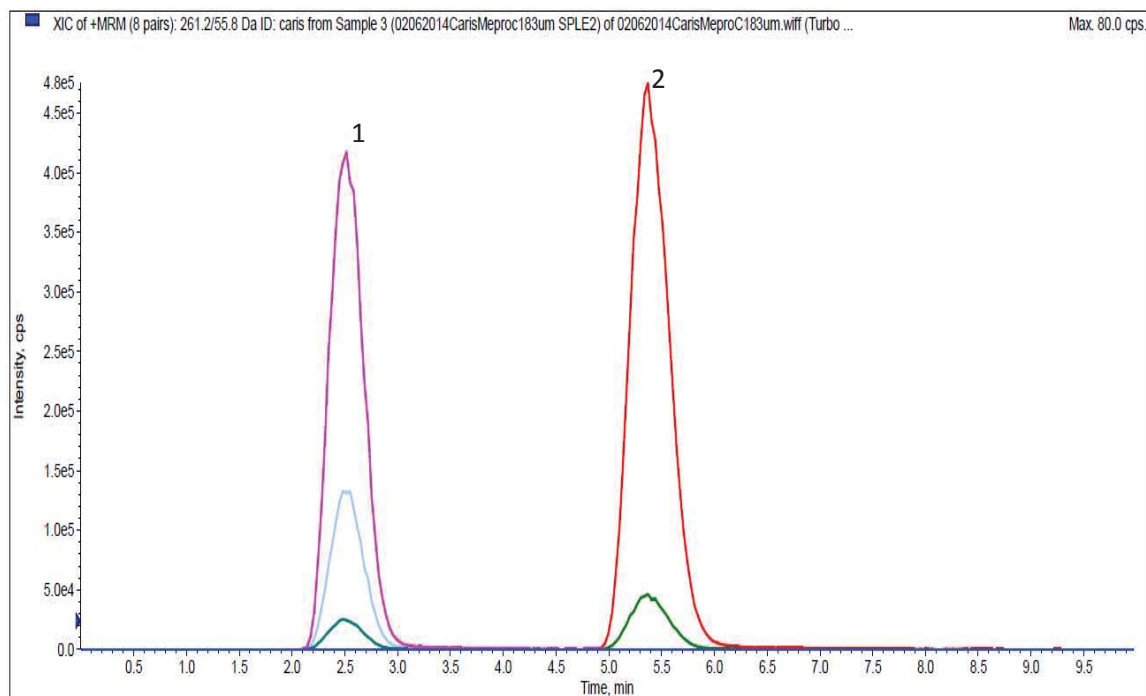
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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. H₂O

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

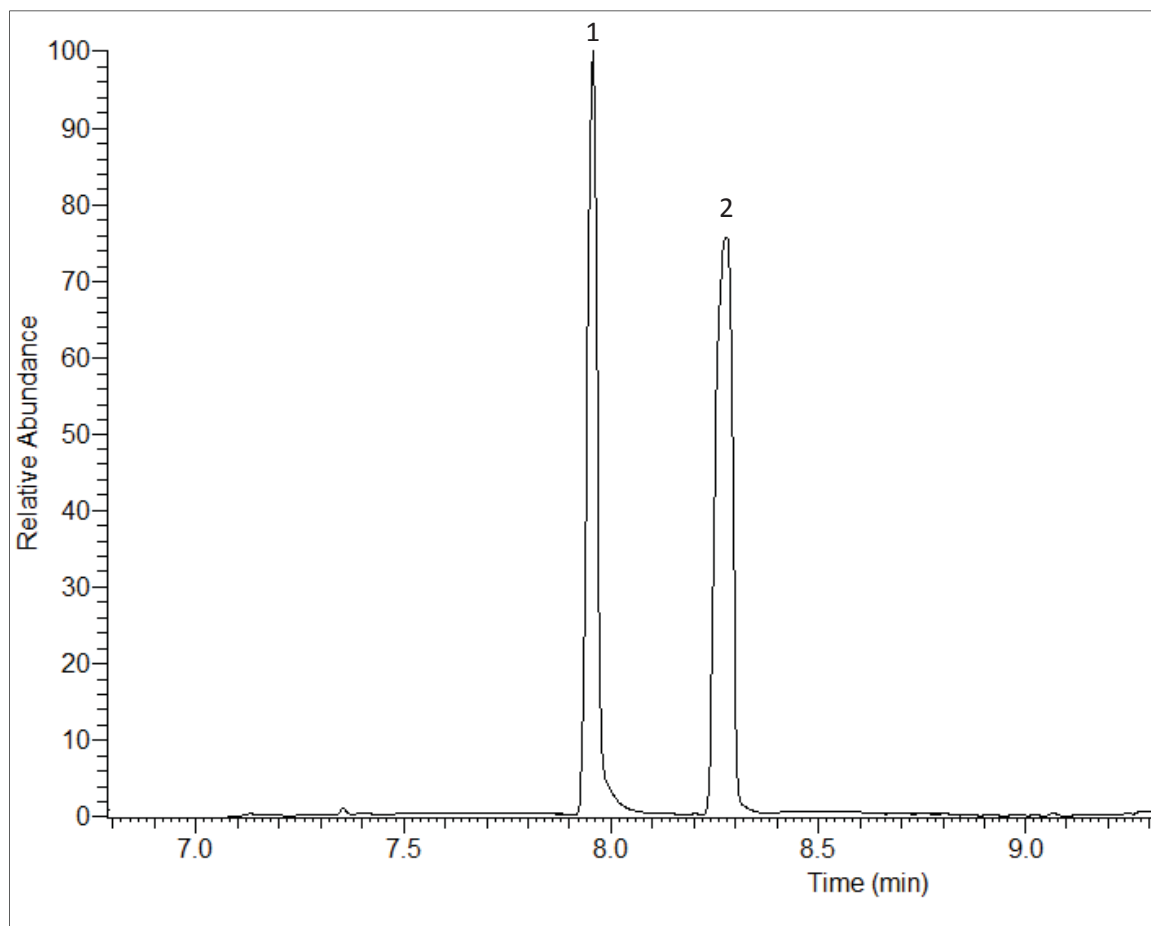
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 °C, MS Source: 350 °C, MS Quad: 150 °C



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. H₂O

Mix/vortex

2. CONDITION CLEAN-UP[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH

1 x 3 mL D.I. H₂O

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

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:

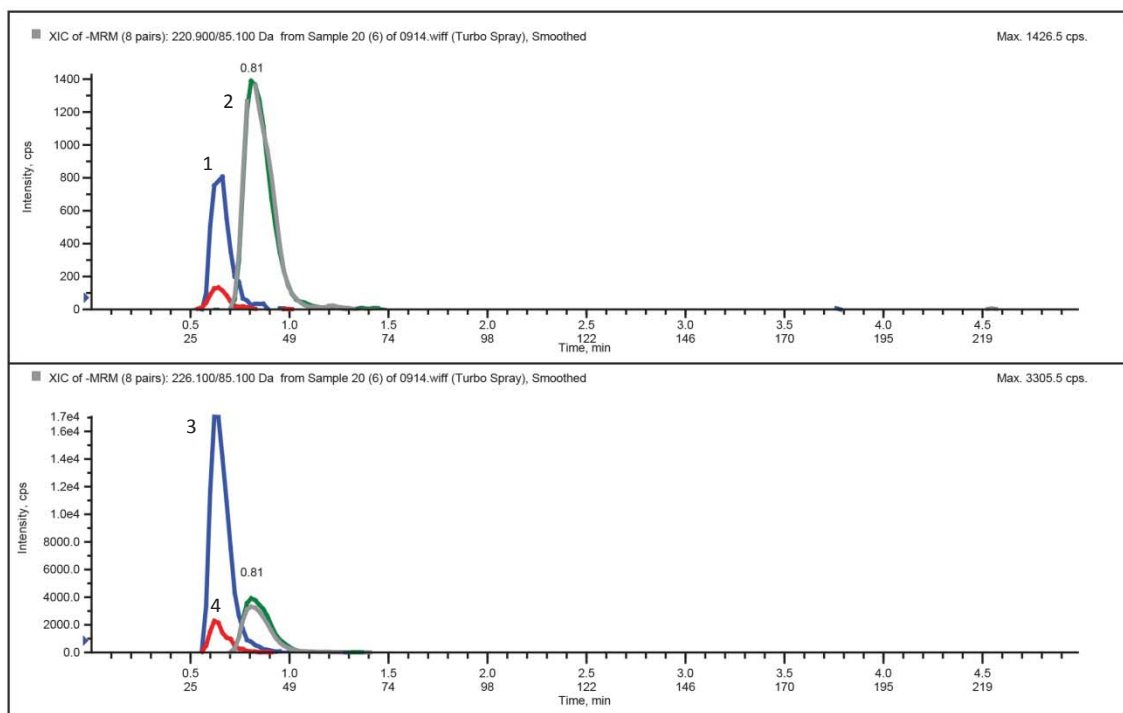
Evaporate to dryness at < 40 °C

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. H₂O

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 μ L 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 μ m 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 μ m filter for LC use.

LC Mobile Phase –Ammonium Acetate: Acetonitrile (10: 90) at a flow rate of 0.2 mL/minute.

Injection Volume – 10 μ L.

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 μ L/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).

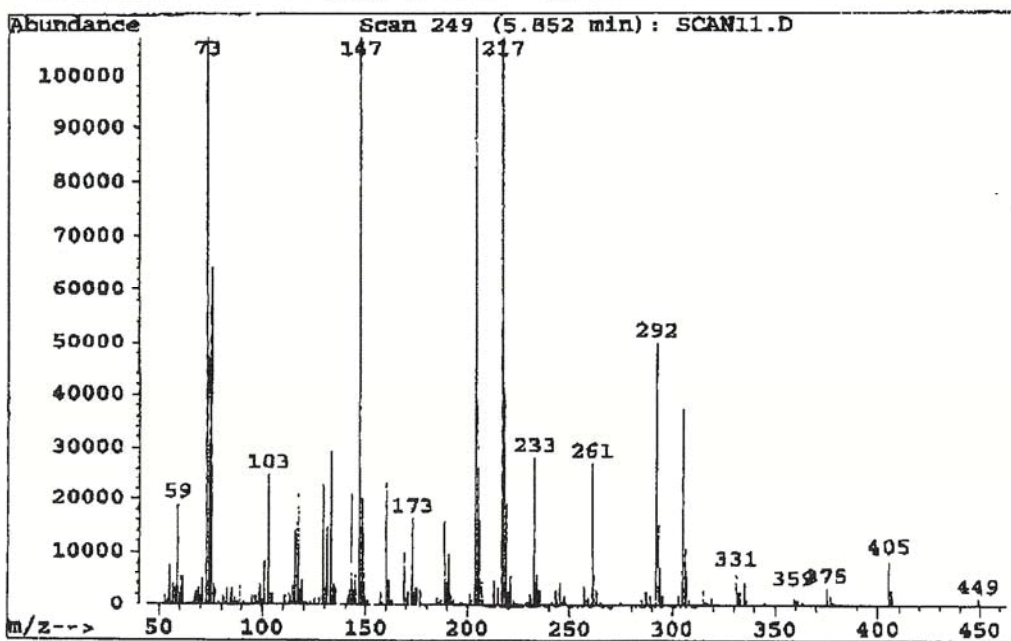
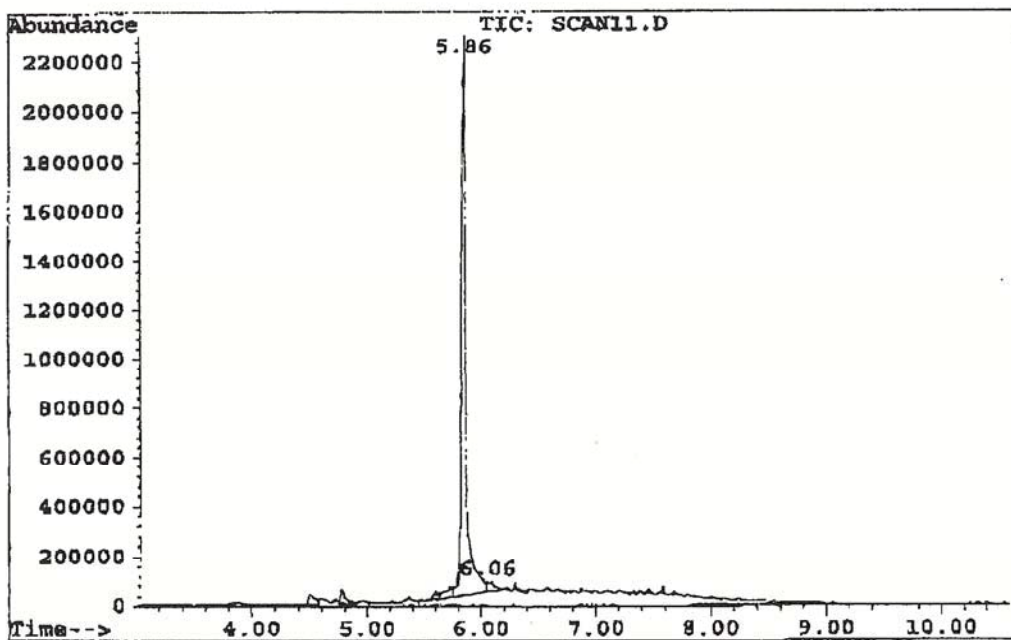
NOTES:

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 μ L of internal standard D₃-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 CH₃OH.

1 x 3 mL D.I. H₂O.

3. APPLY SAMPLE:

Decant supernatant onto SPE column.

4. WASH COLUMN:

1 x 10 mL of D.I. H₂O.

Dry with vacuum for 3 min.

1 x 5 mL of CH₃OH.

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 μ L of MSTFA + 1% TMCS and 25 μ L Ethyl Acetate.

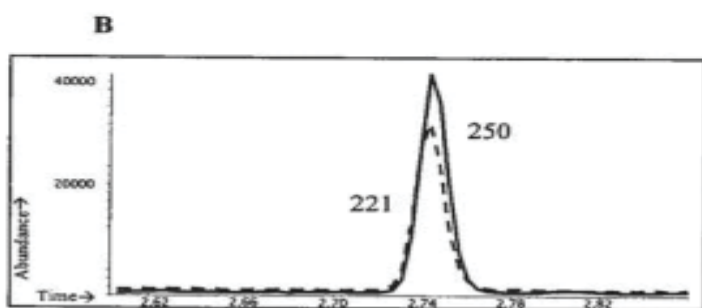
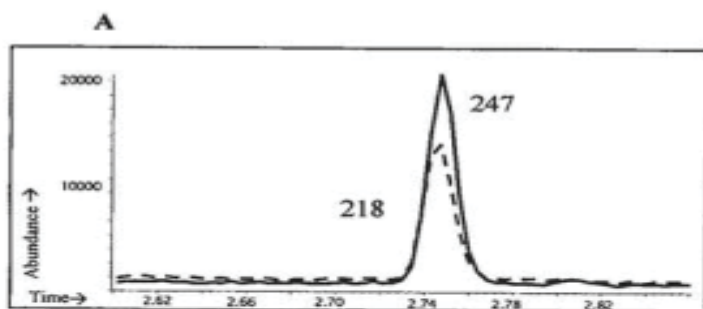
Heat for 20 min at 60 °C.

8. QUANTITATE:

Inject 1 to 2 μ L onto gas chromatograph.

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion	Relative Retention Time (minutes)
1. Methylmalonic Acid	247	218	2.76
2. Methylmalonic Acid-D ₃	250	221	2.74

PARAMETERS

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

GC capillary column: RtxR-200 MS 20m x 0.18mm, 0.4 μ m

Injector: 1 μ L Split 1:20 270 $^{\circ}$ C

Oven temperature program: 100 $^{\circ}$ C for 0.5min; 18 $^{\circ}$ C/min to 160 $^{\circ}$ C; 50 $^{\circ}$ C/min to 300 $^{\circ}$ C for 2.50minutes

Carrier gas: Helium

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 250 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C

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 CH₃OH
1 x 3 mL D.I. H₂O
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

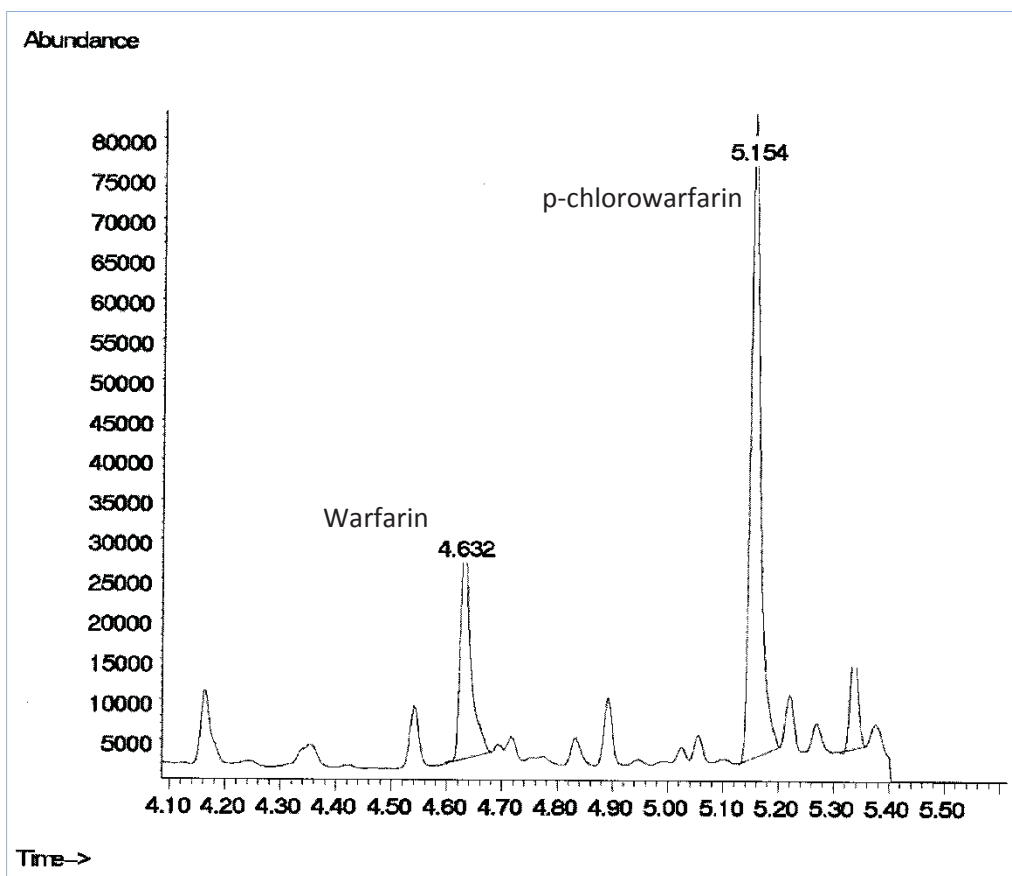
7. Dry samples:

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:

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

WARFARIN CHROMATOGRAM

GC-MS (methylation)





CLINICAL



FORENSICS



UCT

Basic Drugs





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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM acetic acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE ANTIDEPRESSANTS:

1 x 3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2 v/v)
Collect eluate at 1 to 2 mL/minute
or
1 x 3 mL Ethyl Acetate/ IPA/ NH₄OH (78:20:2 v/v)

NOTE: Prepare elution solvent daily
Add IPA/ NH₄OH, 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

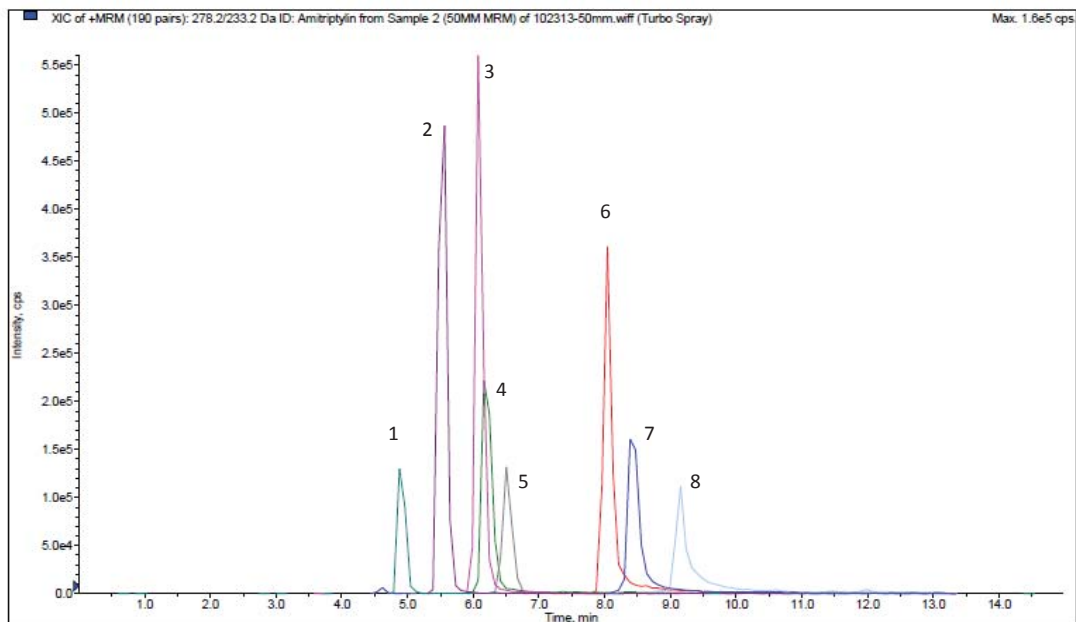
Alternate Derivatization

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. H₂O

Flow Rate: 0.5 mL/minute

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

Mobile Phase B: 0.1% Formic Acid in Methanol

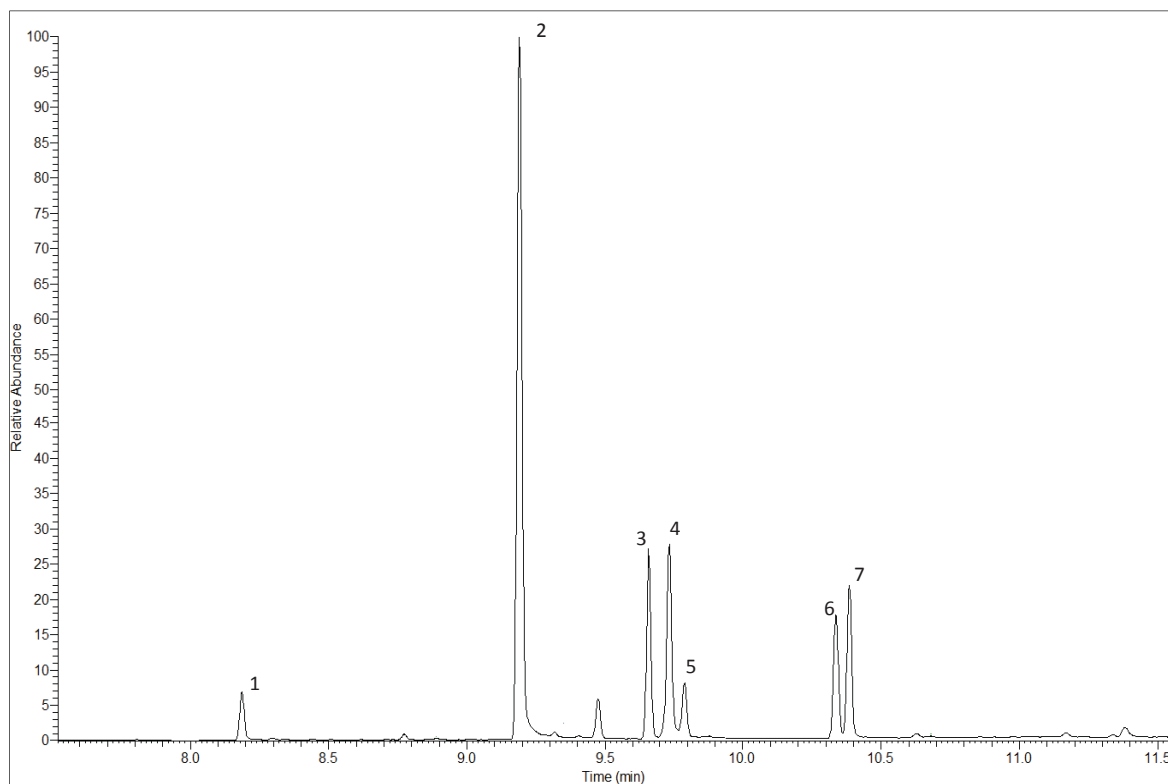
Polarity: Positive

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. Nortriptyline	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 (0.25 μ m) TG-1MS

Injector: 1 μ L Splitless, 250 $^{\circ}$ C

Oven temperature program: 70 $^{\circ}$ C (0.5) to 320 $^{\circ}$ C (25 $^{\circ}$ C/minute): hold (2 minutes)

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

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 350 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM acetic acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE BASIC ANALYTES:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

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

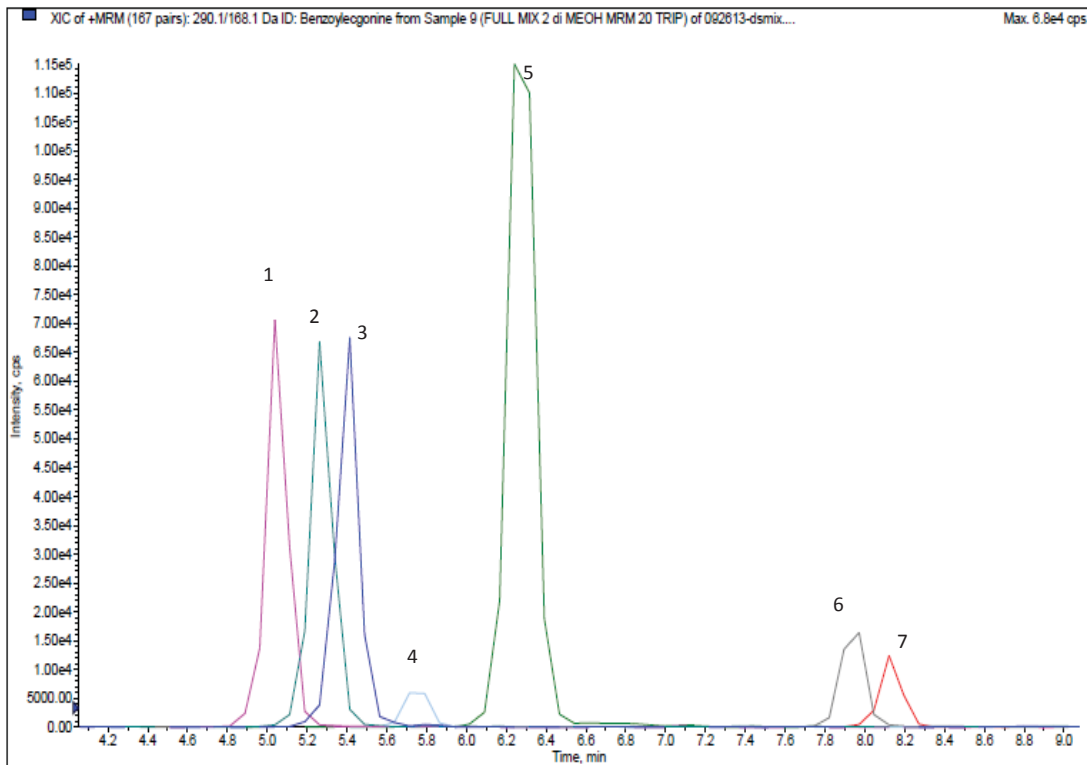
Alternate Derivatization

Dissolve residue in 50 μ L of Ethyl Acetate and 50 μ L of derivatizing reagent and react at 70 $^{\circ}$ C for 30 minutes; Cool and inject 1-2 μ L

INSTRUMENT CONDITIONS (LC-MS/MS):

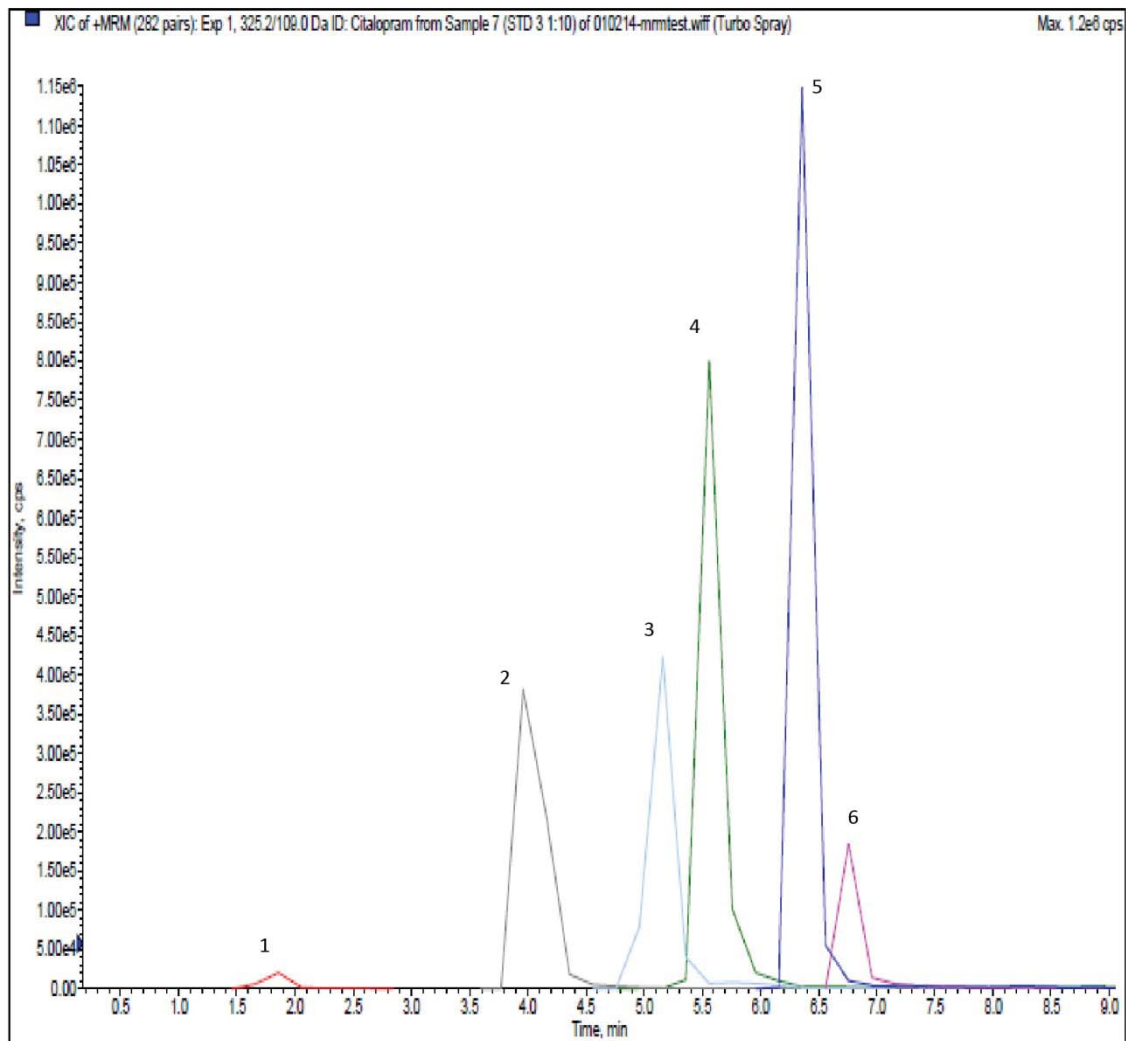
CHROMATOGRAMS

Basic Panel 1



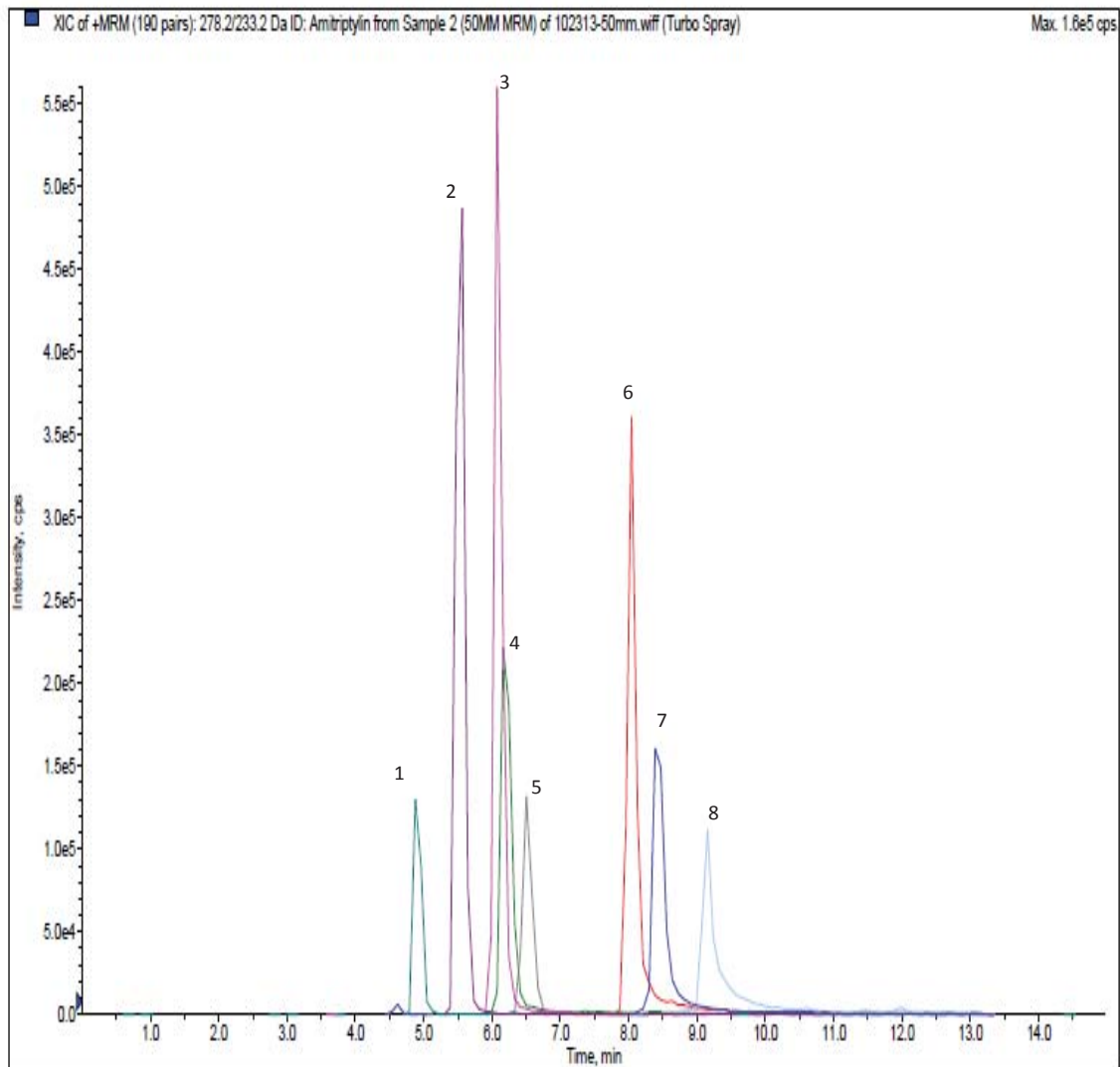
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.Tapentadol	222.2	107.2	5.10
2.Tramadol	264.2	58.0	5.25
3.Benzoyllecgonine	290.1	168.1	5.40
4.Meperidine	248.2	220.0	5.75
5.Cocaine	304.1	182.1	6.30
6.Fentanyl	337.2	188.2	7.90
7.Buprenorphine	468.3	396.3	8.15

Basic Panel 2



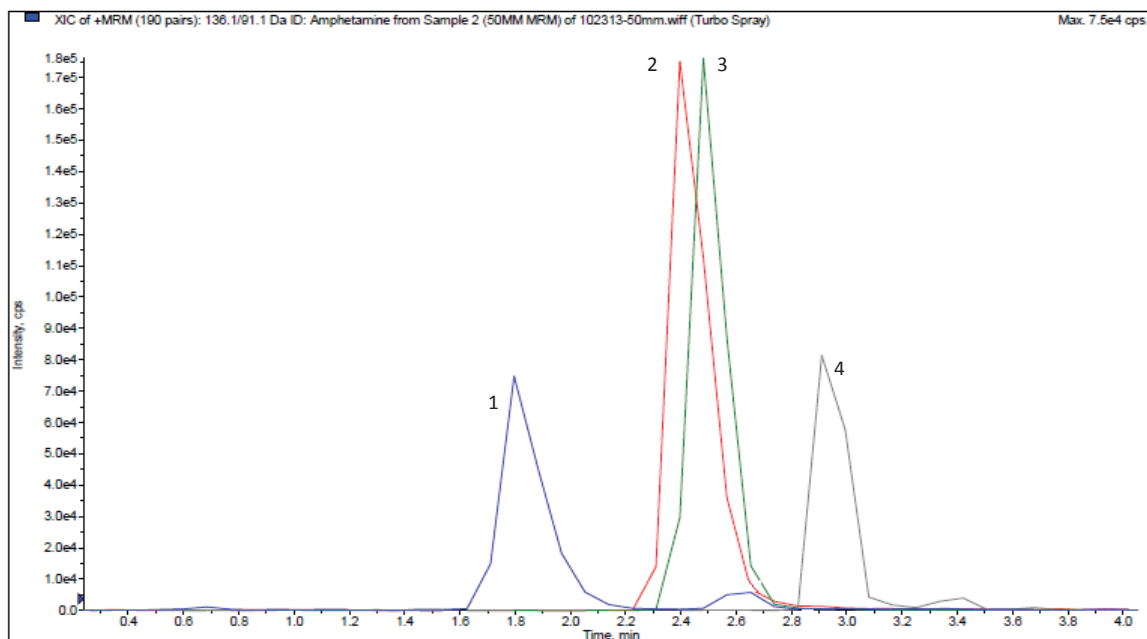
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

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. Quetiapine	384.2	253.1	6.50
6. Imipramine	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. H₂O

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 μ m

1. PREPARE SAMPLE:

To 1-2 mL whole blood, plasma/ serum or urine add 500 μ L 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 μ L of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme[®] β -glucuronidase. Optionally, add 500 μ L of acetate buffer and 25 μ L 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 CH₂Cl₂/ IPA/ NH₄OH (78:20:2)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (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 μ L of mobile phase
Inject 20 μ L.
- **GC-MS:** Dissolve residue in 100 μ L of Ethyl Acetate

Alternate Derivatization

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

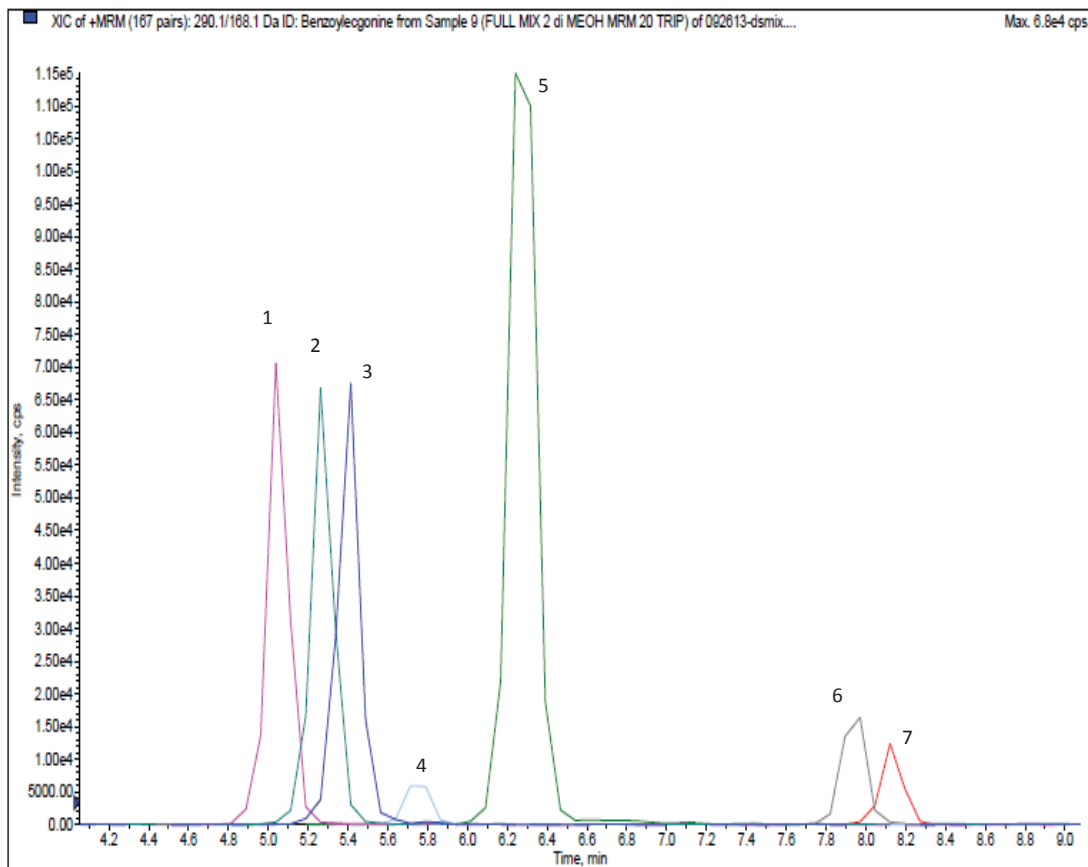
NOTES

(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):

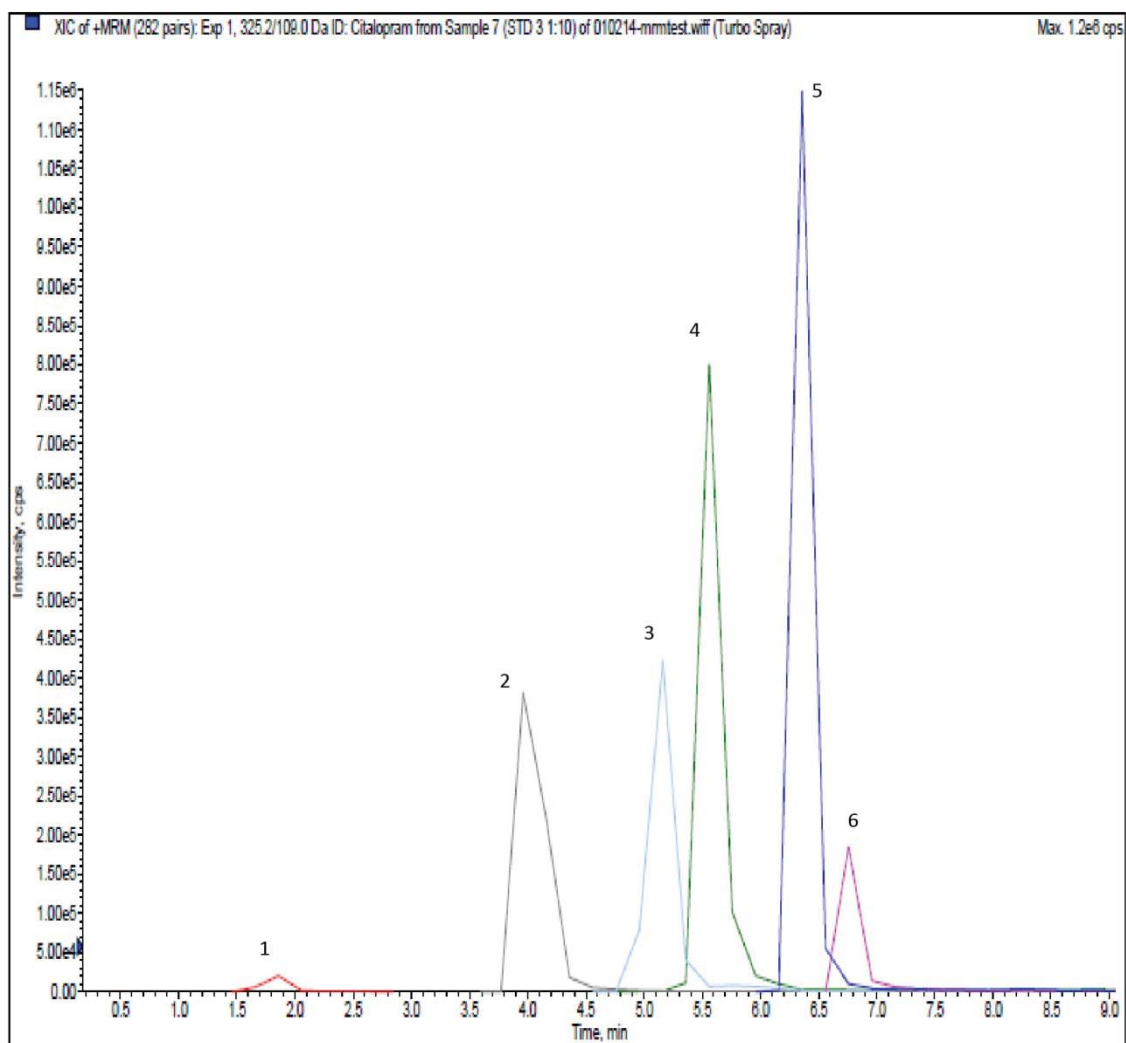
CHROMATOGRAMS

Basic Panel 1



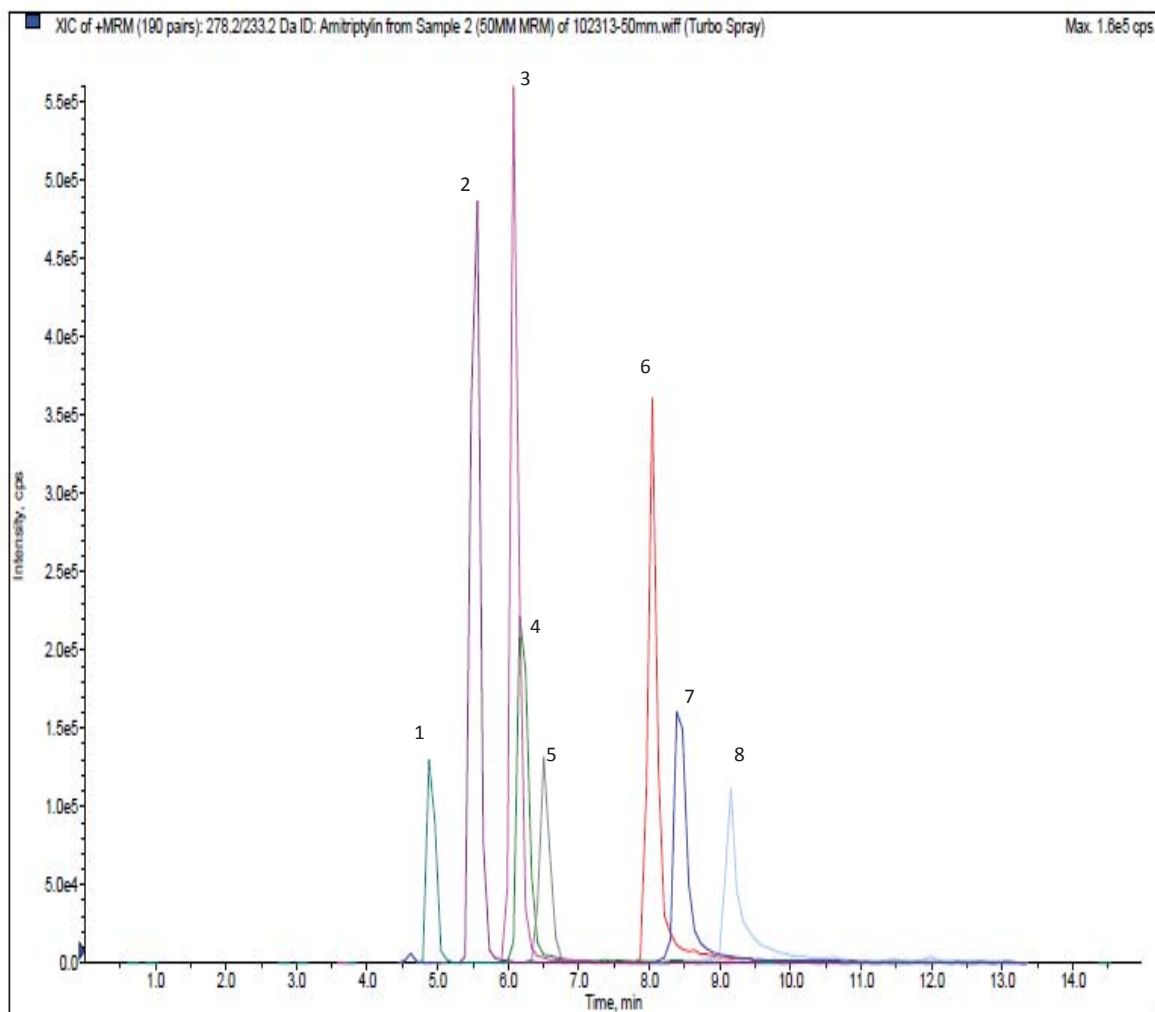
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylcegonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



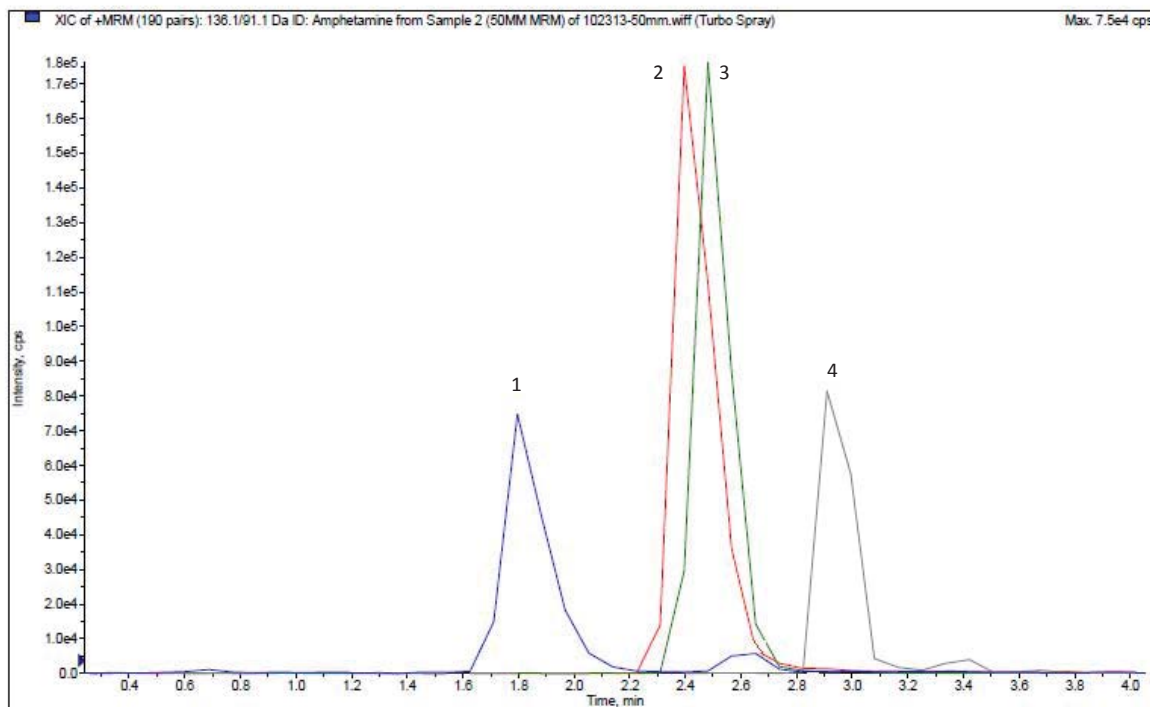
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

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. Quetiapine	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. H₂O

Flow Rate: 0.5 mL/minute

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

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 μ m

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
Mix/vortex and let stand for 5 minutes
Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
Sample pH should be 6.0 \pm 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge for 10 minutes at 2000 rpm and discard pellet
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 μ L of concentrated β -glucuronidase. Vortex and heat for 1-2 hours at 65 $^{\circ}$ 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 CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

5. DRY ELUTE

Evaporate fraction to complete dryness under stream of dry air or nitrogen at \sim 35 $^{\circ}$ C.

6. RECONSTITUTE / DERIVATIZE

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

Alternate Derivatization

Dissolve residue in 50 μ L of Ethyl Acetate and 50 μ L of derivatizing reagent and react at 70 $^{\circ}$ C for 30 minutes; Cool and inject 1-2 μ L

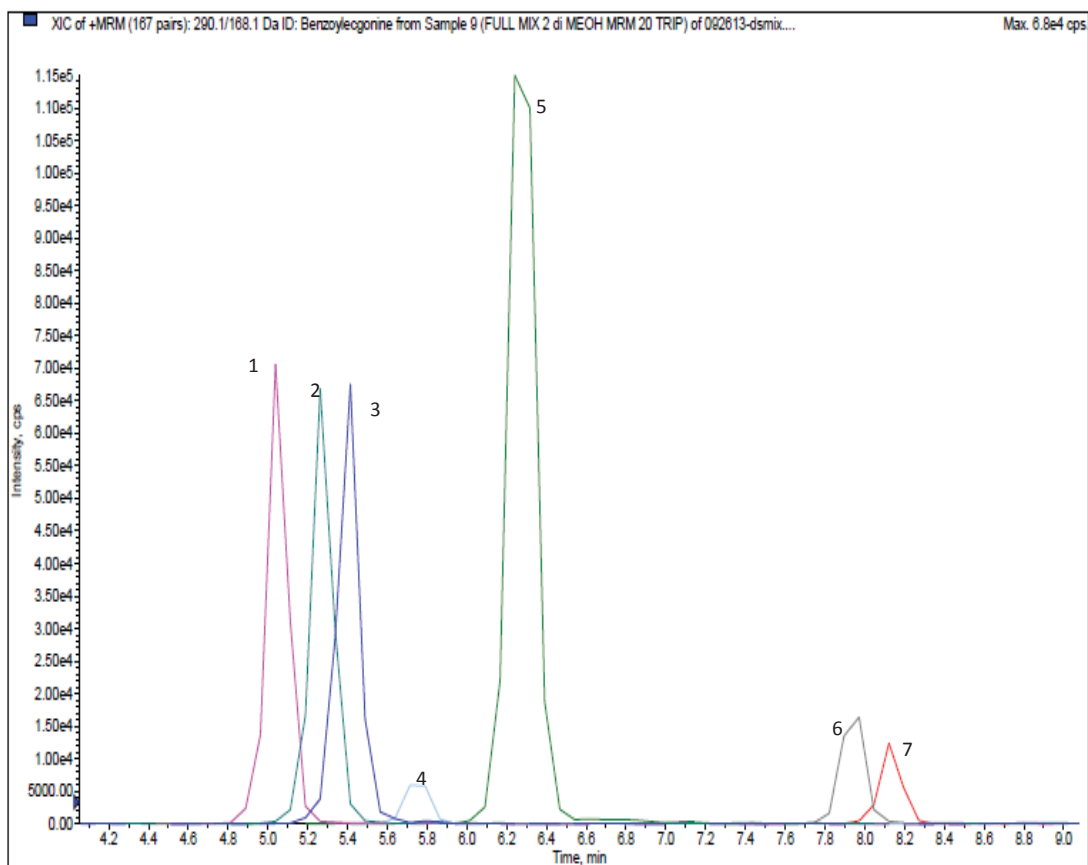
NOTES

(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):

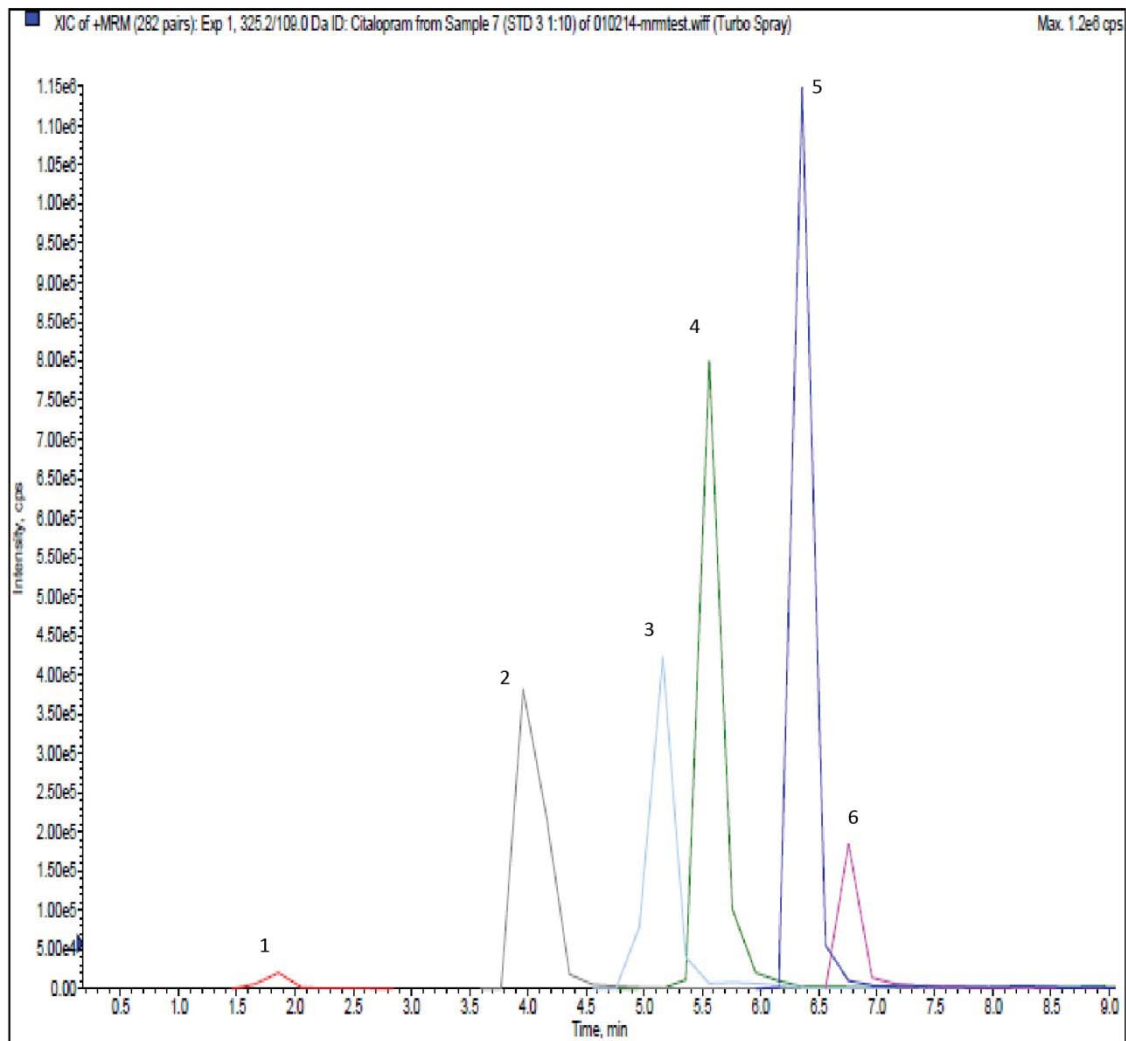
CHROMATOGRAMS

Basic Panel 1



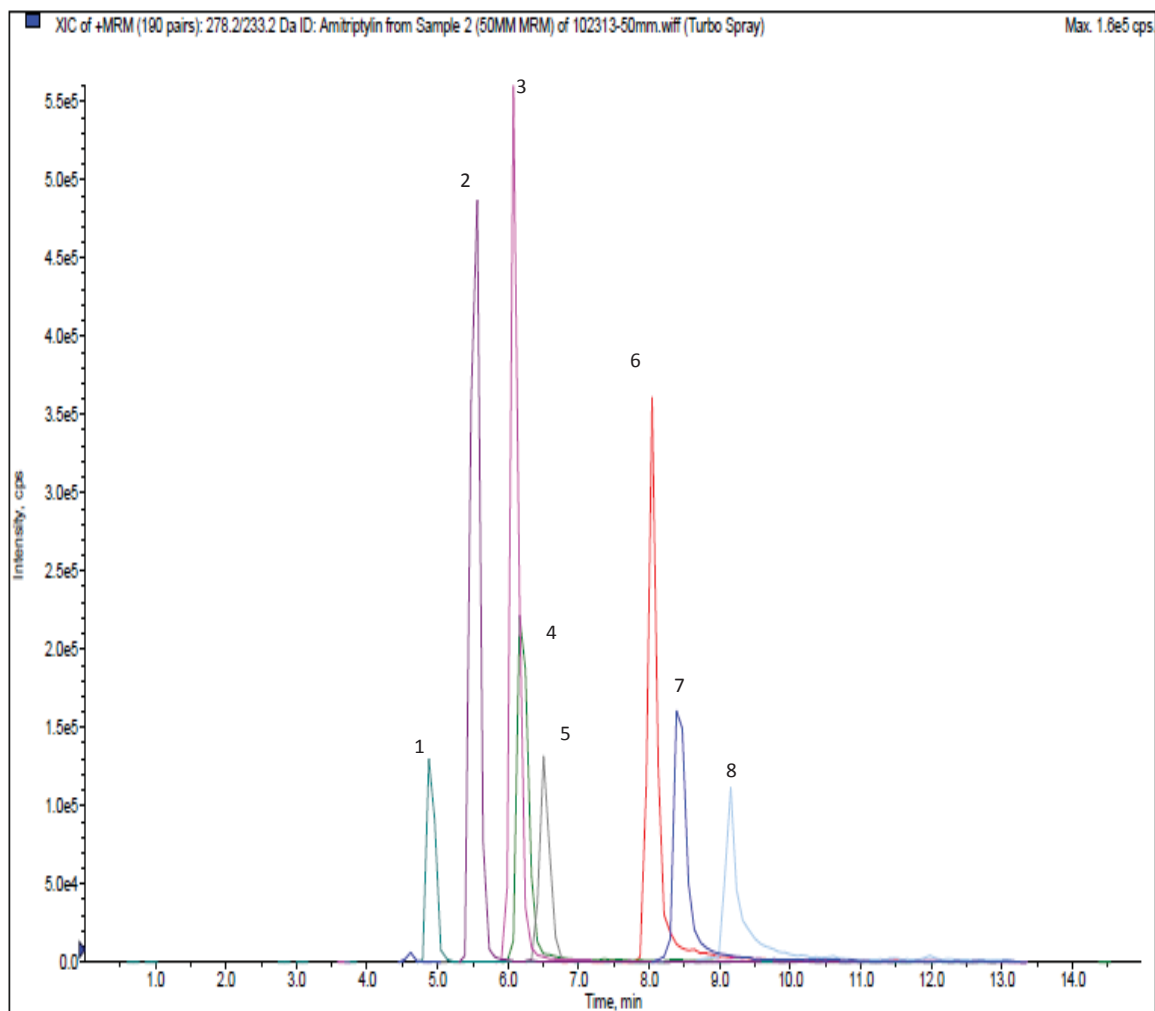
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylcegonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



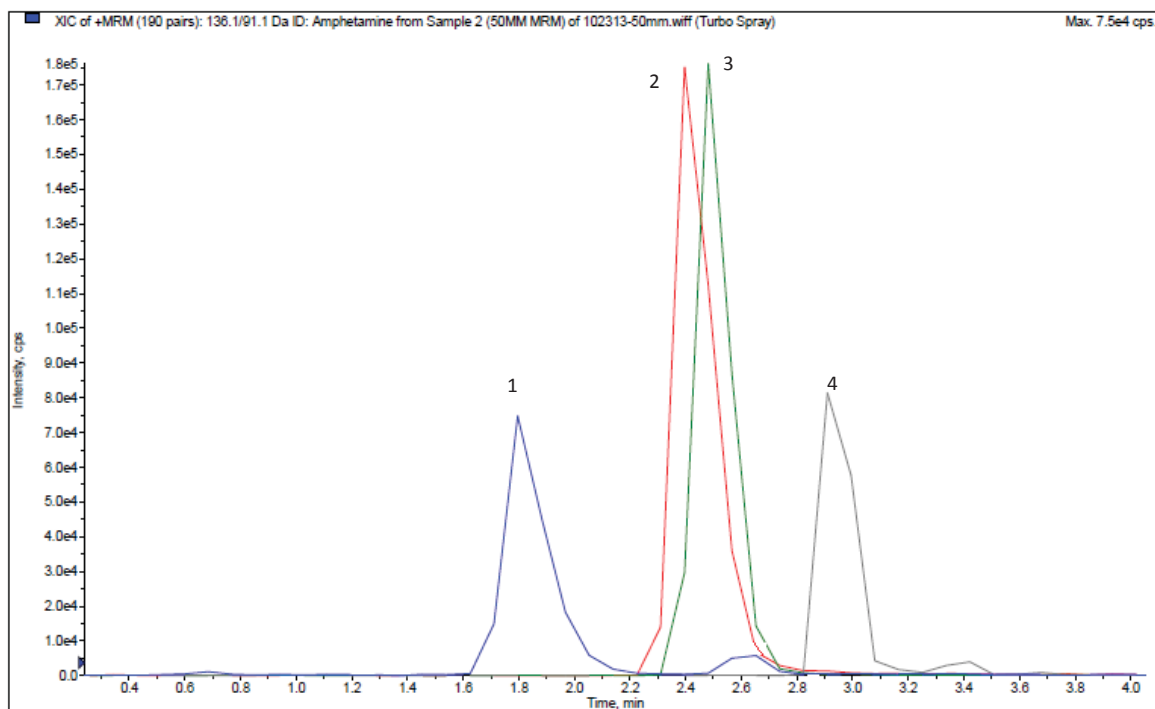
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

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. Quetiapine	384.2	253.1	6.50
6. Imipramine	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. H₂O

Flow Rate: 0.5 mL/minute

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

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 CH₃OH.
1 x 3 mL D.I. H₂O.
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 CH₂Cl₂/ IPA/NH₄OH (78:20:2).
Collect the eluate by gravity.
NOTE: Prepare elution solvent fresh daily. Add IPA/NH₄OH, mix, then add CH₂Cl₂ (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.

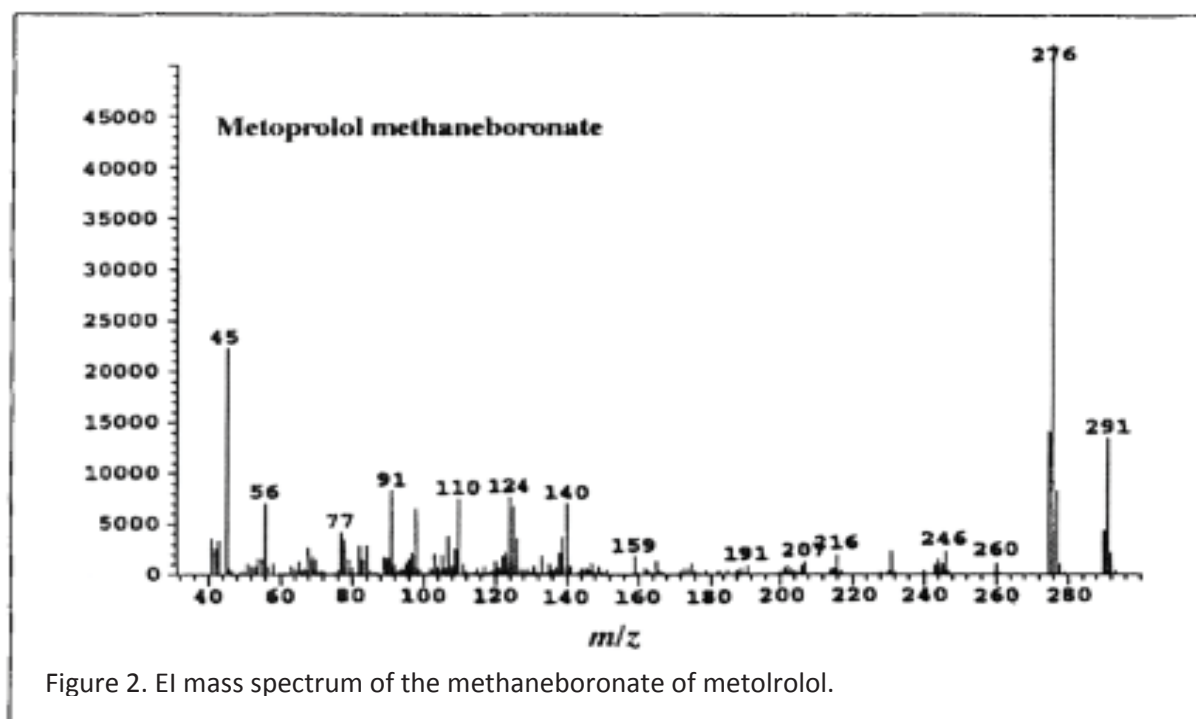
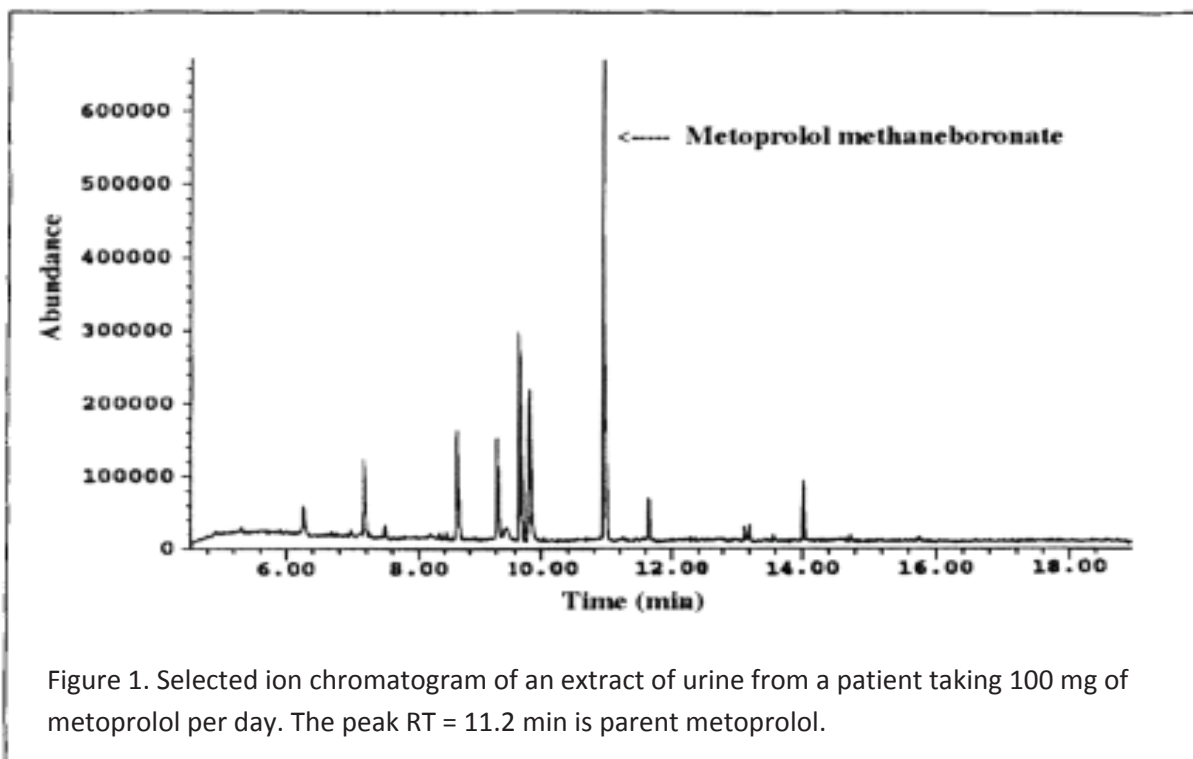
NOTE: Do not evaporate this solution.
- 8. ANALYSIS:**
Inject 1 to 2 µL sample.

Reference:

Branum G, Sweeney S, Palmeri A, Haines L and Huber C
The Feasibility of the Detection and Quantitation of β 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



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 μ L 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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

1 x 3 mL 100 mM acetate buffer (pH 5.0)

1 x 3 mL CH₃OH

Dry column (5-10 minutes at full vacuum or pressure)

5. ELUTE BUPRENORPHINE/NORBUPRENORPHINE:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2 v/v)

Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

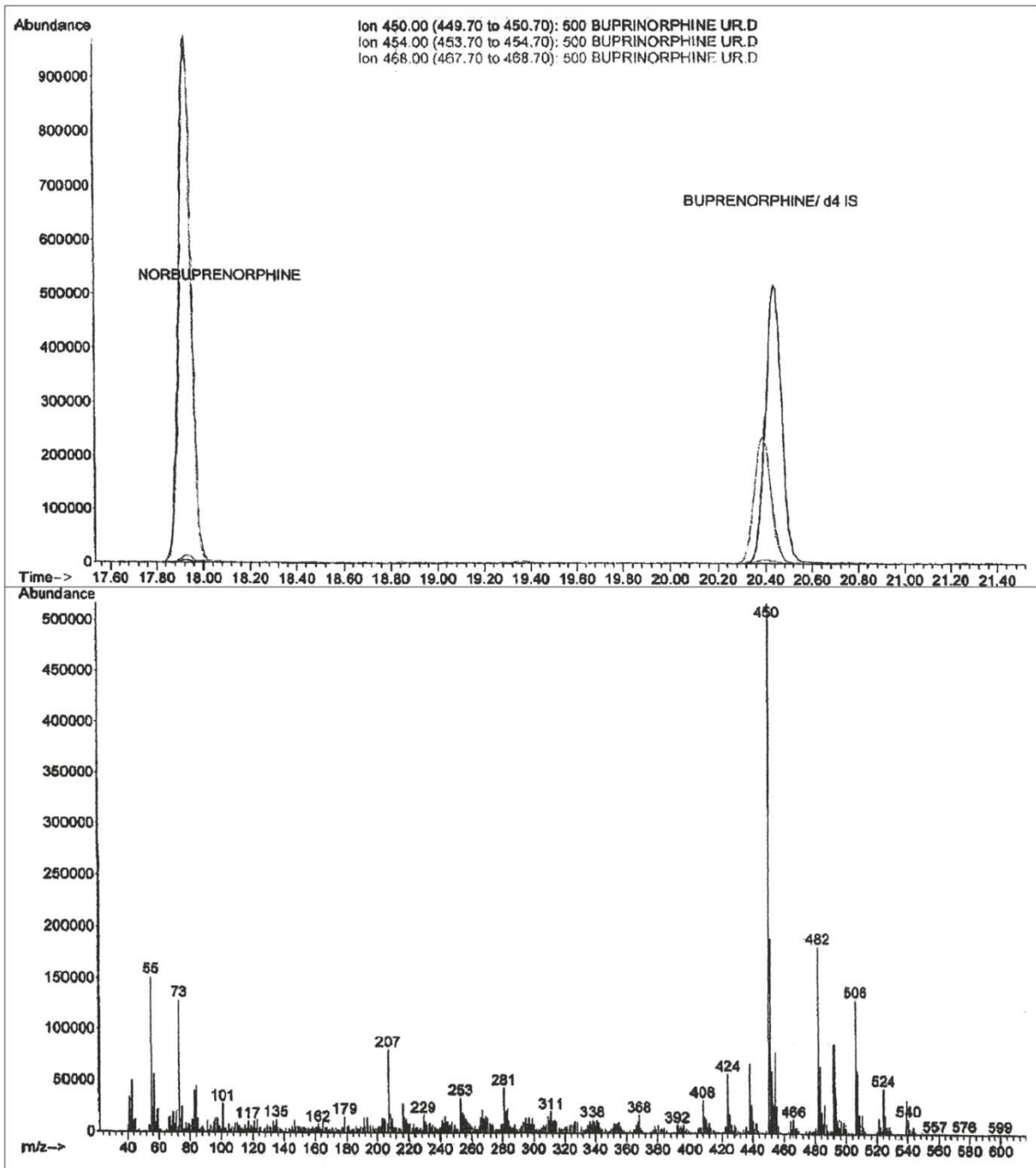
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 10 µL.
- **GC-MS:** Dissolve residue in 100 µL of Ethyl Acetate

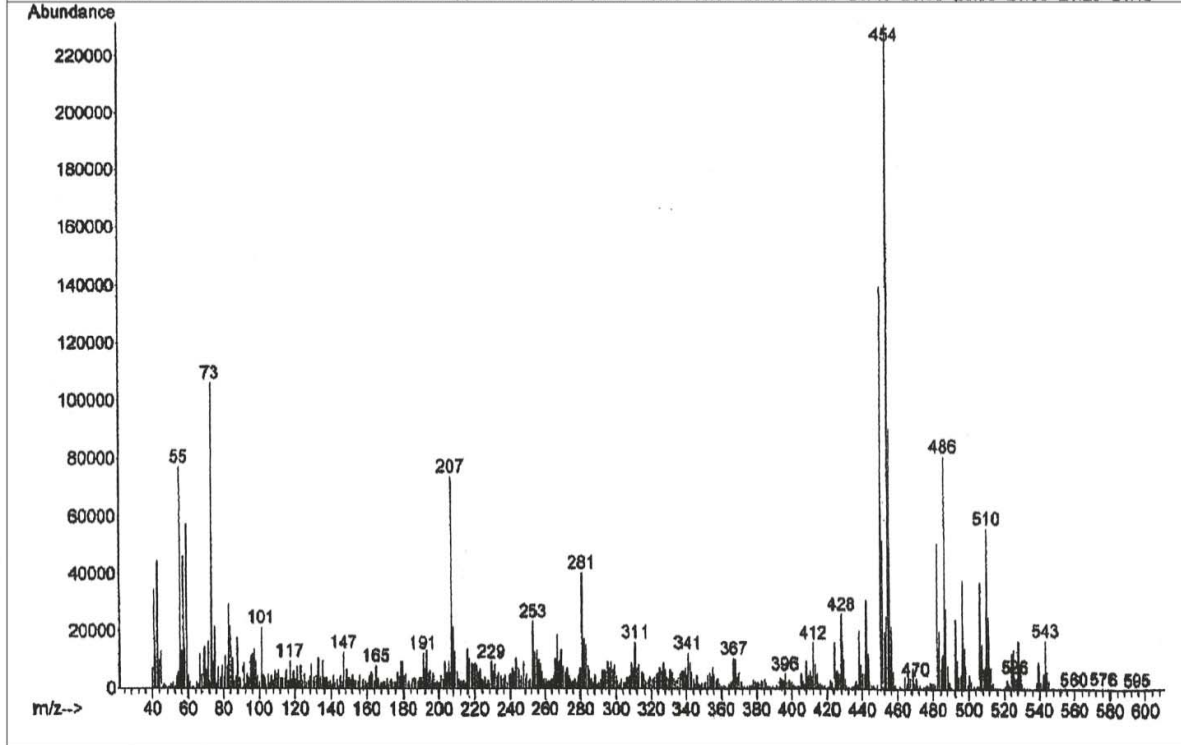
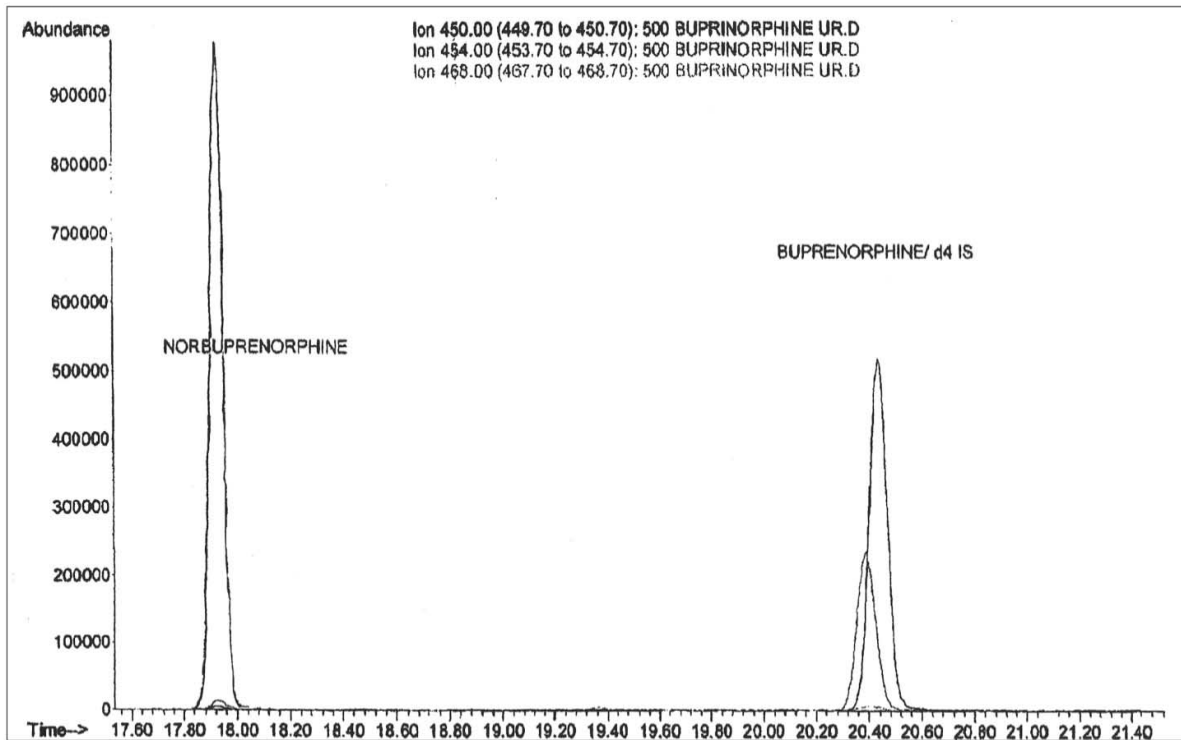
Alternate Derivatization

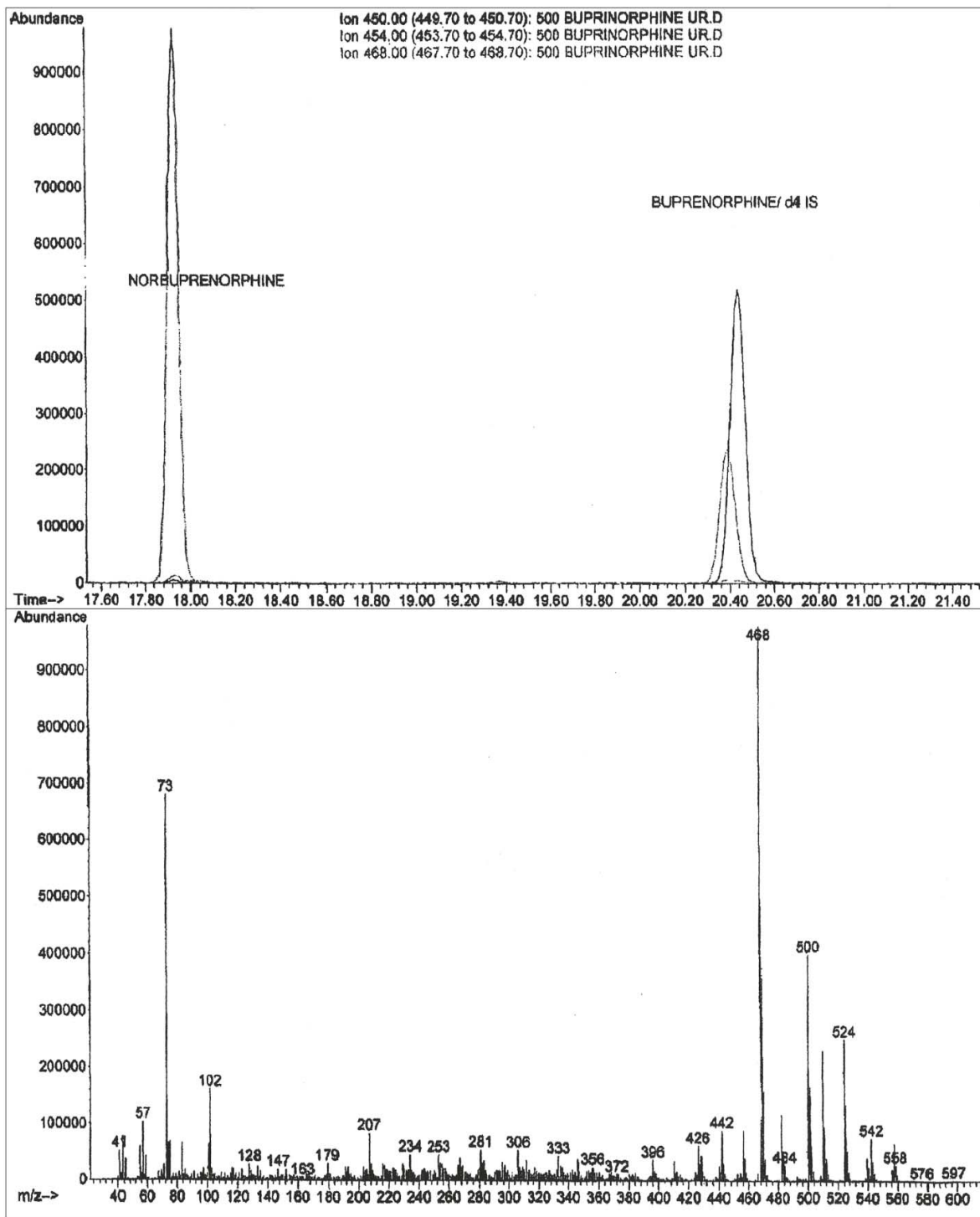
- Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of 50 µL BSTFA w/
1% TMCS react at 70 °C for 30 minutes; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAMS







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 phosphate 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 H₂O.

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. H₂O

1 x 0.5 mL 1% NH₄OH in D.I. H₂O.

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

5. ELUTE CLOZAPINE:

1 x 0.2 mL 1% NH₄OH 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 μ m) Zorbax: Agilent Technologies.

Mobile phase: Acetonitrile: 0.1% Formic Acid (33:67).

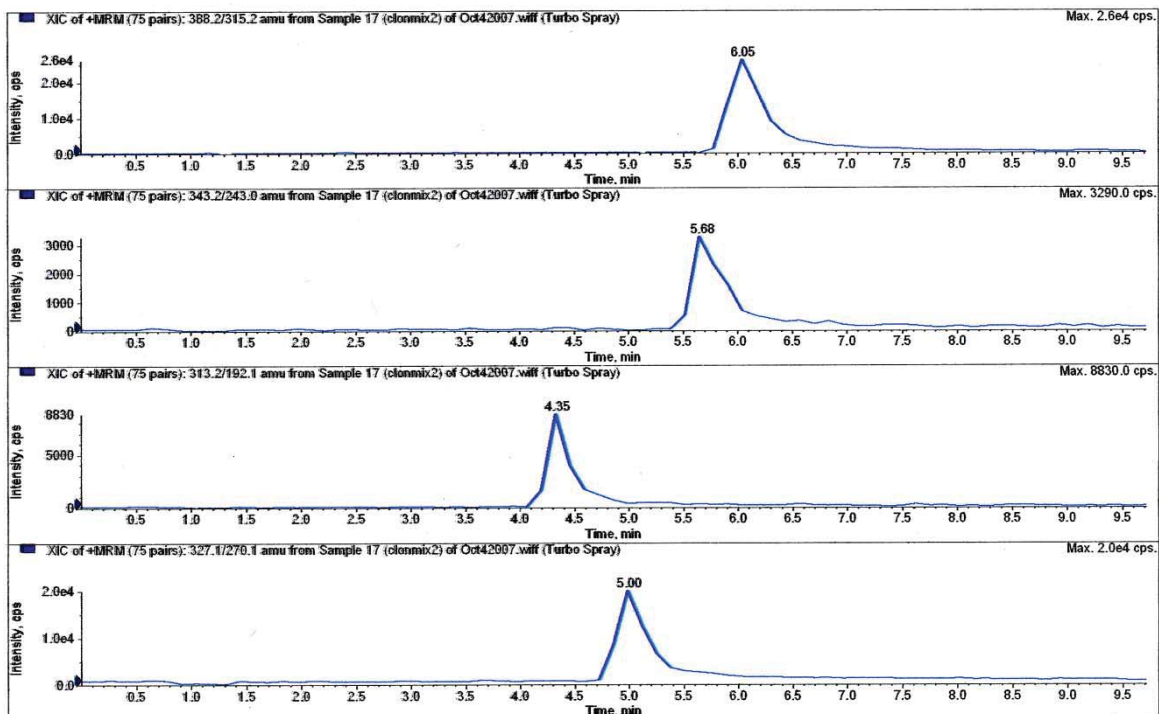
Flowrate: 0.35 mL/min.

Column Temperature: ambient.

Detector: API 2000 MS/MS.

HP1100 Diode-Array (230 nm).

<u>Compound</u>	<u>MRM Transition</u>
Clozapine	327.1/270.1
Desmethylozapine	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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

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 CH₃OH containing 2% NH₄OH

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

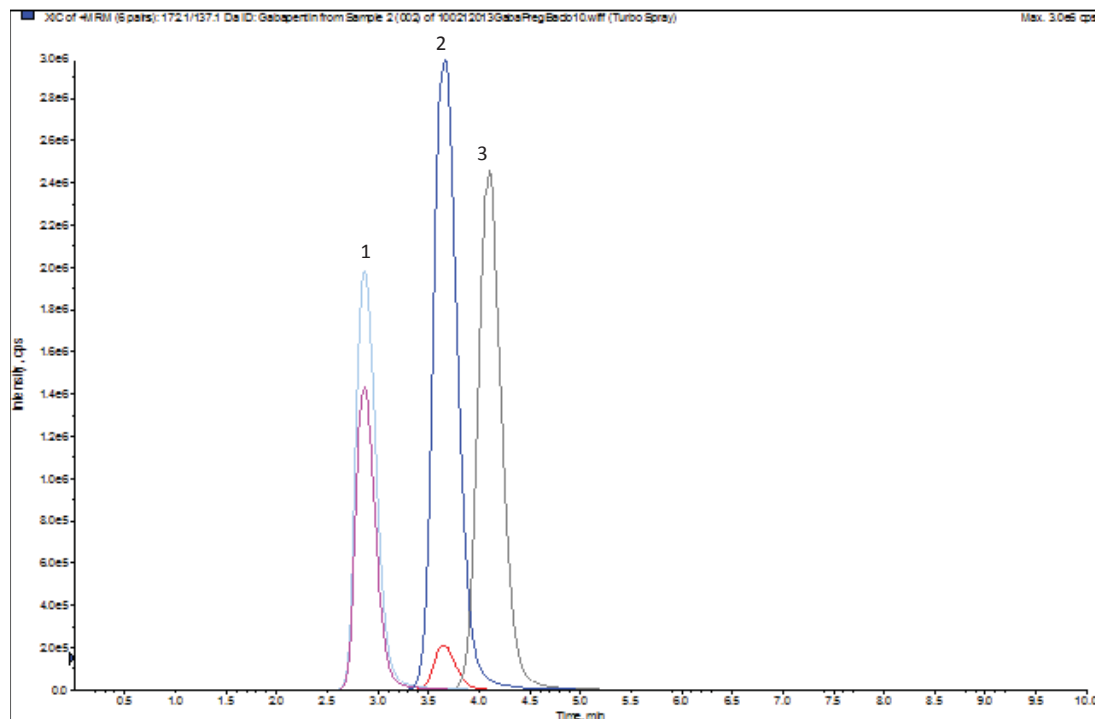
- 50 µL of Ethyl Acetate and 50 µL of MTBSTFA w/1% TBDMCS

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. H₂O

Flow Rate: 0.5 mL/minute

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

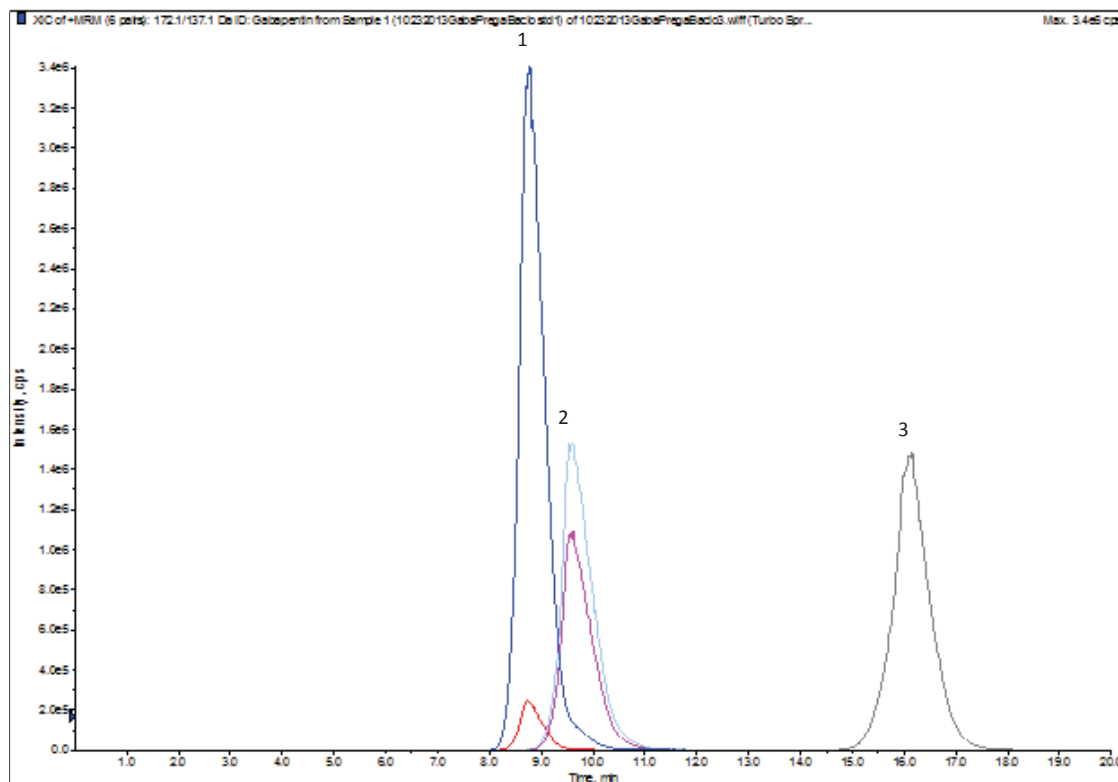
Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

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. H₂O

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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O.

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 CH₂Cl₂/ IPA/ NH₄OH (78:20:2)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

6. DRY ELUATE:

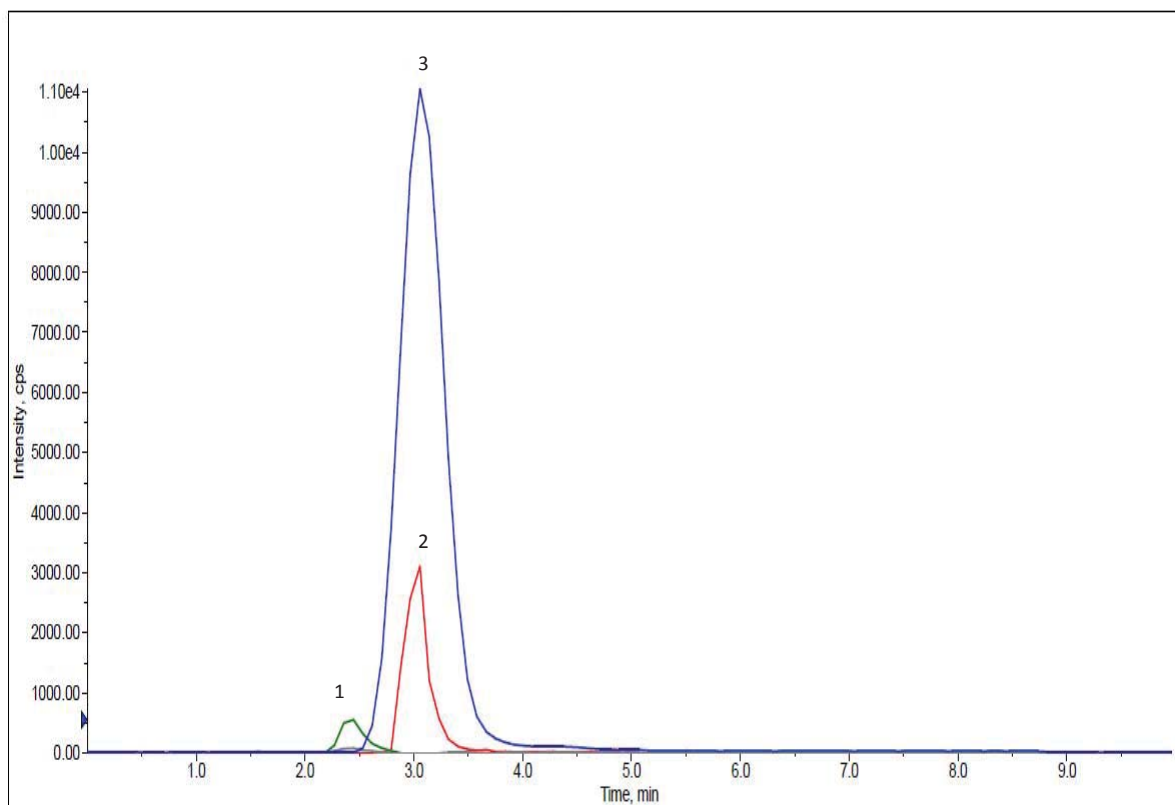
Evaporate to dryness at < 40 °C.

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. H₂O

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

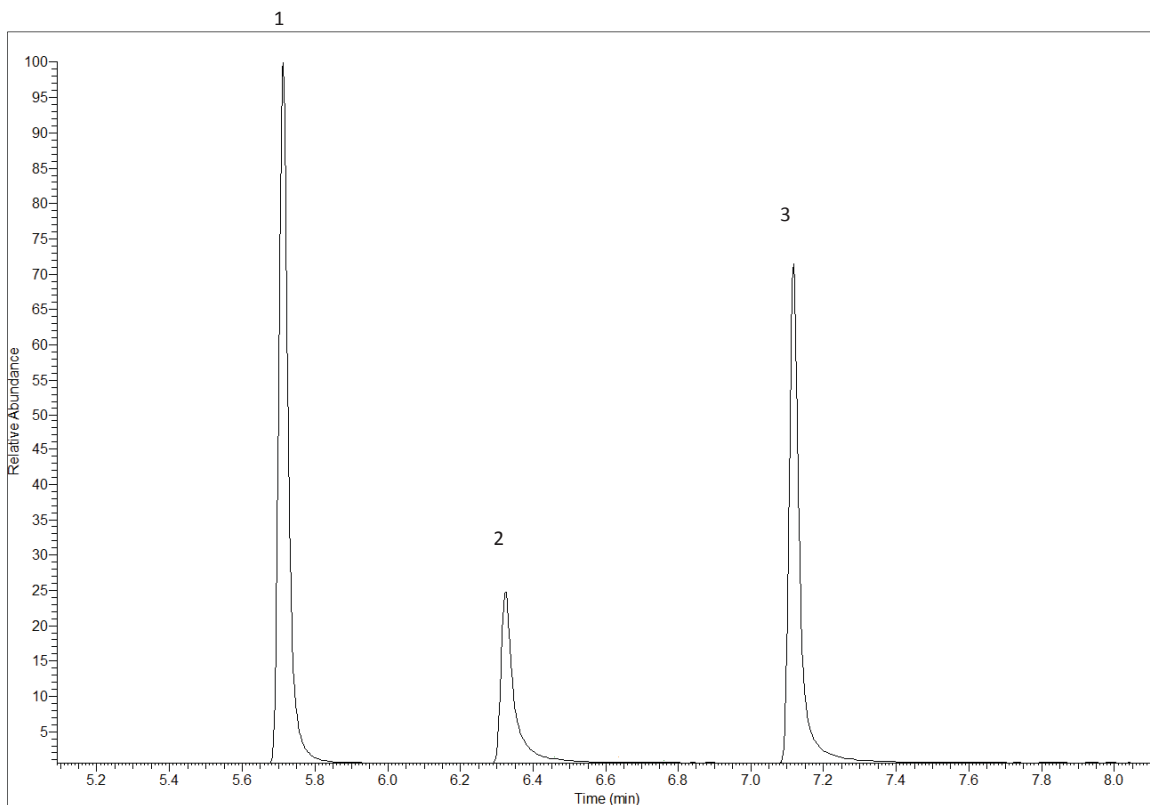
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 °C, MS Source: 300 °C, MS Quad: 150 °C



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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM acetic acid
1 x 3 mL CH₃OH
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE PAROXETINE:

1 x 3 mL Ethyl Acetate: Acetonitrile: NH₄OH (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:	<u>Time</u>	<u>Acetonitrile</u>	<u>0.1% Formic Acid aq</u>
	0	10	90
	15	50	50
	16	10	90
	20	10	10

Flow rate: 0.35 mL/ minute

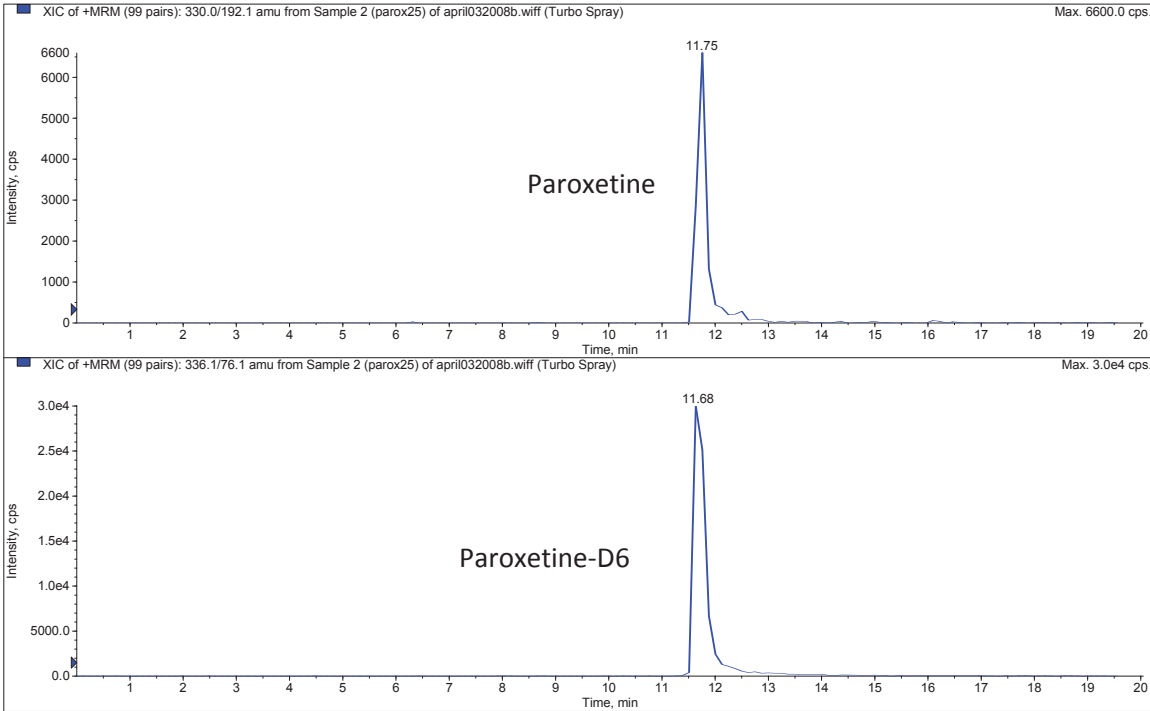
Injection Volume: 5 µL

Column Temperature: ambient

Detector: API 2000 MS/MS.

<u>Compound</u>	<u>MRM Transition</u>
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 CH₃OH.

1 x 3 mL D.I. H₂O.

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 CH₃OH.

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/ NH₄OH (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. Reconstitue sample in 100 µL 0.1% trifluoroacetic acid (aq).

Inject 50 µL.

INSTRUMENT CONDITIONS:

Column: C₁₈ 150 x 4.6 mm (3 μm) Zorbax (Agilent Technologies).

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

Flowrate: 1 mL / min.

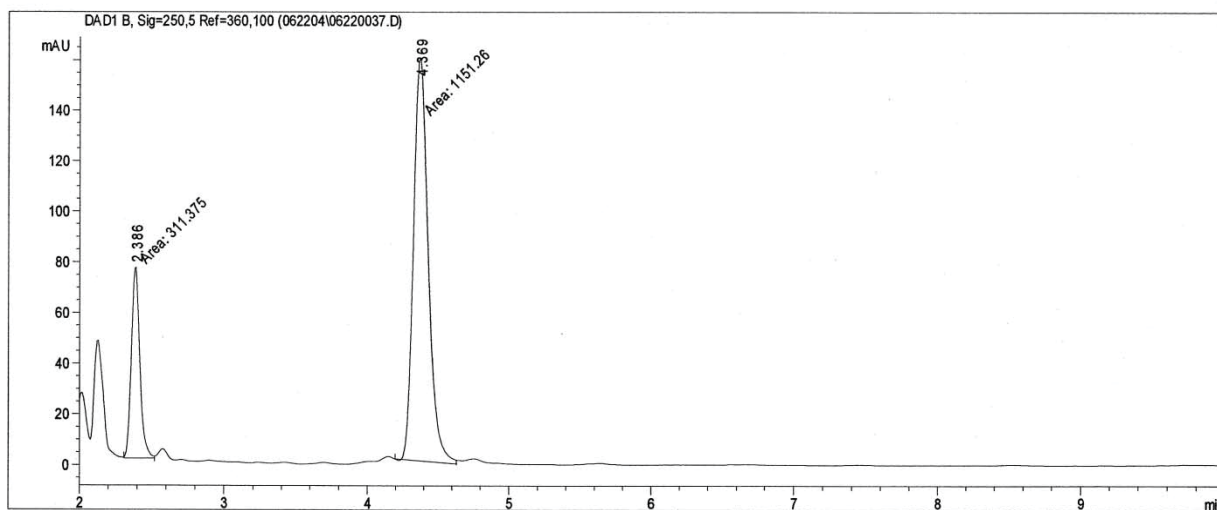
Column Temperature: 35 °C.

Detector: Diode Array (250 nm).

Chromatogram:

Quetiapine

Quinidine (internal standard)





CLINICAL

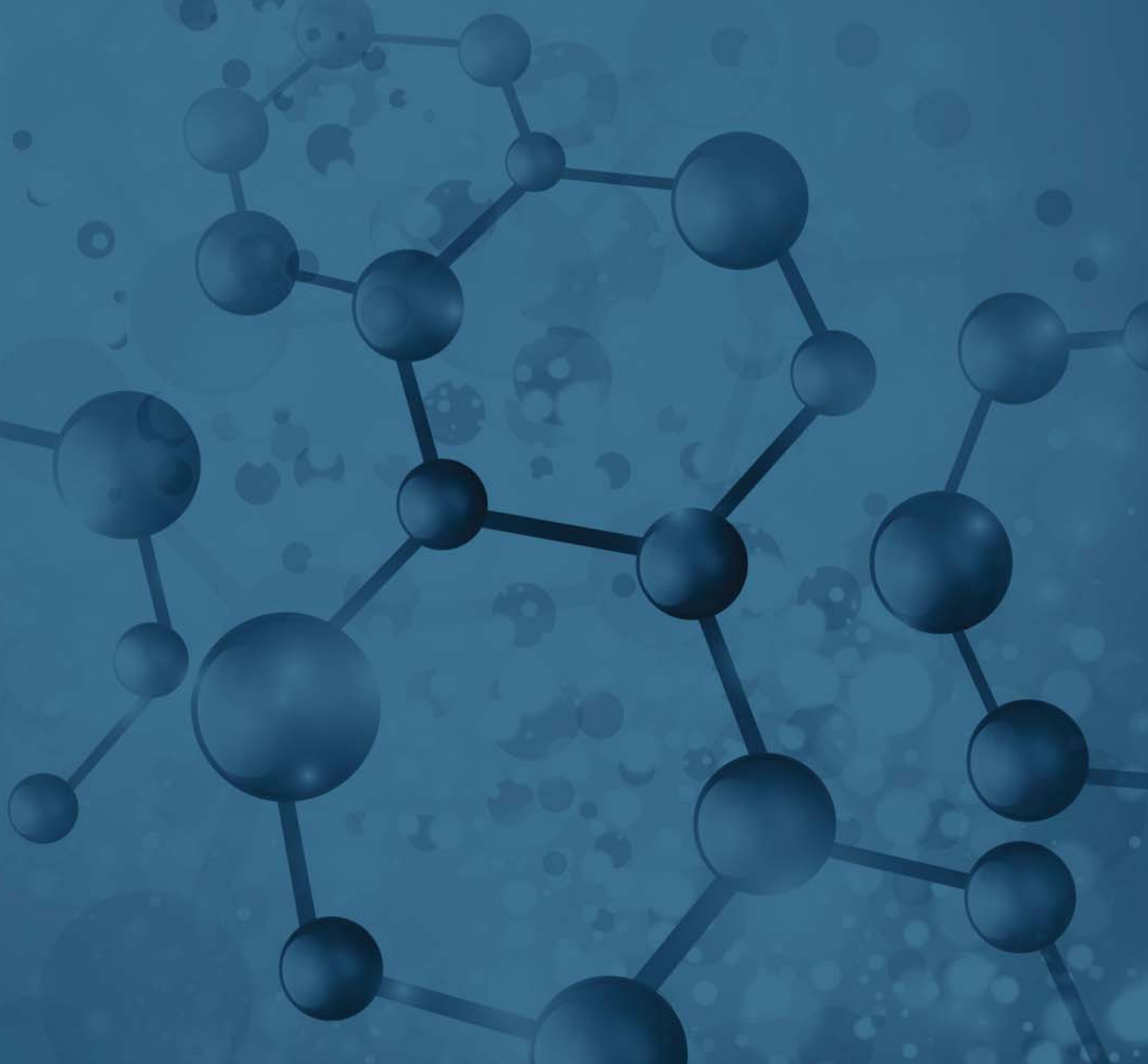


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COCAINE AND BENZOYLECGONINE IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

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 CH₃OH.
1 x 3 mL D.I. H₂O.
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. H₂O.
1 x 3 mL 100 mM HCl
1 x 3 mL CH₃OH.
Dry column (10 minutes at full vacuum or pressure).

5. ELUTE COCAINE/METABOLITES:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

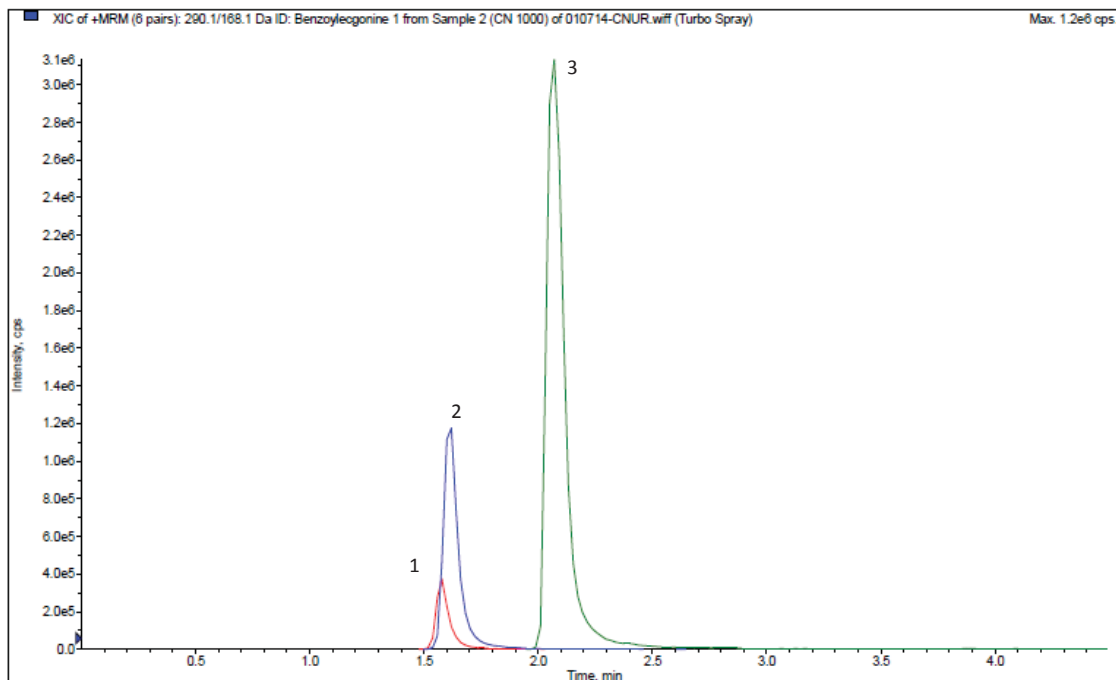
6. DRY ELUATE:

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 N₂ 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. Benzoylcegonine D ₈	298.1	171.1	1.58
2. Benzoylcegonine	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. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

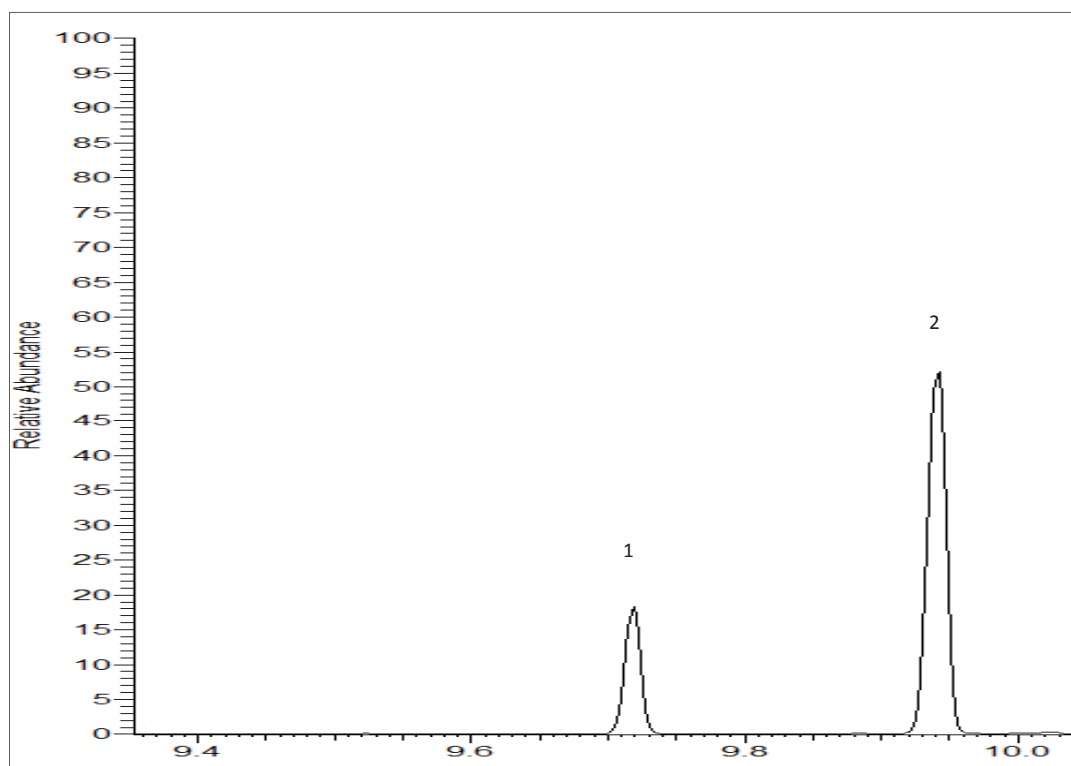
LC Column: Selectra[®] DA HPLC Column 50 x 2.1 mm 5 µm

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. Benzoyecgonine TMS	240	256	361	9.94
Benzoyecgonine 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 °C, MS Source: 350 °C, MS Quad: 150 °C



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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O.

1 x 1 mL 100 mM HCl

1 x 3 mL CH₃OH.

Dry column (10 minutes at full vacuum or pressure).

5. ELUTE COCAINE/METABOLITES

1 x 3 mL CH₂Cl₂/ IPA /NH₄OH (78:20:2)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

6. DRY ELUATE

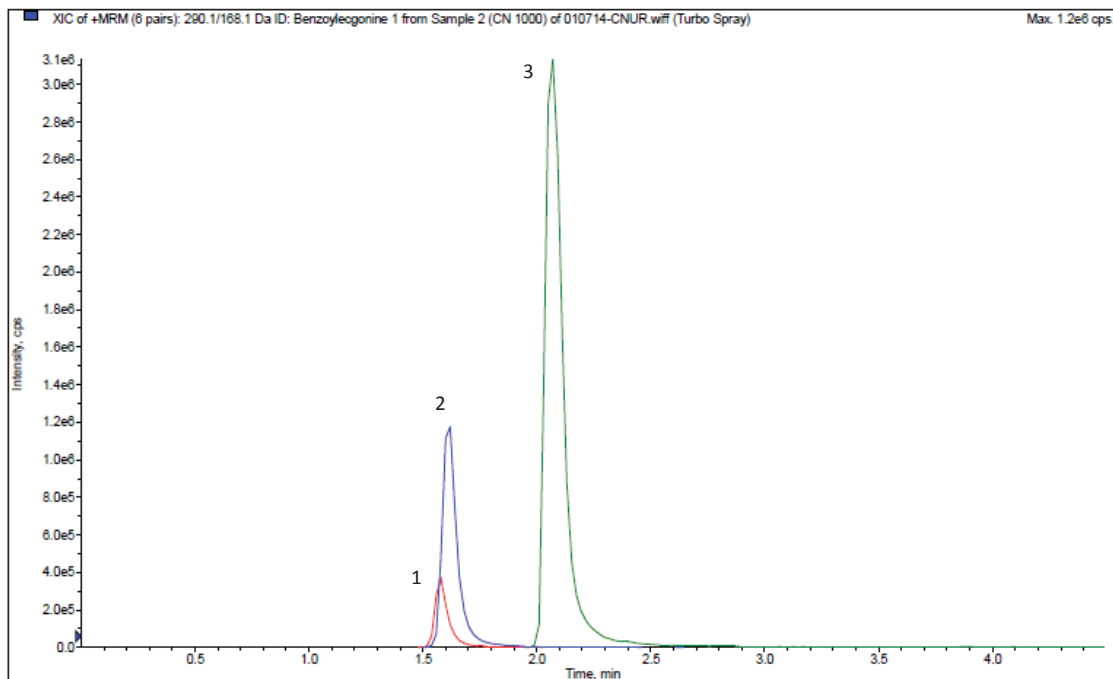
Evaporate to dryness at < 40°C.

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 N₂ 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. Benzoylcegonine D ₈	298.1	171.1	1.58
2. Benzoylcegonine	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. H₂O

Flow Rate: 0.7mL/minute

Reconstitute: 100 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:

Mobile Phase B: 0.1% Formic Acid in Methanol

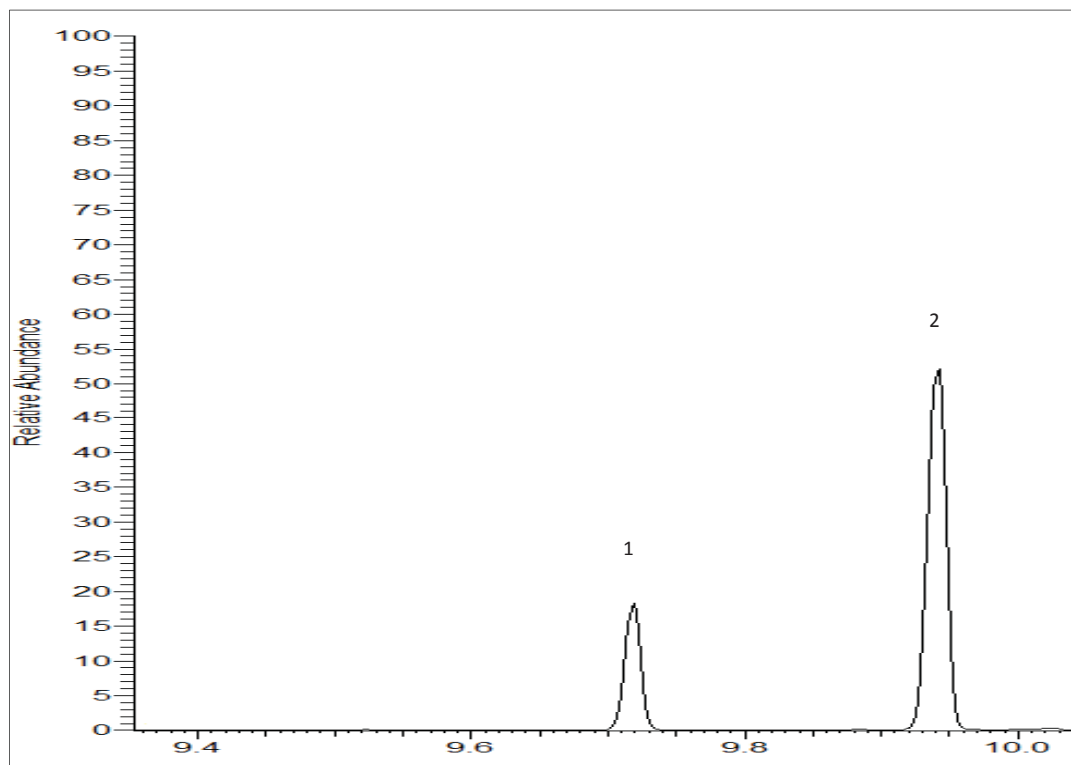
Polarity: Positive

Injection Volume: 10 µL

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. Benzoyecgonine TMS	240	256	361	9.94
Benzoyecgonine 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 °C, MS Source: 350 °C, MS Quad: 150 °C



COCAINE AND BENZOYLECGONINE IN URINE BY LC-MS/MS OR GC-MS STRYRE 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, 5 μ m

1. PREPARE SAMPLE:

To 1 mL of urine add internal standard(s) and 300 μ L 100mM HCl.
Mix/Vortex.

2. APPLY SAMPLE:

Load at 1 to 2 mL/minute.

3. WASH COLUMN:

1 x 1 mL D.I. H₂O.

1 x 1 mL 100 mM HCl

1 x 1 mL CH₃OH.

Dry column (10 minutes at full vacuum or pressure).

4. ELUTE COCAINE/METABOLITE:

2 x 0.5 mL CH₂Cl₂/ IPA /NH₄OH (78:20:2)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA /NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

5. DRY ELUATE:

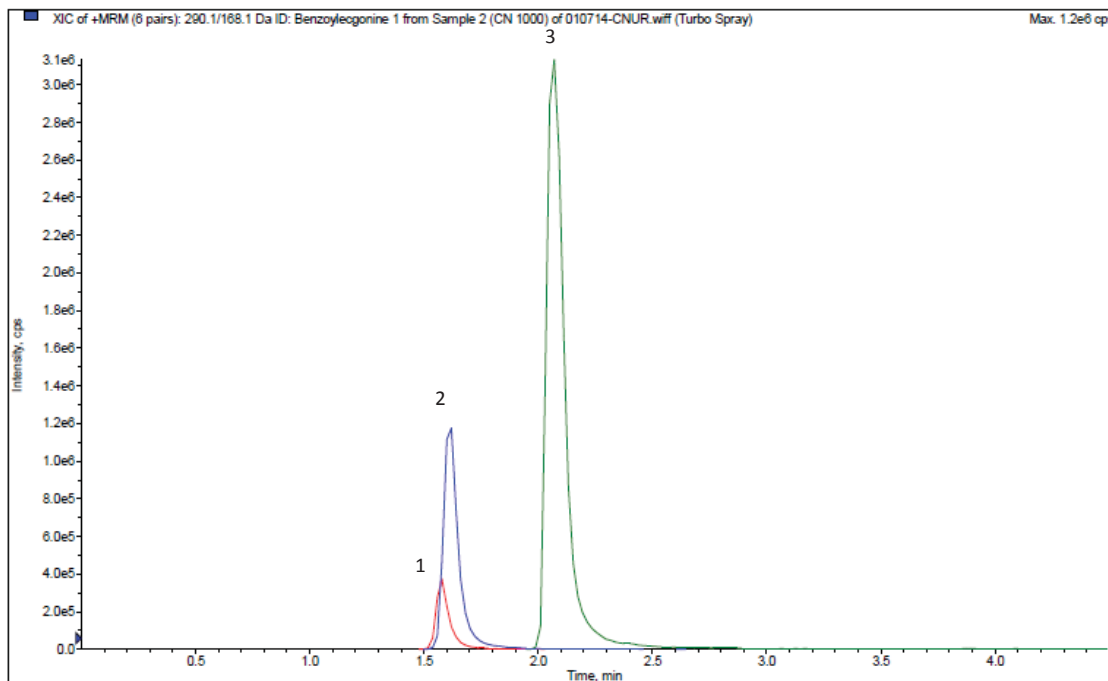
Evaporate to dryness at < 40 °C.

6. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of Ethyl Acetate and 50 μ L BSTFA w/ 1%
TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70°C; Cool and inject 1 μ L

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Benzoylcegonine D ₈	298.1	171.1	1.58
2. Benzoylcegonine	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. H₂O

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

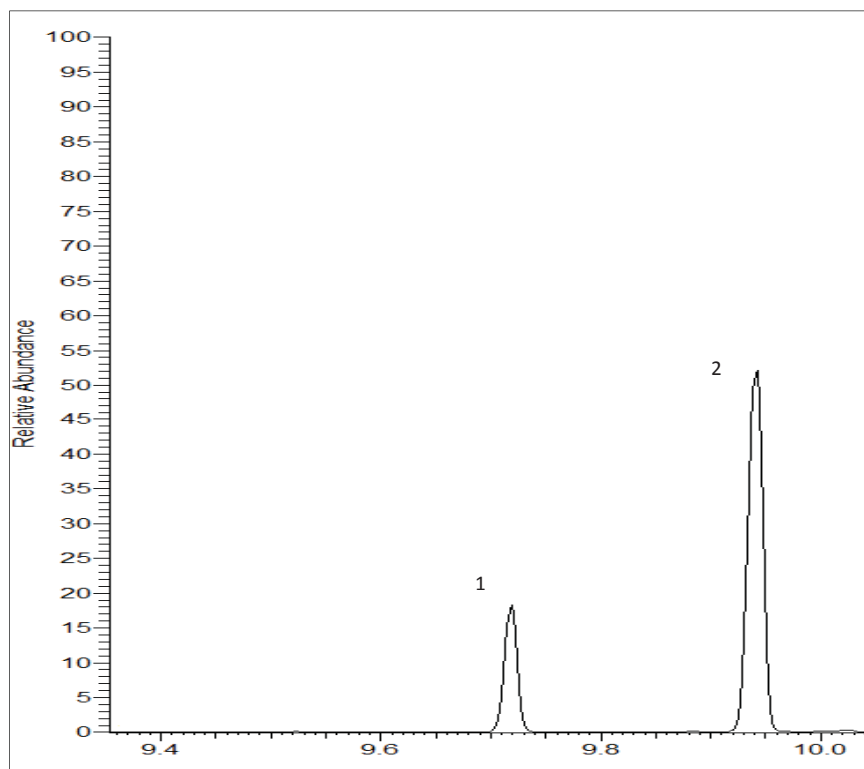
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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. Benzoyecgonine TMS	240	256	361	9.94
Benzoyecgonine 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 °C, MS Source: 350 °C, MS Quad: 150 °C



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, 5 μ m

1. PREPARE SAMPLE

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

2. 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 CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

5. DRY ELUTE

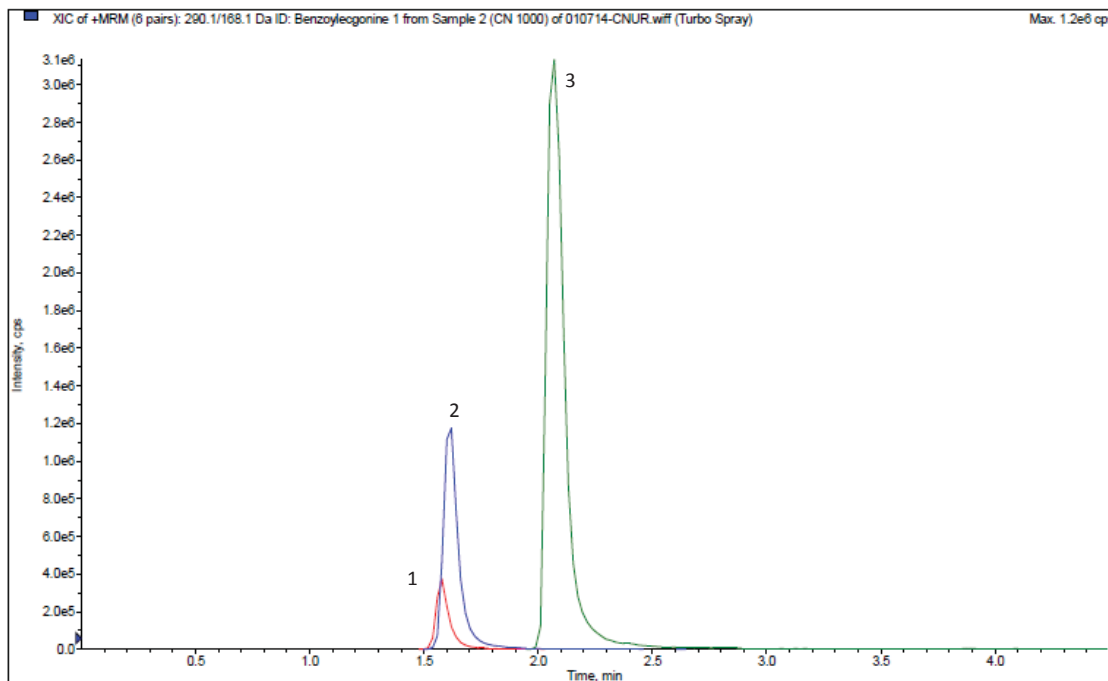
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of Ethyl Acetate and 50 μ L BSTFA w/
1%TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 μ L

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Benzoylcegonine D ₈	298.1	171.1	1.58
2. Benzoylcegonine	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. H₂O

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

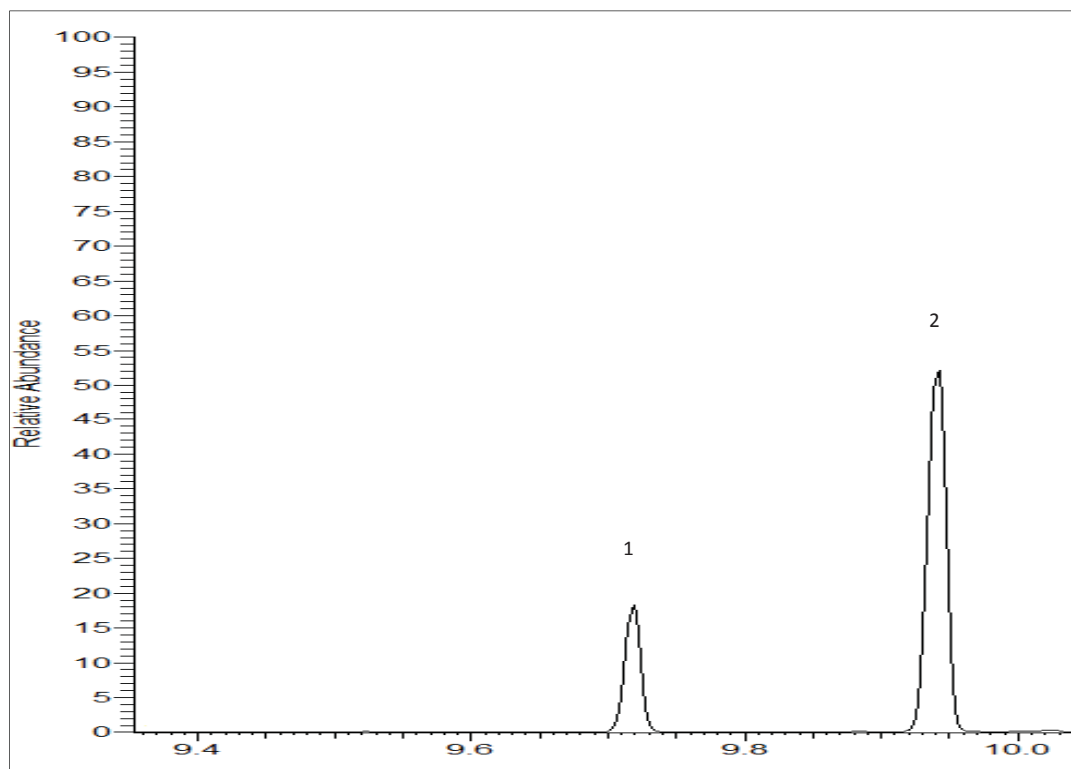
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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. Benzoyecgonine TMS	240	256	361	9.94
Benzoyecgonine TMS D ₃	243	259	369	-

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

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

Injector: 1 μ L 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 °C, MS Source: 350 °C, MS Quad: 150 °C



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

SPPHP06001-10 - 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) 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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

1 x 2 mL 100 mM HCl

1 x 3 mL CH₃OH

Dry column (5 minutes at full vacuum or pressure)

5. ELUTE COCAINE AND BENZOYLECGONINE:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)

Collect eluate at 1 to 2 mL/ minute

NOTE: Prepare elution solvent daily. Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (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

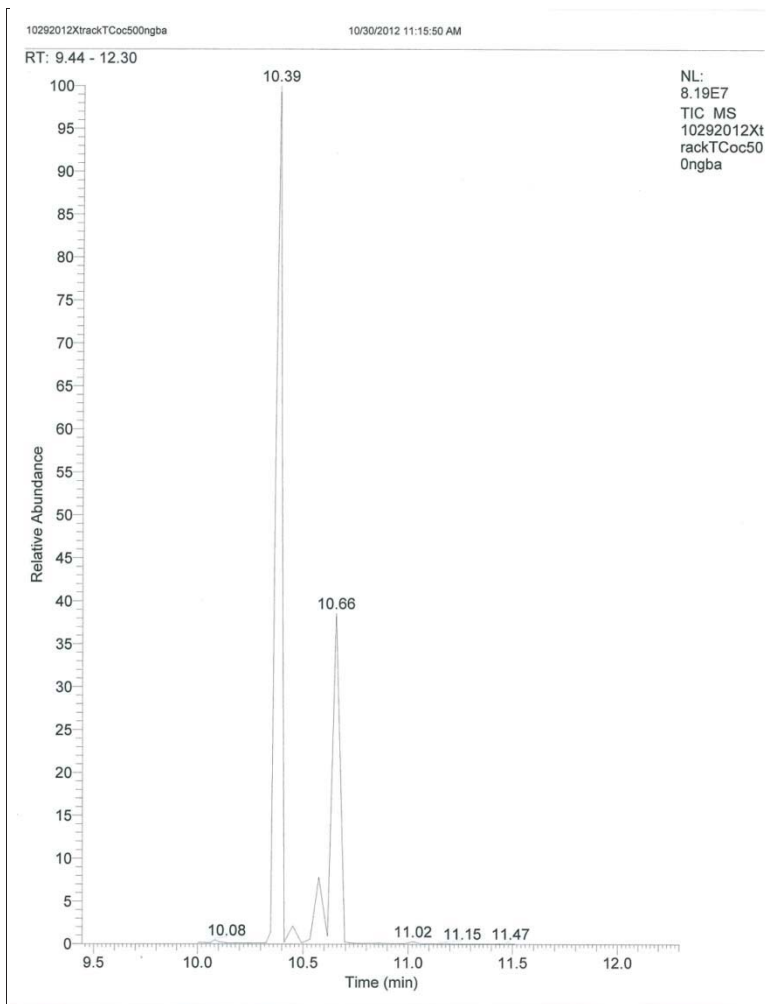
React 20 minutes at 70 °C

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
Benzoyllecgonine-TMS	240	256	361
Benzoyllecgonine-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 phosphate 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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O.

1 x 3 mL 100 mM acetic acid.

1 x 3 mL CH₃OH.

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

5. ELUTE DULOXETINE:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20: 2 v/v). Collect eluate at 1-2 mL /minute.

6. EVAPORATION:

Evaporate eluate under a gentle stream of nitrogen < 40 °C.

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

Inject 5 µL.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (5 µm) C₁₈

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/minute. Column

Temperature: ambient. Detector: API

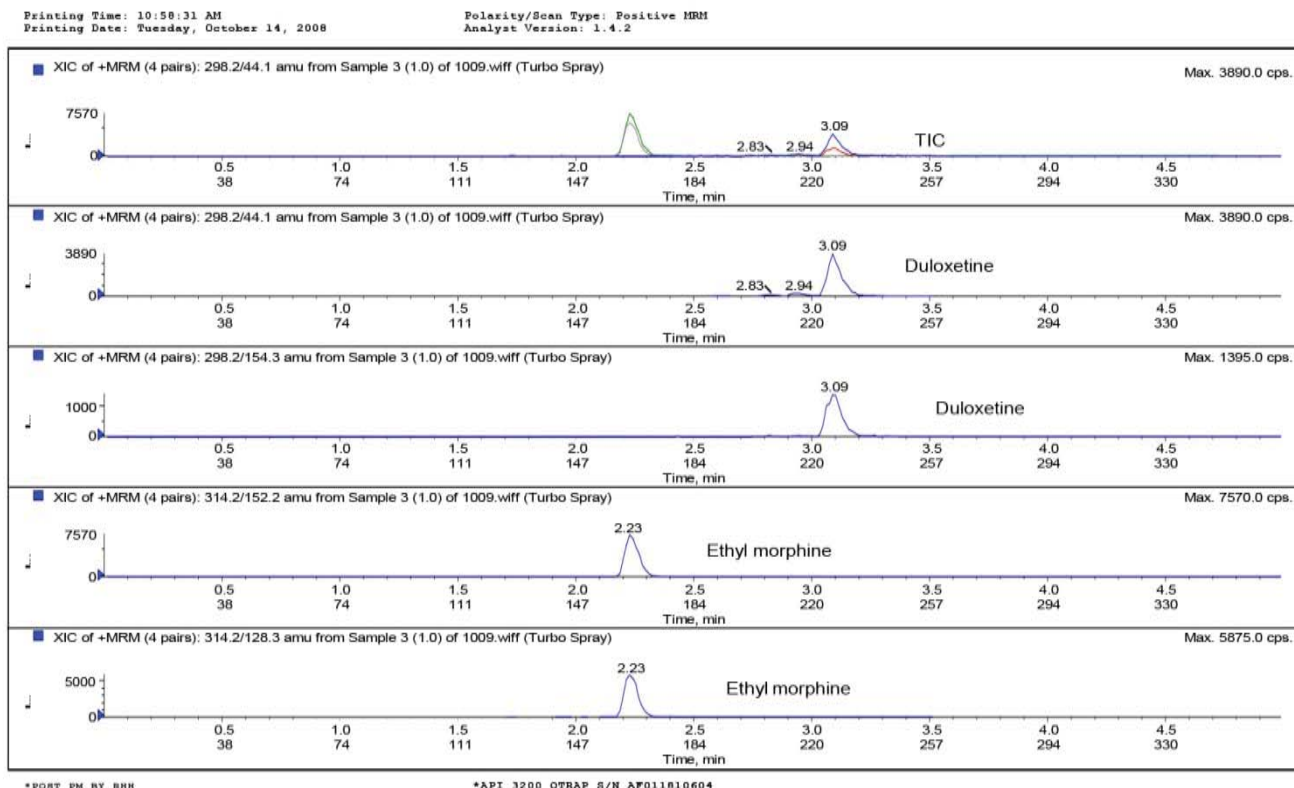
3200 Q-Trap MS/MS.

Compound

MRM Transition

* 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-BUTYRLACTONE (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. H₂O; prepared from Cerilliant stock 1 mg/mL.

GHB –D6 working internal standard; 100 µg/mL; use as supplied Cerilliant stock (0.1 mg/mL).

<u>Working Standard</u>	<u>Whole Blood</u>	<u>Concentration</u>
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 CH₃OH.
- 1 x 3 mL of D.I. H₂O.
- 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 CH₃OH/ NH₄OH (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.

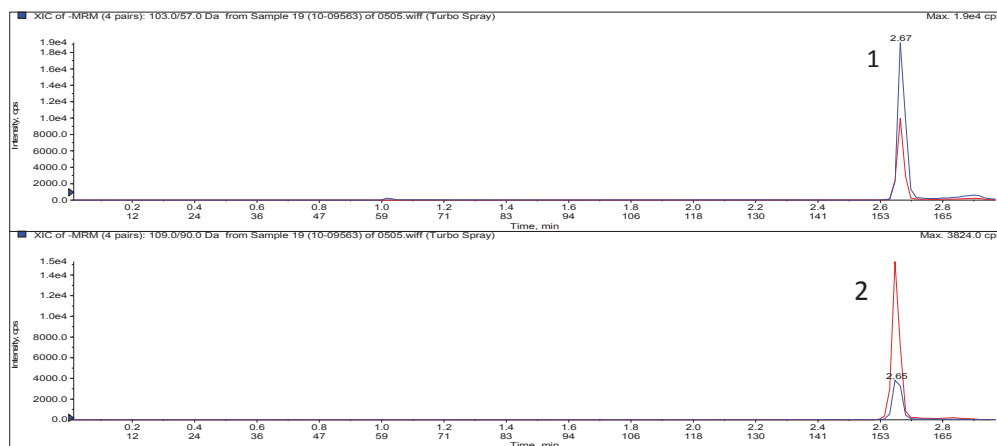
Evaporate to dryness at 70 °C using a stream of nitrogen or air.

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. H₂O

Flow Rate: 1.25mL/minute

Reconstitute: 100µl

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

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

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Polarity: Negative

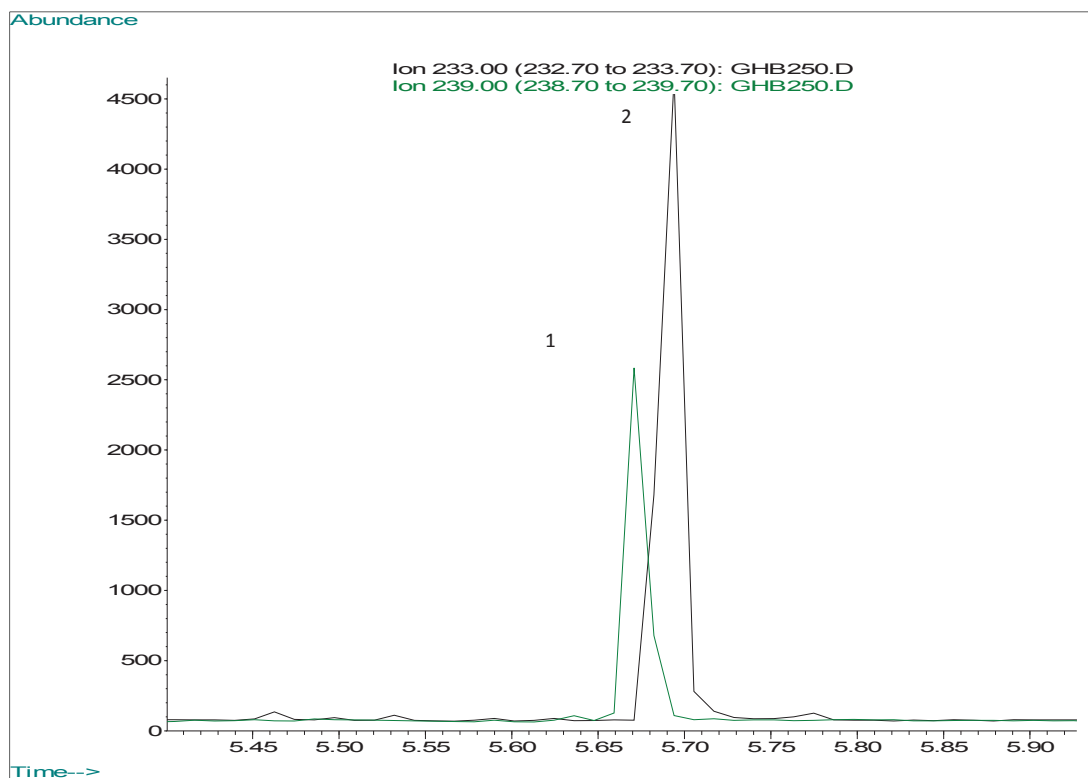
Injection Volume: 20µl

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 °C, MS Source: 250 °C, MS Quad: 150 °C

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-BUTYRLACTONE (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 CH₃OH.

1 x 3 mL D.I. H₂O.

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/ NH₄OH (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

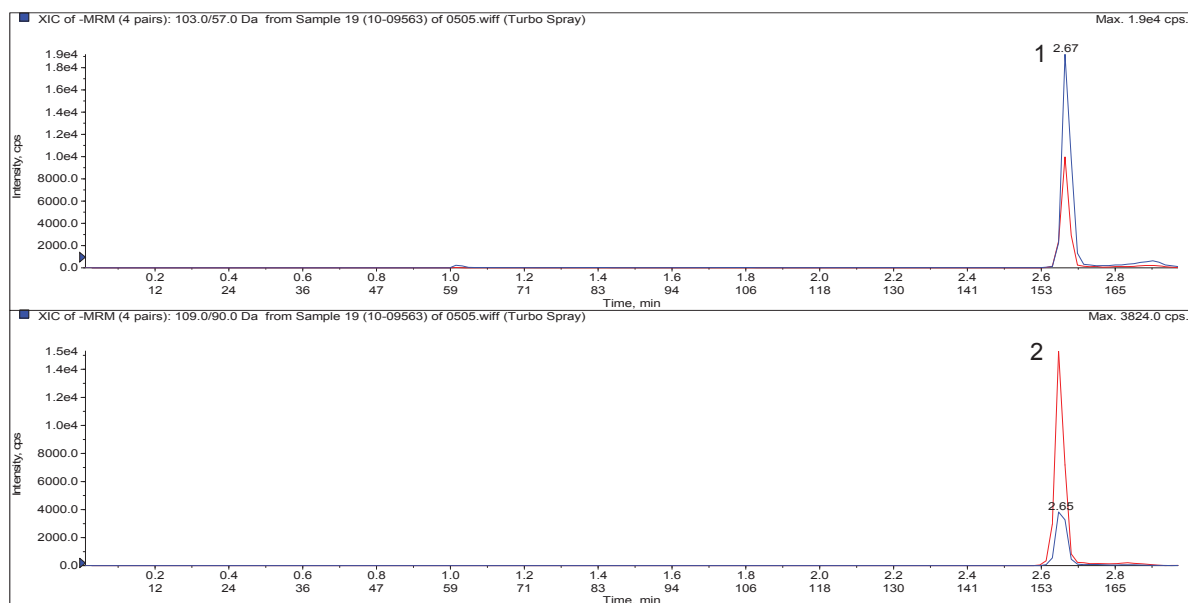
Overlay with N₂ and cap. Mix/vortex.

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. H₂O

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

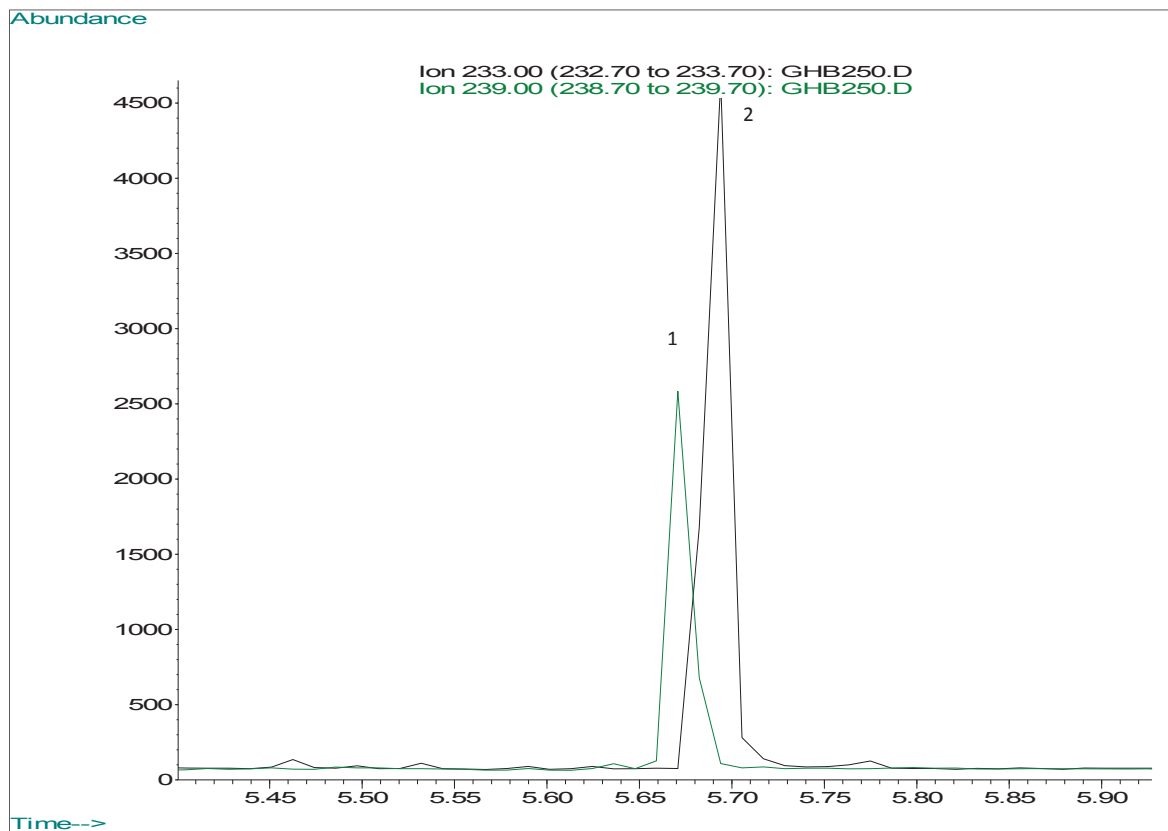
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 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 °C, MS Source: 250 °C, MS Quad: 150 °C



GHB IN URINE WITHOUT CONVERSION TO GAMMA-BUTYRLACTONE (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:

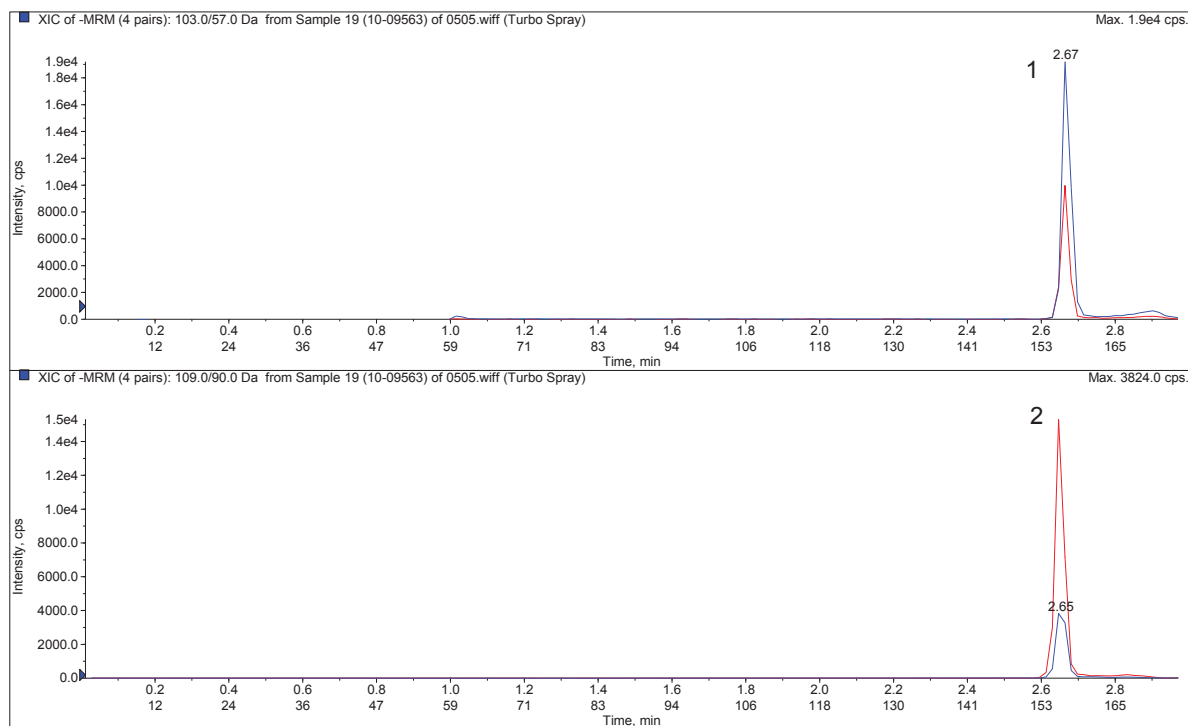
Evaporate to dryness at < 40°C.

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. H₂O

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

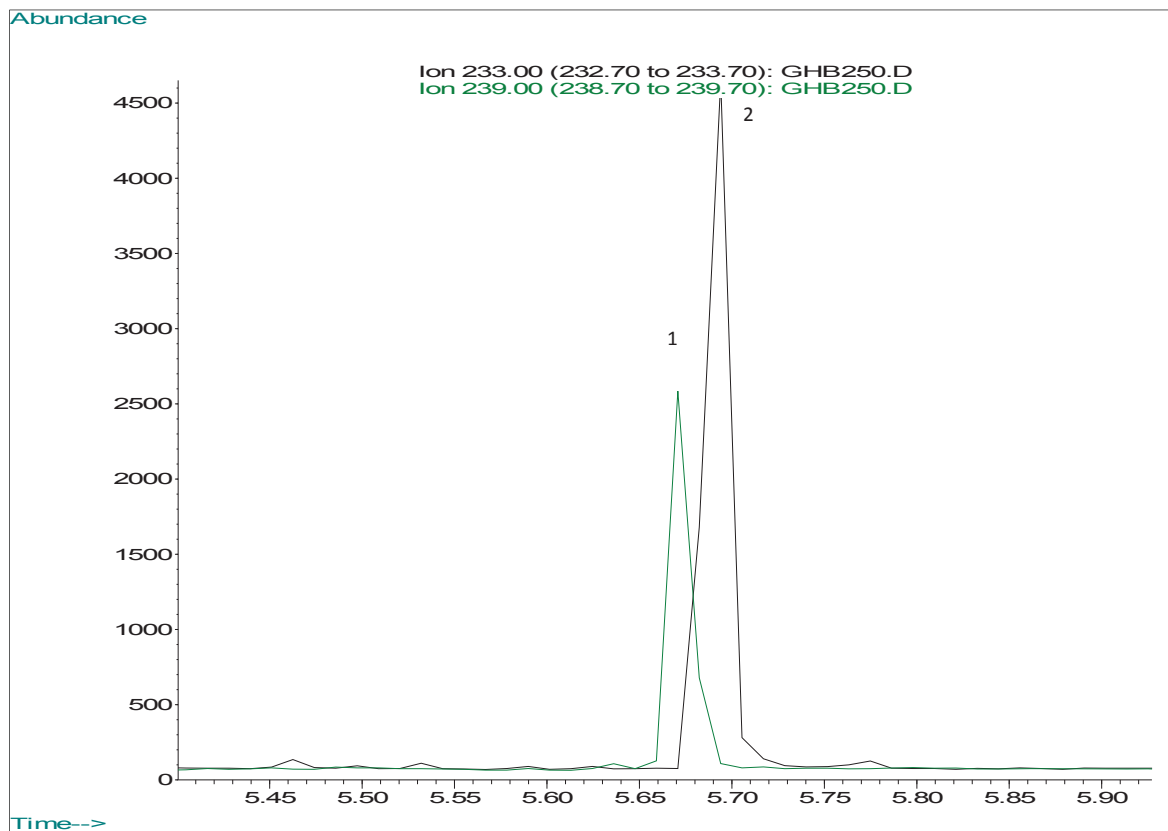
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 °C, MS Source: 250 °C, MS Quad: 150 °C



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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM acetic acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE LSD AND METABOLITES:

1 x 3 mL Ethyl Acetate/ Acetonitrile: NH₄OH (78: 20: 2 v/v)

or

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2 v/v) Collect eluate at 1-2 mL /minute.

NOTE: Prepare elution solvent daily

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

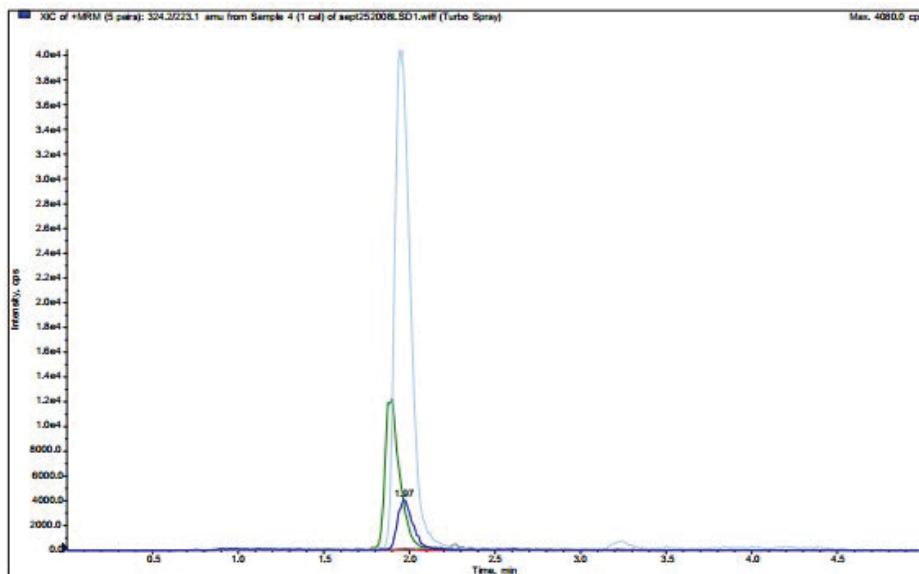
6. DRY ELUATE:

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. H₂O

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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM acetic acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE NARCOTICS/METABOLITES:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2 v/v)
Collect eluate at 1 to 2 mL/minute
or
1 x 3mL Ethyl Acetate/ IPA/ NH₄OH (78:20:2 v/v)

NOTE: Prepare elution solvent daily

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

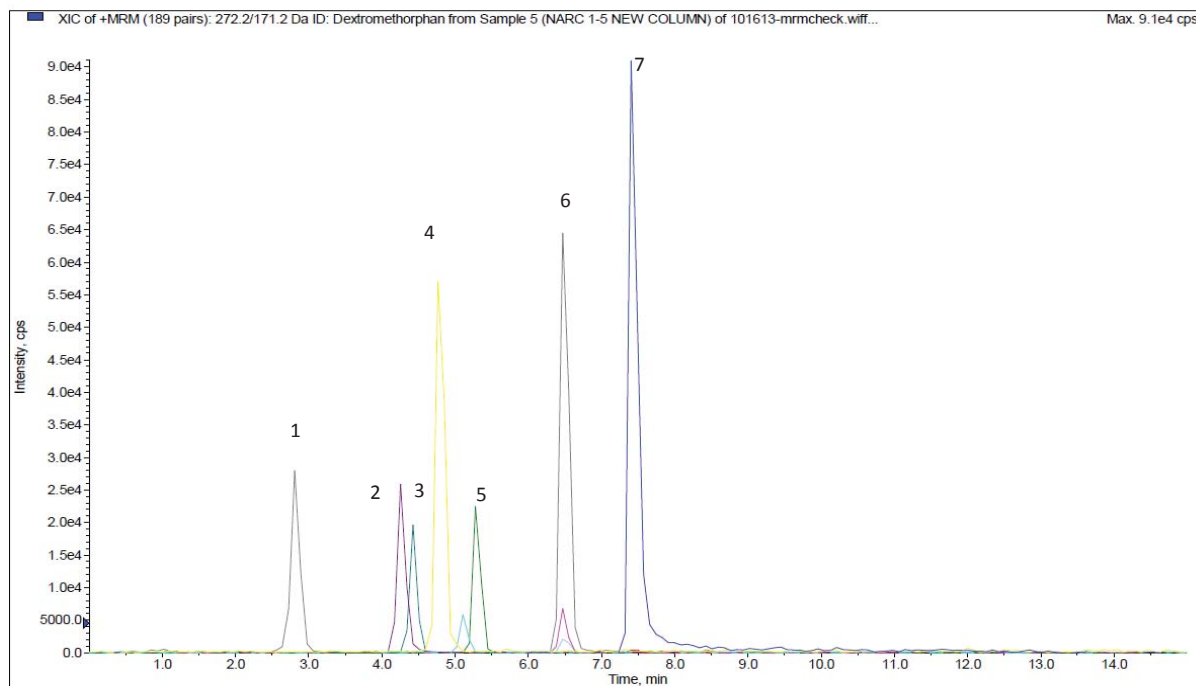
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 20 µL.
- **GC-MS:** Dissolve residue in 100 µL of Ethyl Acetate

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. H₂O

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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM acetic acid
1 x 3 mL CH₃OH
Dry column (5 minutes at > 10 inches Hg).

5. ELUTE PAROXETINE:

1 x 3 mL Ethyl Acetate/ Acetonitrile/ NH₄OH (78:20:2)
Collect eluate at 1-2 mL / minute.

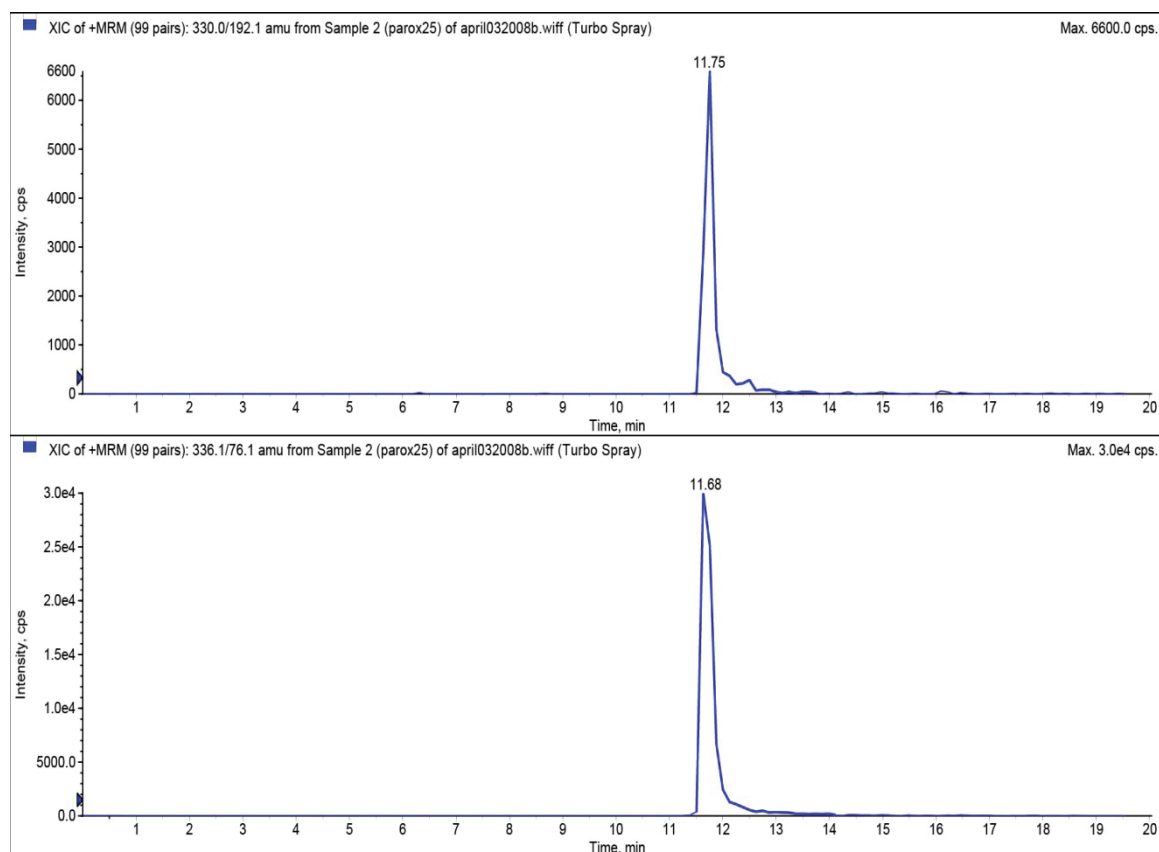
6. DRY ELUATE:

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. H₂O

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, 5 μ m

1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards

Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate

Mix/vortex and let stand for 5 minutes

Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex

Sample pH should be 6.0 \pm 0.5.

Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. APPLY SAMPLE:

Load at 1 to 2 mL/minute

3. WASH COLUMN:

1 x 1 mL D.I. H₂O

1 x 1 mL 100 mM acetic acid

1 x 1 mL CH₃OH

Dry column (5 minutes at full vacuum or pressure)

4. ELUTE PHENCYCLIDINE:

2 x 0.5 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)

Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (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:

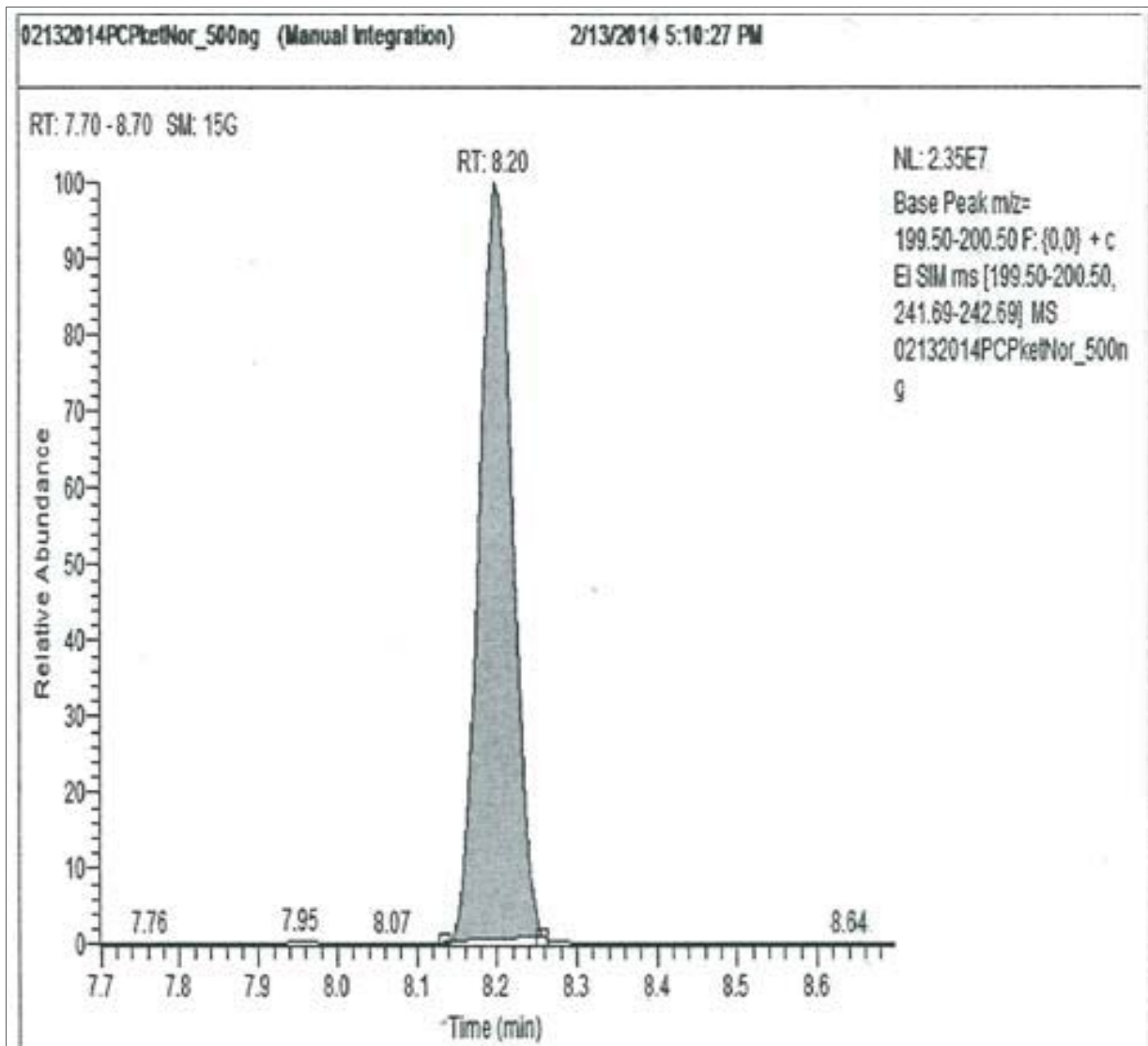
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 20 µL.
- **GC-MS:** Dissolve residue in 100 µL of Ethyl Acetate

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 °C, MS Source: 350 °C, MS Quad: 150 °C



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

SLDA501D21-3UM – SELECTRA[®] DA HPLC Column 50 x 2.1mm, 3 μ m

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 \pm 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 μ L 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. H₂O

1 x 3 mL 100 mM acetic acid

1 x 3 mL of CH₃OH

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

4. ELUTE PSILOCIN:

1 x 3 mL Ethyl Acetate/ Acetonitrile/ NH₄OH (78: 20: 2 v/v)

Or

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2 v/v)

Collect eluate at 1-2 mL /minute.

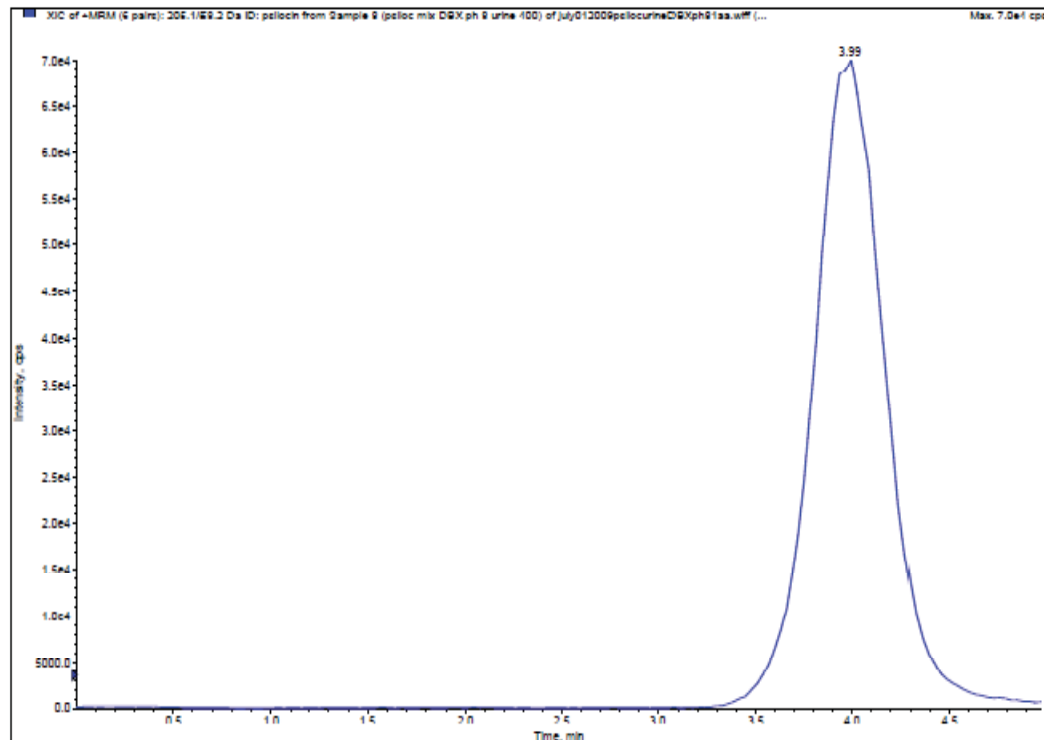
5. DRY ELUATE:

Evaporate to dryness at < 40 °C.

6. RECONSTITUTE:

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

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. H₂O

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 μ L of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme[®] β -glucuronidase.

Optionally, add 500 μ L of acetate buffer and 25 μ L 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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O

1 x 3 mL of CH₃OH

Aspirate at full vacuum or pressure for 5 minutes

5. ELUTE PSILOCIN:

1 x 3 mL Ethyl Acetate containing 2% NH₄OH

Collect eluate at 1-2 mL /minute

5b. ELUTE PSILOCYBIN:

1 x 3 mL CH₃OH containing 2% NH₄OH

Collect eluate at 1-2 mL /minute

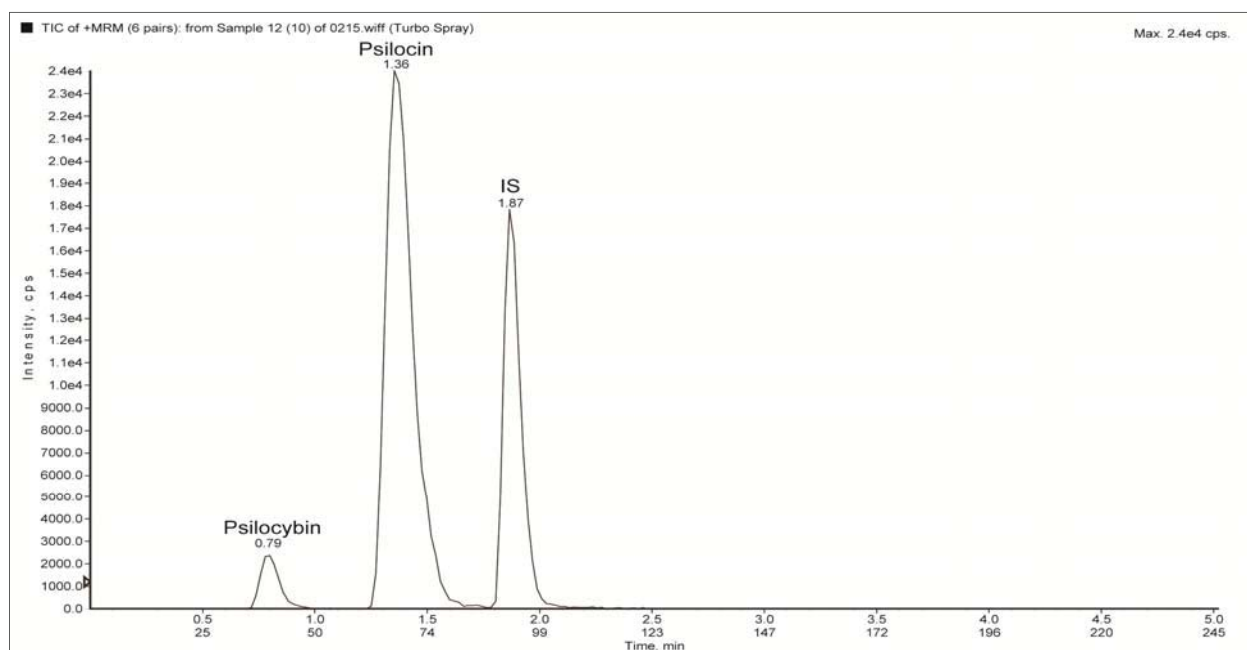
6. DRY ELUATE:

Evaporate to dryness at < 40 °C.

7. RECONSTITUTE / DERIVATIZE:

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

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. H₂O

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) C₁₈

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



UCT

Amphetamines





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

SLPFP50ID21-5UM – SELECTRA® PFP 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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM Acetic Acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE AMPHETAMINES:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (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 N₂ 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

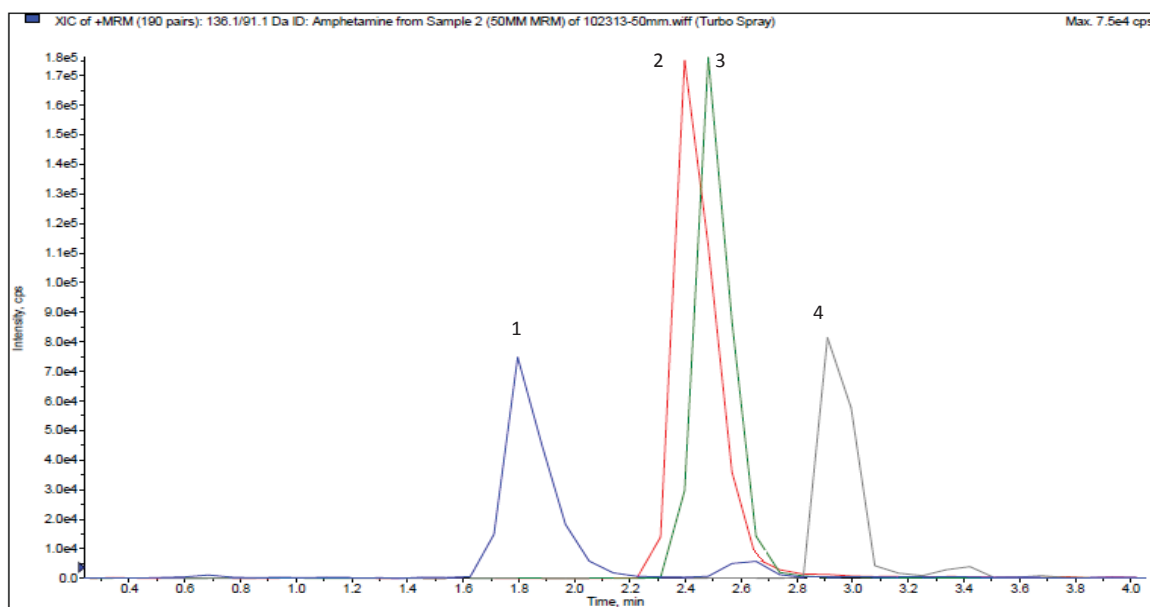
Alternate Derivatization

1. Fluoroacylate with HFPA (HFAA)
Add 50 μ L HFPA. Over lay with N₂ and cap
*Improved derivatization by addition of PFPOH
React 20 minutes at 70 °C. Evaporate to dryness < 40 °C
Reconstitute with 100 μ L Ethyl Acetate
2. Form TMS Derivatives by adding 50 μ L BSTFA w/ 1% TMCS and 50 μ L 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 \pm 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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

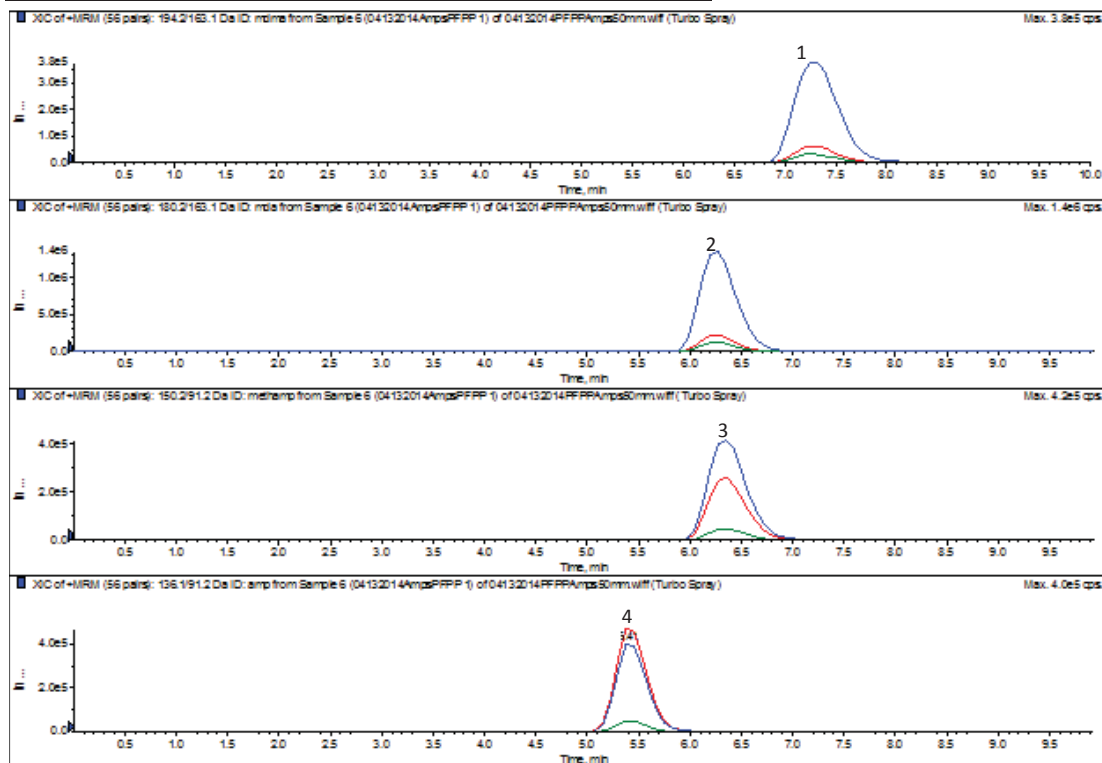
Polarity: Positive

Injection Volume: 20 µL

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. H₂O

Flow Rate: 0.3 mL/minute

Reconstitute: 100 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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

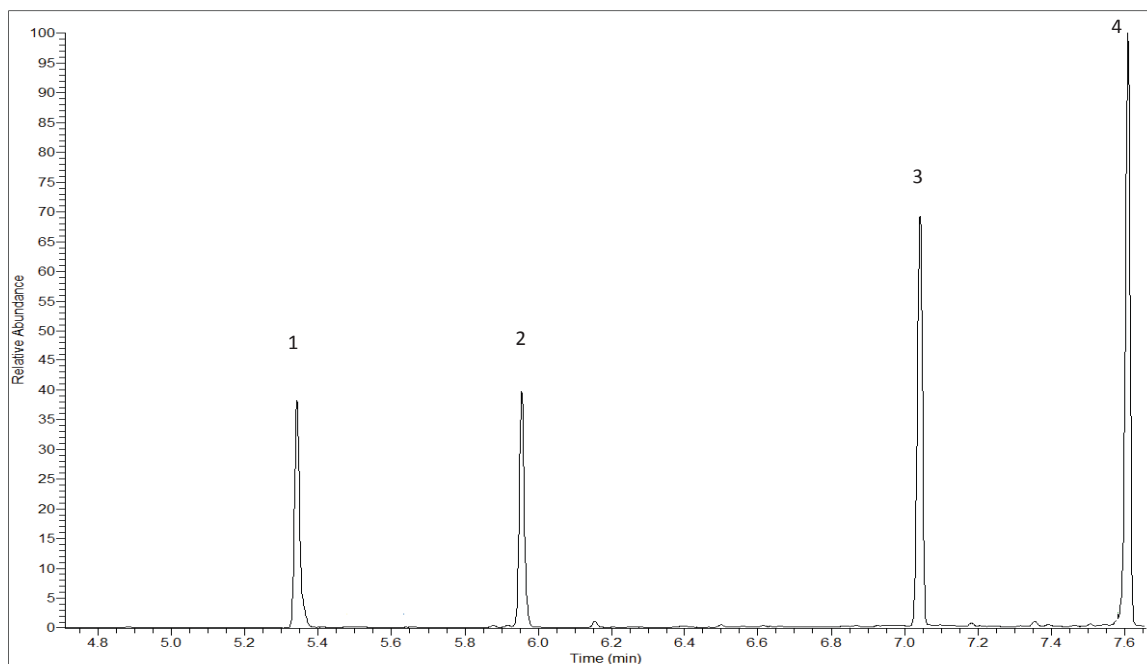
Injection Volume: 10 µL

Isocratic:

Time	%A	%B
0.00	30	70
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

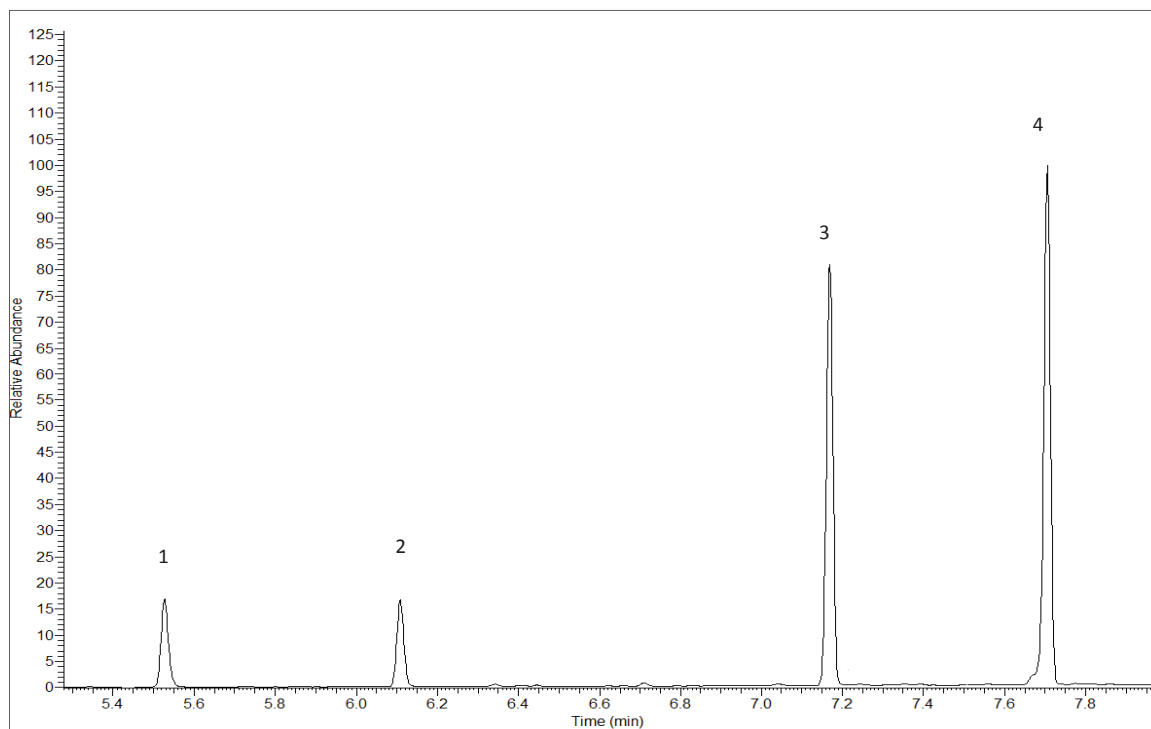
CHROMATOGRAM



Fluoroacylate with PFAA (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 μ m) TG-1MS

Injector: 1 μ L Splitless, 250 $^{\circ}$ C

Oven temperature program: 70 $^{\circ}$ C (0.5) to 320 $^{\circ}$ C (25 $^{\circ}$ C/ minute): hold (2 minutes)

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

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 350 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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 μ m

or

SLPFPP50ID21-5UM – SELECTRA[®] PFPP HPLC Column, 50 x 2.1 mm, 5 μ m

1. PREPARE SAMPLE:

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
Mix/vortex and let stand for 5 minutes
Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
Sample pH should be 6.0 ± 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. H₂O
1 x 1 mL 100 mM Acetic Acid
1 x 1 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

4. ELUTE AMPHETAMINES:

2 x 0.5 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

5. DRY ELUATE:

Add 100 μ L 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 μ L of mobile phase
Inject 10-20 μ L.
- **GC-MS:** Fluoroacetylate with PFPA (PFAA)
Add 50 μ L PFPA. Over lay with N₂ and cap
*Improved derivatization by addition of PFPOH
React 20 minutes at 70 °C. Evaporate to dryness <40 °C
Reconstitute with 100 μ L Ethyl Acetate

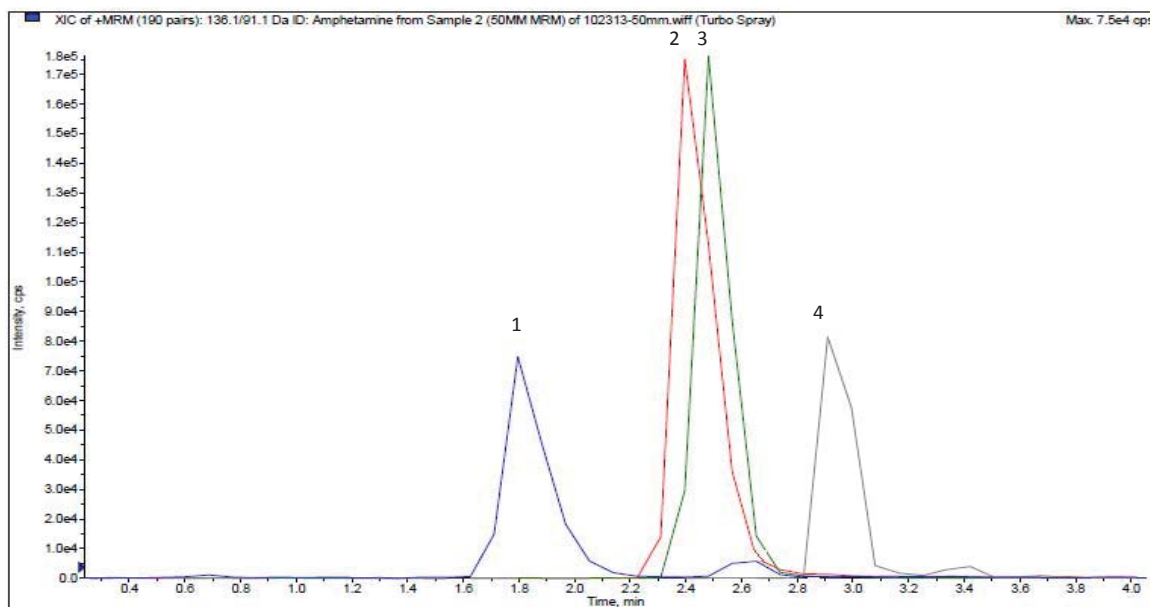
Alternate Derivatization

3. Fluoroacylate with HFPA (HFAA)
Add 50 µL HFPA. Over lay with N₂ 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. H₂O

Flow Rate: 0.5mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

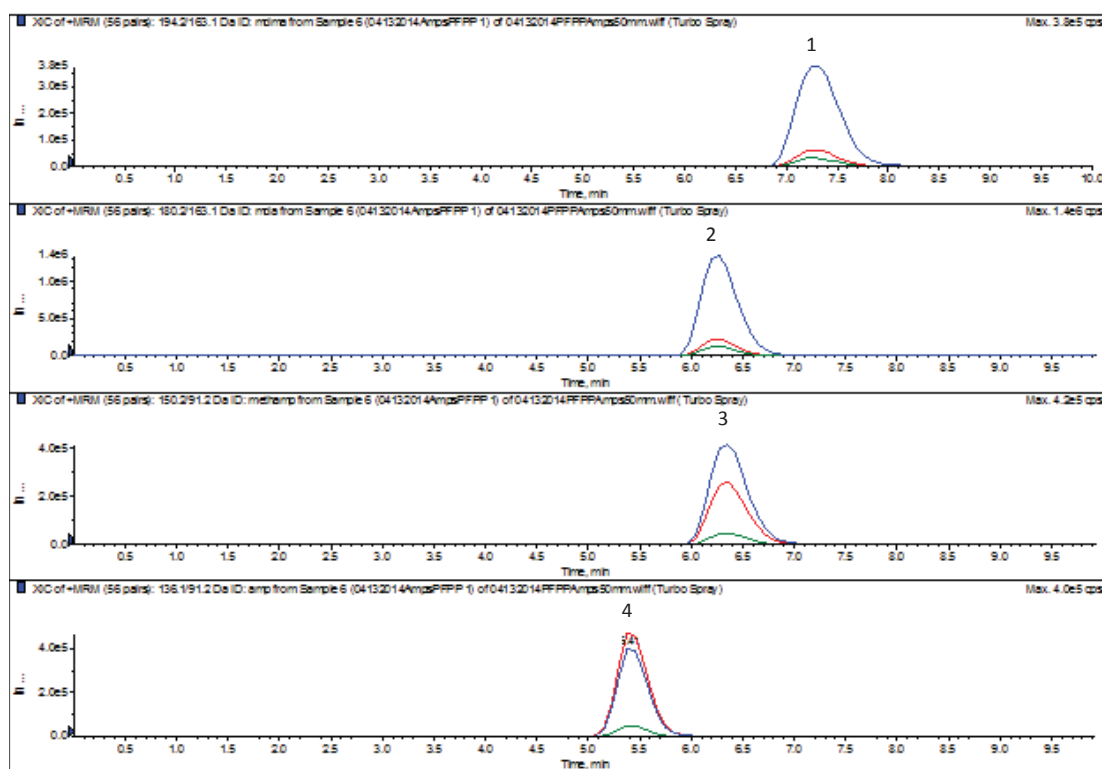
Polarity: Positive

Injection Volume: 20 µL

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. H₂O

Flow Rate: 0.3 mL/minute

Reconstitute: 100 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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

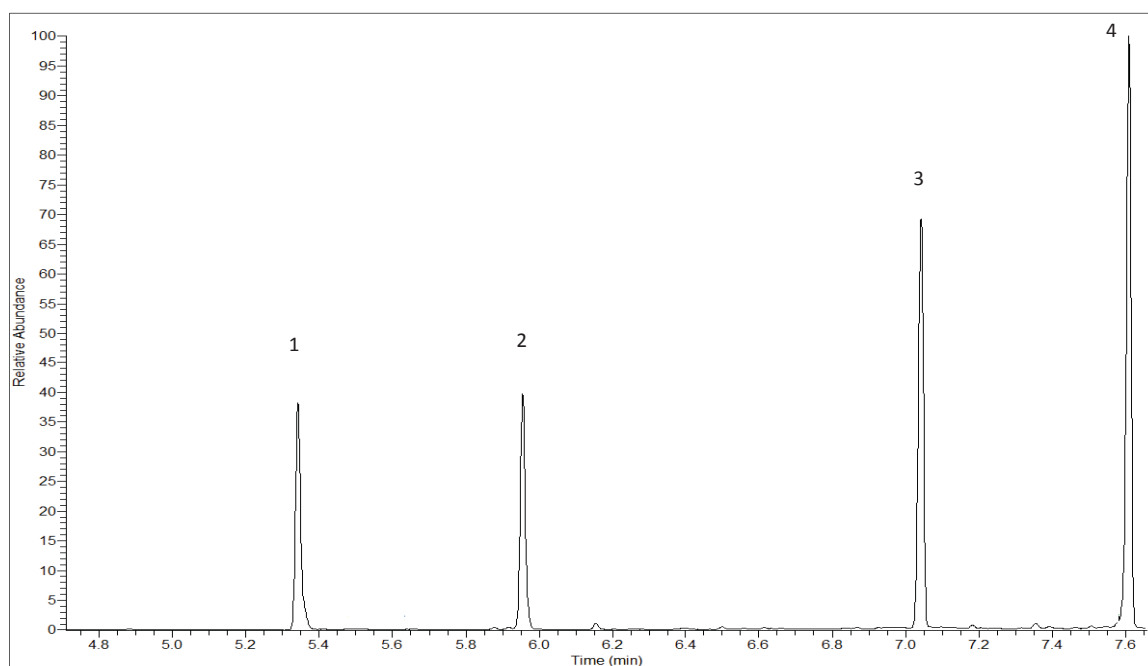
Injection Volume: 10 µL

Isocratic:

Time	%A	%B
0.00	30	70
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

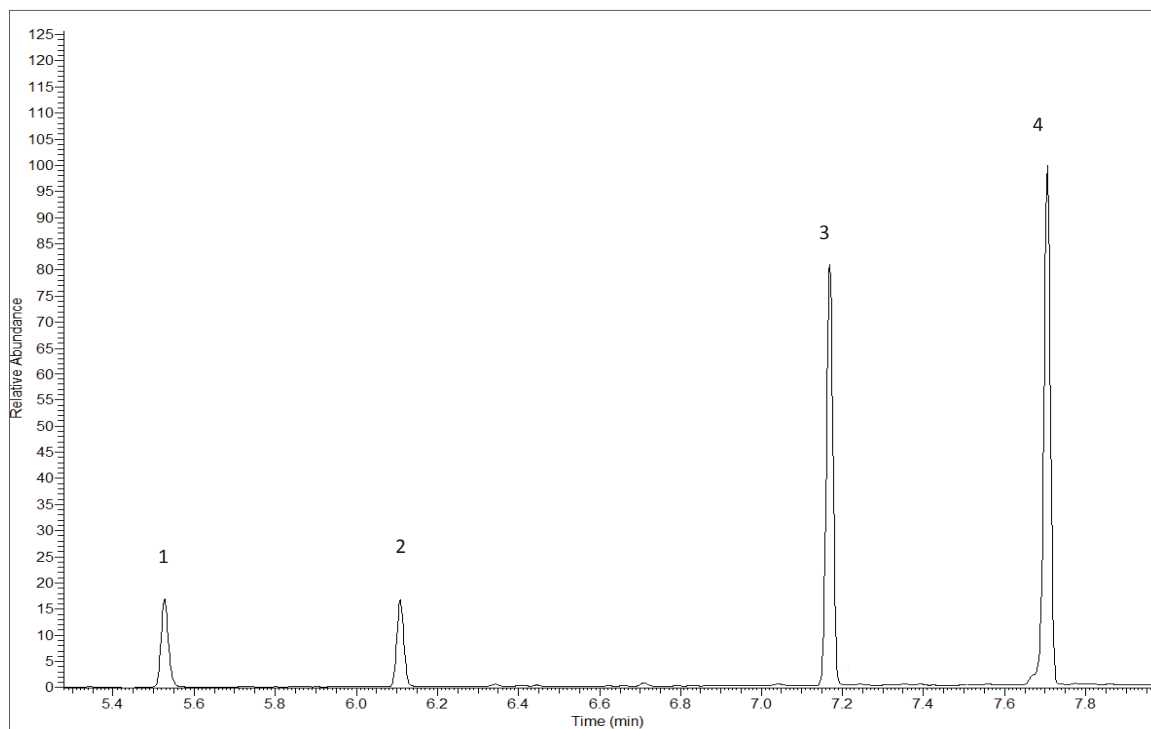
CHROMATOGRAM



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 °C, MS Source: 350 °C, MS Quad: 150 °C



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

SLPFP50ID21-5UM – SELECTRA® PFP 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 CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

5. DRY ELUTE

Add 50 µL of 1% HCl in CH₃OH to each tube
Evaporate fraction to complete dryness under stream of dry air or nitrogen at~ 35 °C.

NOTE: A 1% HCl in CH₃OH solution has been used to prevent volatilization 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 N₂ 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

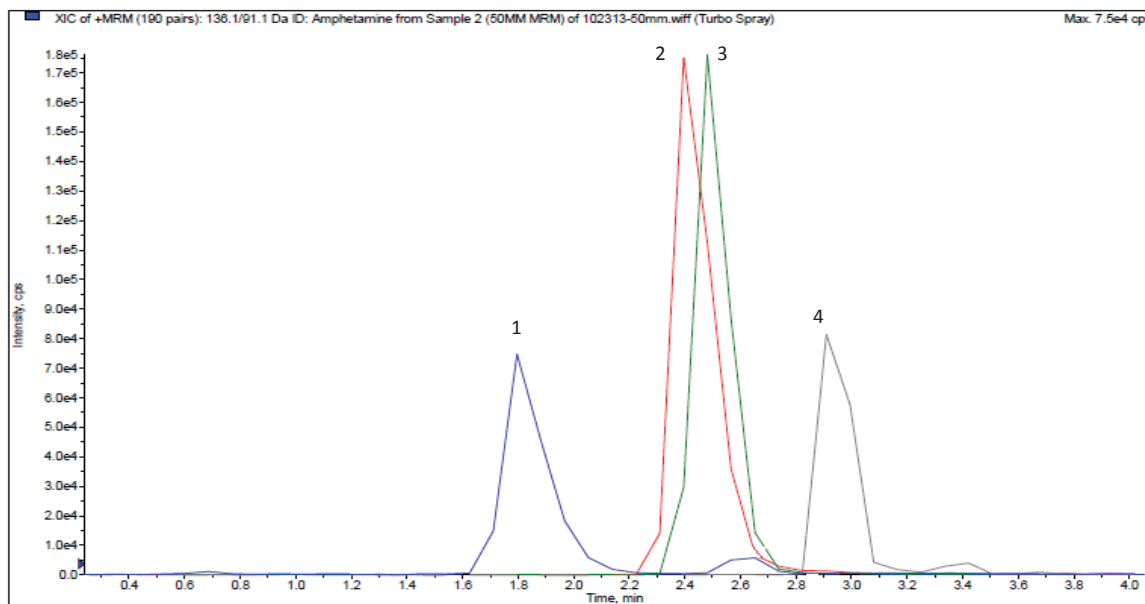
5. Fluoroacrylate with HFPA (HFAA)
 Add 50 µL HFPA. Over lay with N₂ and cap
 *Improved derivatization by addition of PFPOH
 React 20 minutes at 70 °C. Evaporate to dryness <40 °C
 Reconstitute with 100 µL Ethyl Acetate

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

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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

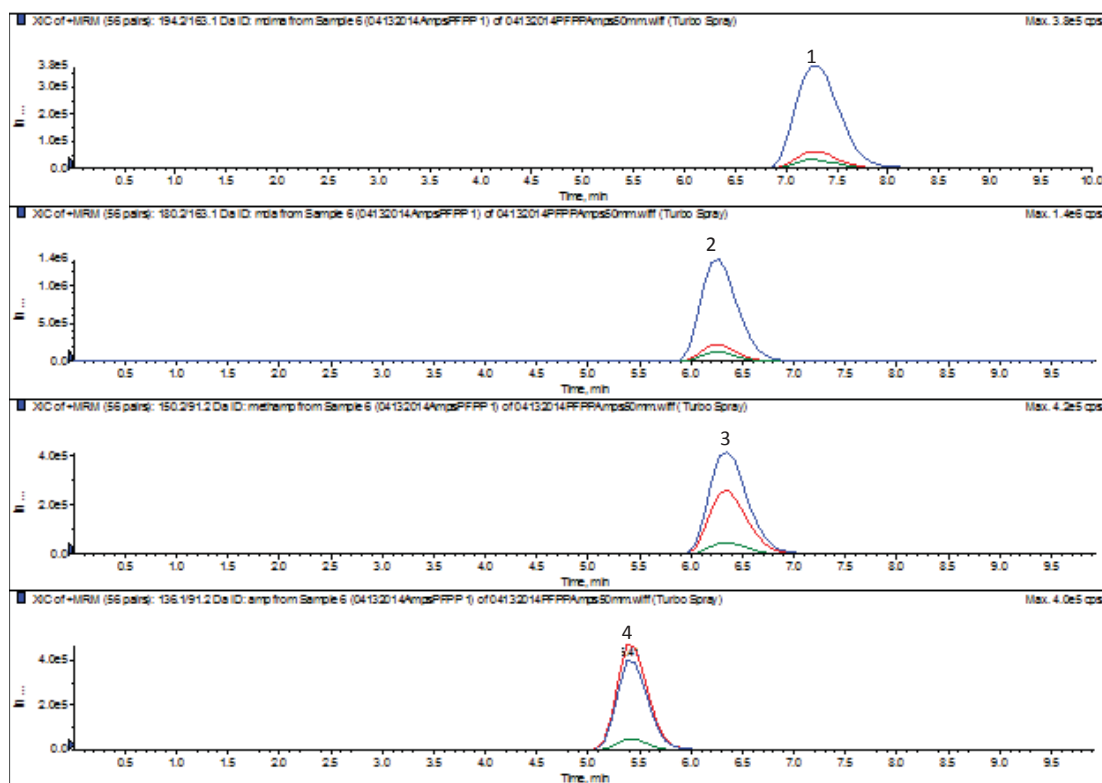
Polarity: Positive

Injection Volume: 20 µL

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. H₂O

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

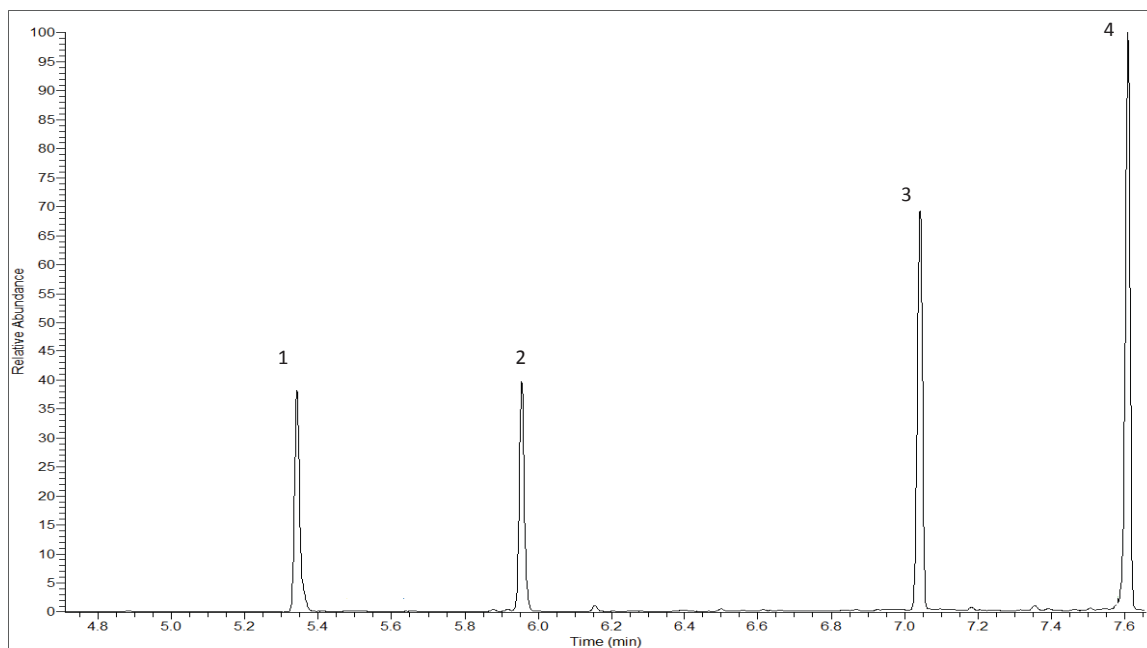
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	30	70
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

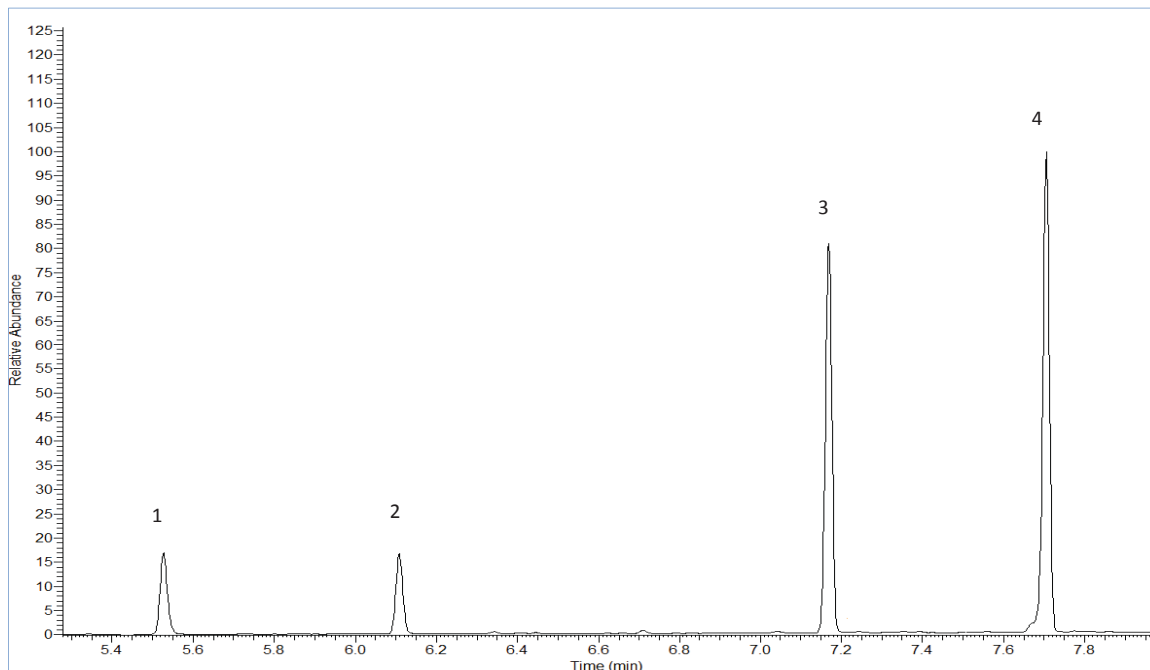
CHROMATOGRAM



Fluoroacrylate 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 μ m) TG-1MS

Injector: 1 μ L Splitless, 250 $^{\circ}$ C

Oven temperature program: 70 $^{\circ}$ C (0.5) to 320 $^{\circ}$ C (25 $^{\circ}$ C/ minute): hold (2 minutes)

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

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 350 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM Acetic Acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE BATH SALTS:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

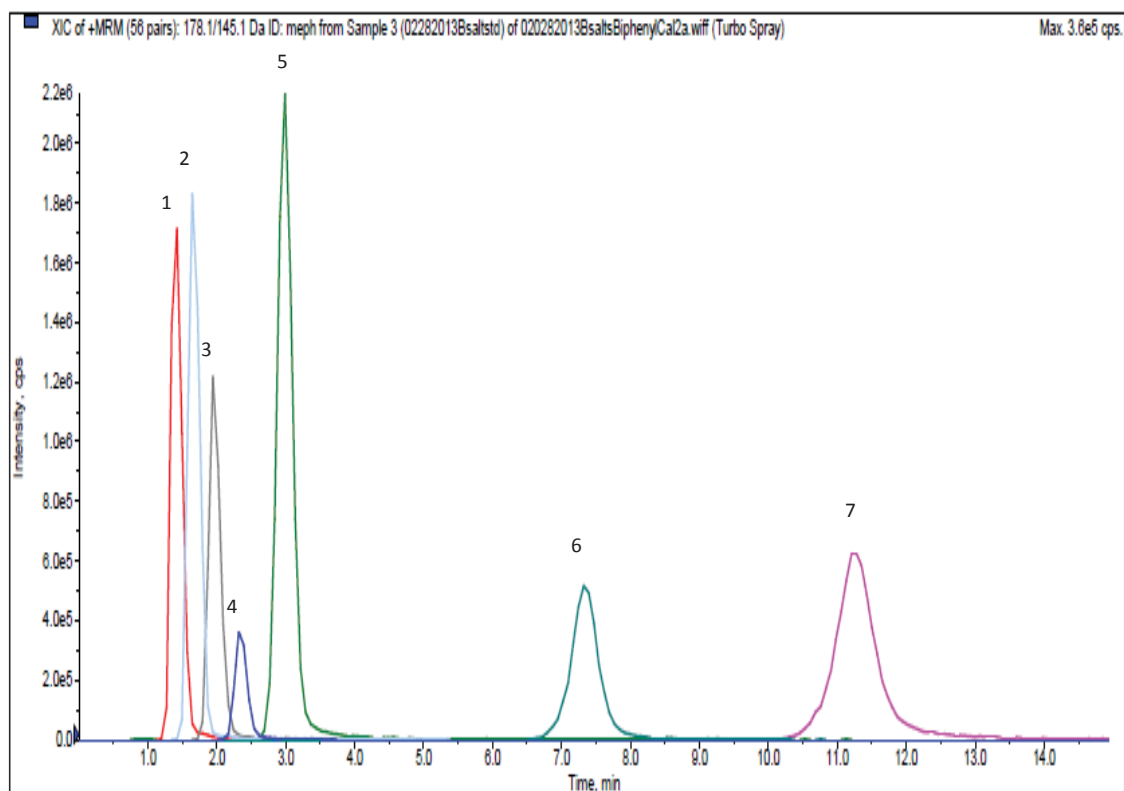
Add 50 µL of 1% HCl in Methanol to each test tube
Evaporate to dryness at < 40 °C

NOTE: A 1% HCl in CH₃OH solution has been used to prevent volatilization 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 N₂ 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.Pyralvalerone	246.2	105.2	11.24

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.7 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 5 µL

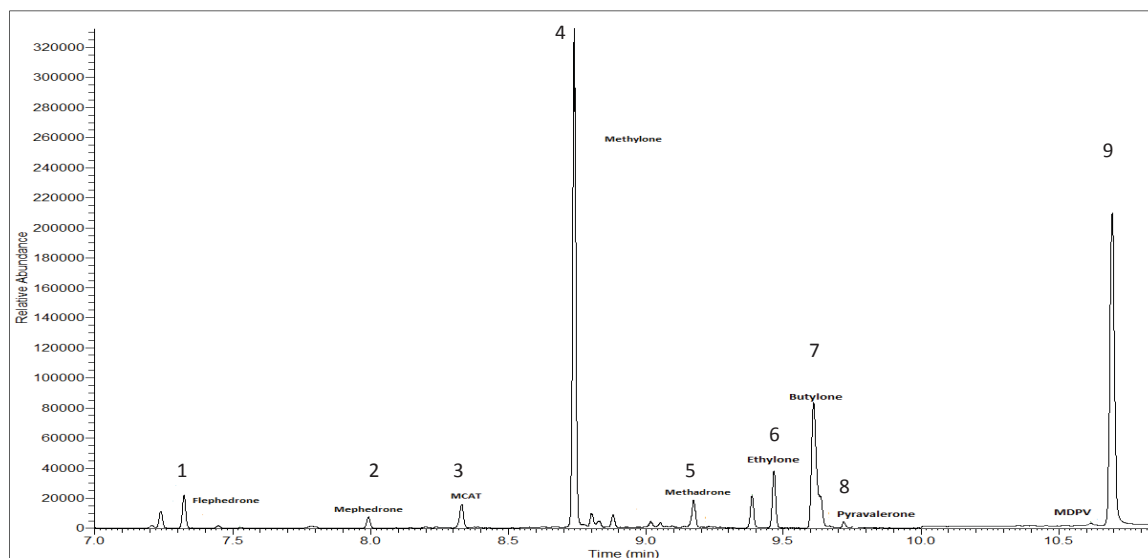
LC Column: Selectra[®] DA HPLC Column 100 x 2.1 mm 5 µm

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):



Fluoroacrylate with PFFA (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.Pyralerone	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 °C, MS Source: 250 °C, MS Quad: 150 °C

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 μ m

1. PREPARE SAMPLE

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

2. 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 CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

5. DRY ELUTE

Add 50 μ L of 1% HCl in CH₃OH to each tube
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

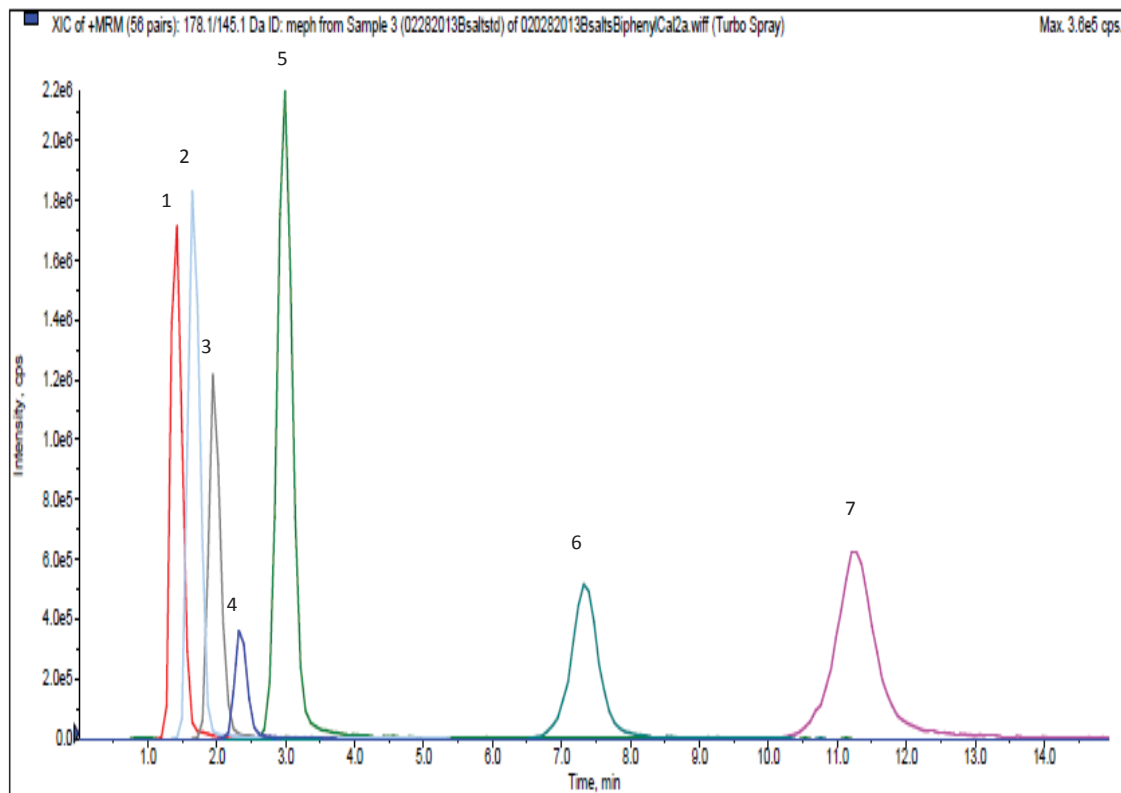
NOTE: A 1% HCl in CH₃OH solution has been used to prevent volatilization by the formation of the hydrochloric salt of the drugs.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 5 μ L.
- **GC-MS:** Fluoroacylate with PFPA (PFAA)
Add 50 μ L PFPA. Over lay with N₂ and cap
*Improved derivatization by addition of PFPOH
React 20 minutes at 70 °C. Evaporate to dryness <40 °C
Reconstitute with 100 μ L 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. H₂O

Flow Rate: 0.7 mL/minute

Reconstitute: 100 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

Mobile Phase B: 0.1% Formic Acid in Methanol

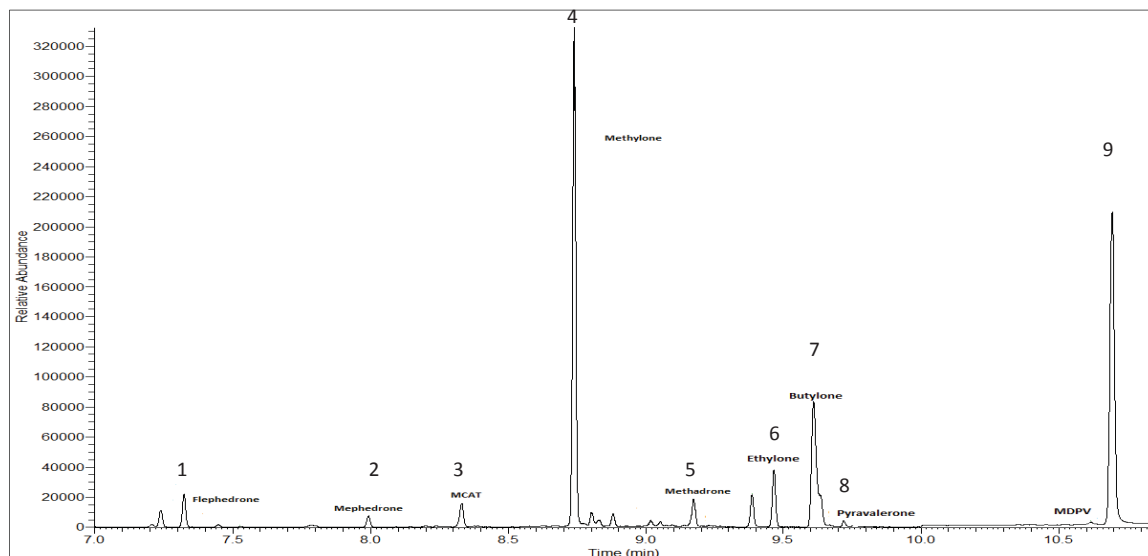
Polarity: Positive

Injection Volume: 5 µL

Isocratic:

Time	%A	%B
0.00	70	30
15.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):



Fluoroacrylate with PFAA (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.Pyralverone	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 μ m) TG-1MS

Injector: 1 μ L 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 °C, MS Source: 250 °C, MS Quad: 150 °C

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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM Acetic Acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE SMA'S:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

Add 100 µL of 1% HCl in Methanol to each test tube
Evaporate to dryness at < 40 °C

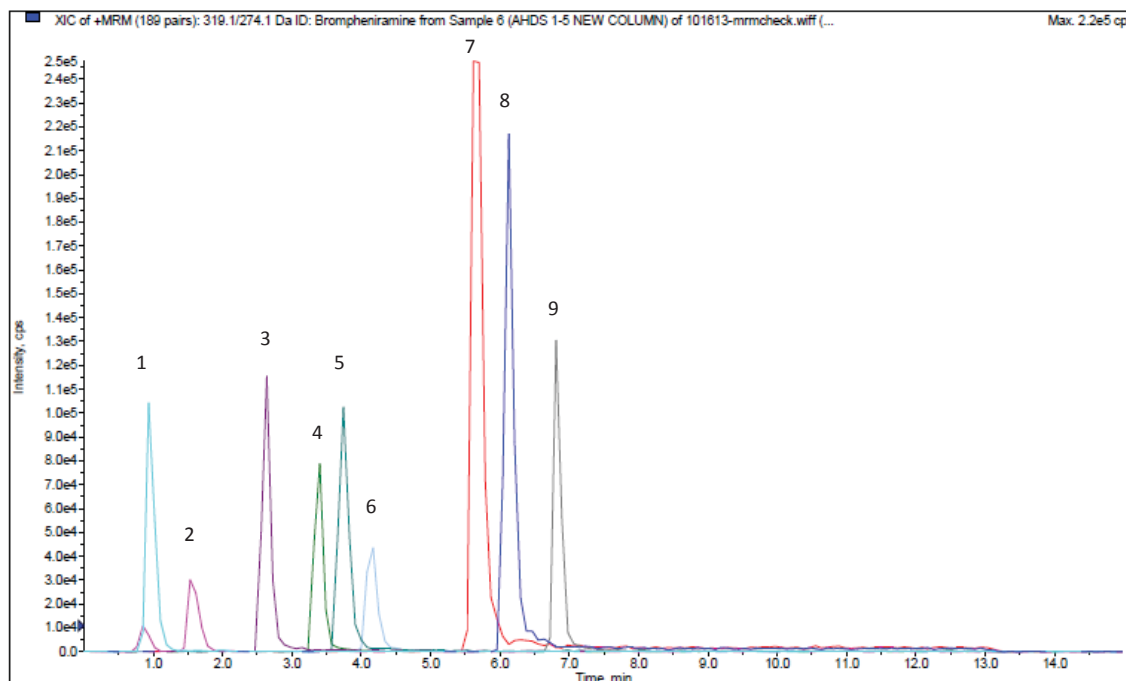
7. RECONSTITUTE / DERIVATIZE:

- **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 N₂ 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 μ L HFPA. Over lay with N₂ and cap
*Improved derivatization by addition of PFPOH
React 20 minutes at 70 °C. Evaporate to dryness <40 °C
Reconstitute with 100 μ L Ethyl Acetate
2. Form TMS Derivatives by adding 50 μ L BSTFA w/ 1% TMCS and 50 μ L 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. Diethylpropion	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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 μ L

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

Injection Volume: 20 μ L

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



CLINICAL



FORENSICS



UCT

Benzodiazepines





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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O.

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% NH₄OH

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

6. DRY ELUATE:

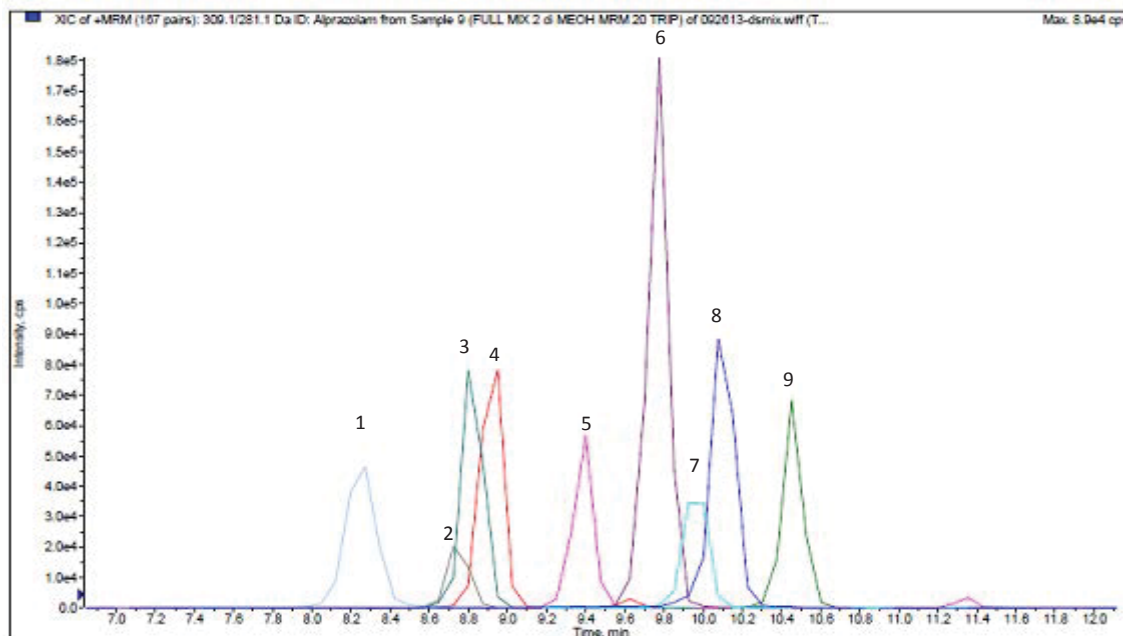
Evaporate to dryness at < 40 °C.

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 N₂ 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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

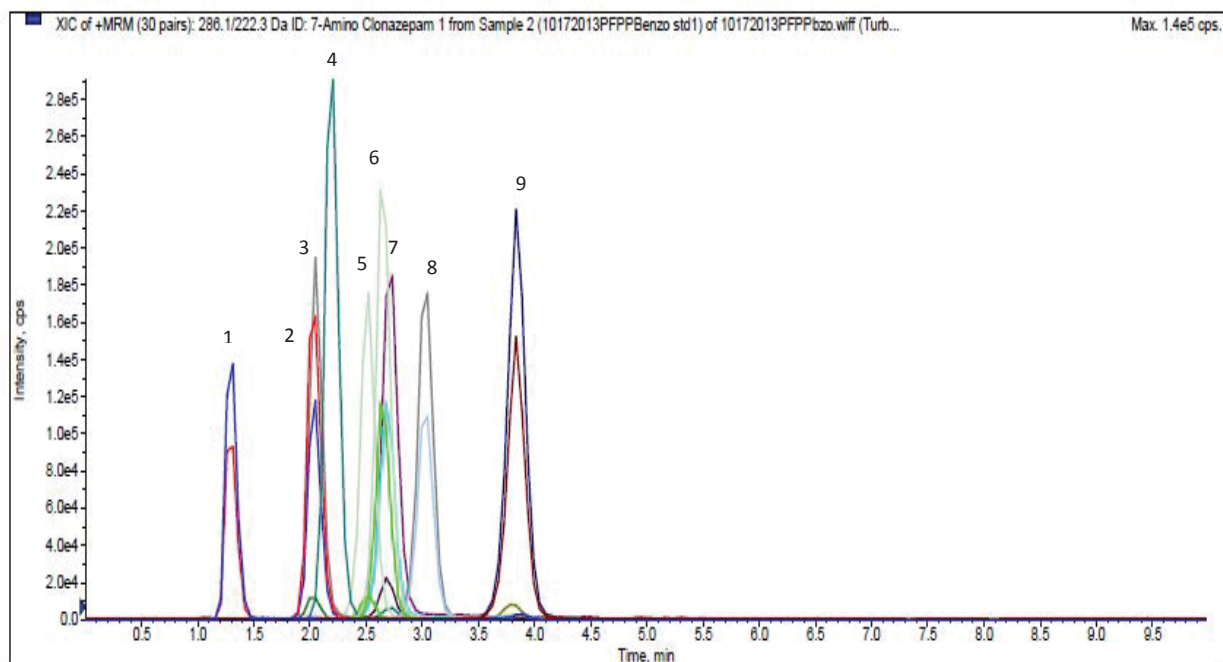
Polarity: Positive

Injection Volume: 20 µL

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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

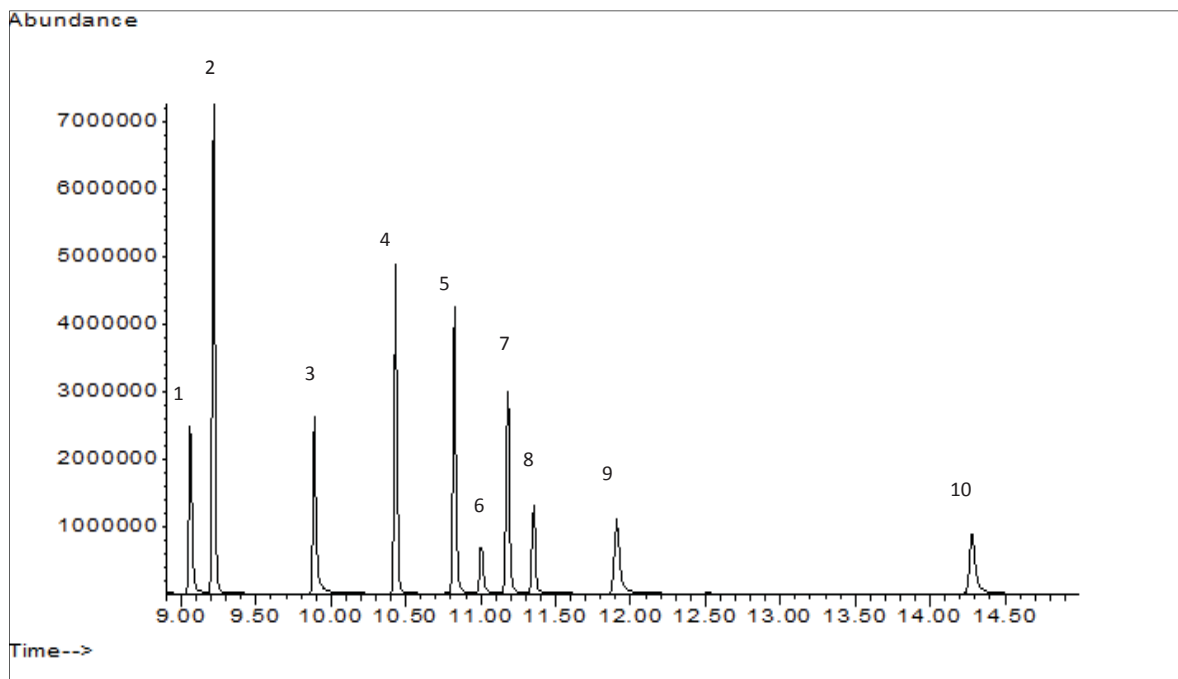
Injection Volume: 10 µL

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. Midazolam	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 μ m

Injector: 1 μ L Splitless 250 $^{\circ}$ C

Oven temperature program: 160 $^{\circ}$ C for 0.5 min; 15 $^{\circ}$ C/min to 310 $^{\circ}$ C for 4.50 minutes

Carrier gas: Helium

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 250 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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

SLPFPF100ID21-5UM - SELECTRA® PFPF 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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O.

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% NH₄OH

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

6. DRY ELUATE:

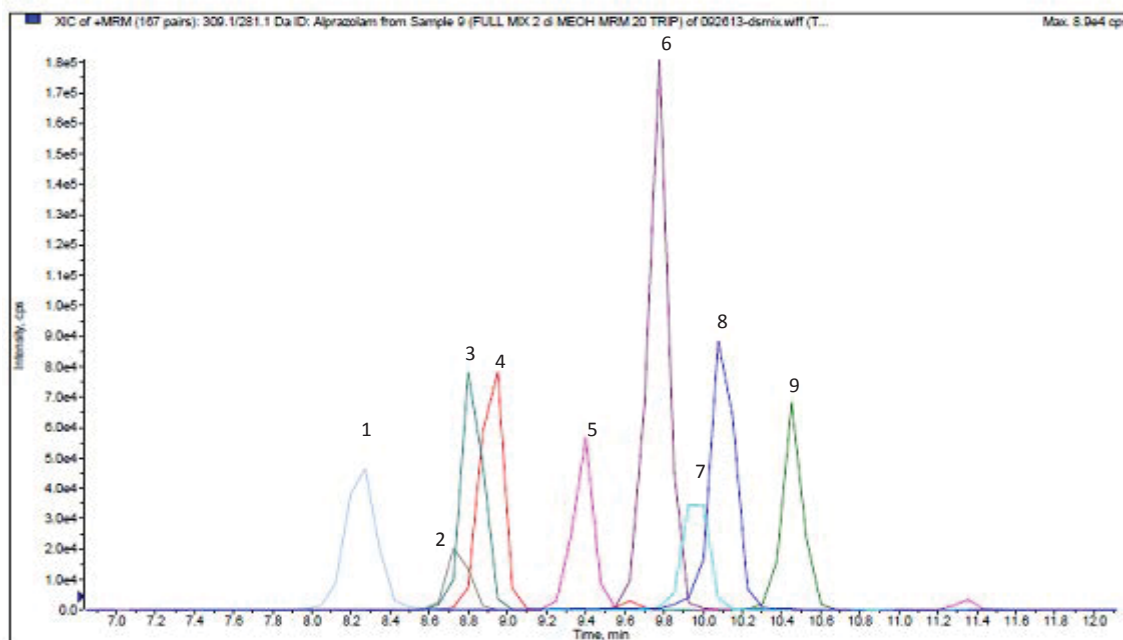
Evaporate to dryness at < 40°C.

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 N₂ 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. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 20 µL

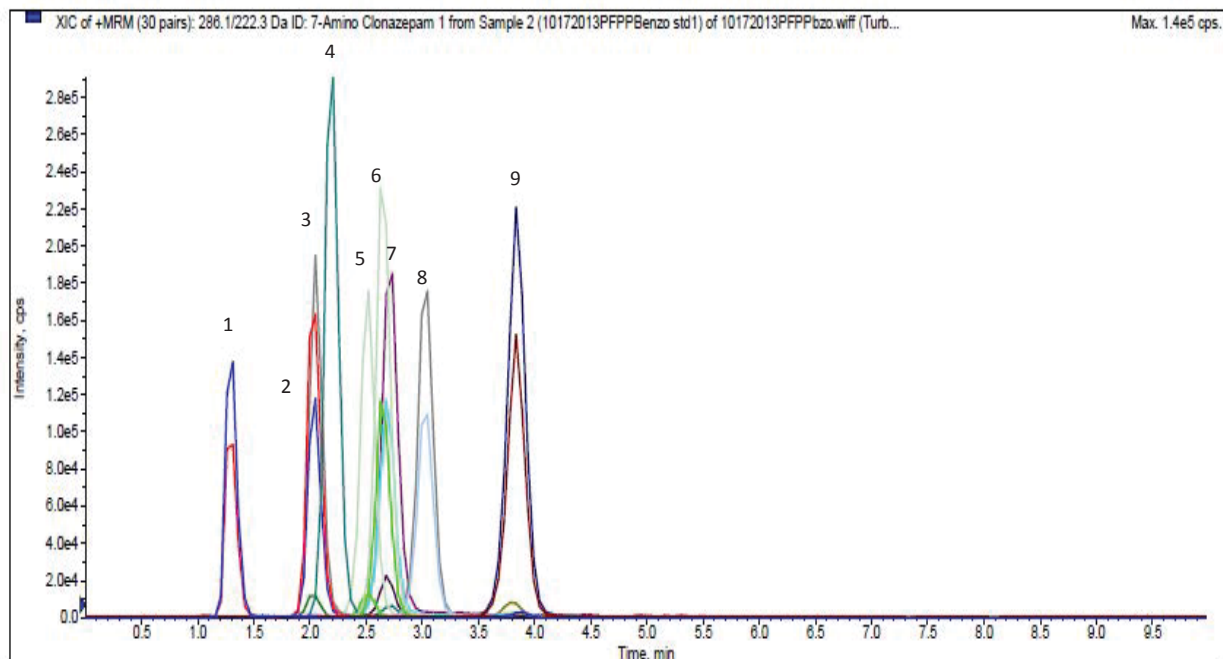
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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. H₂O

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

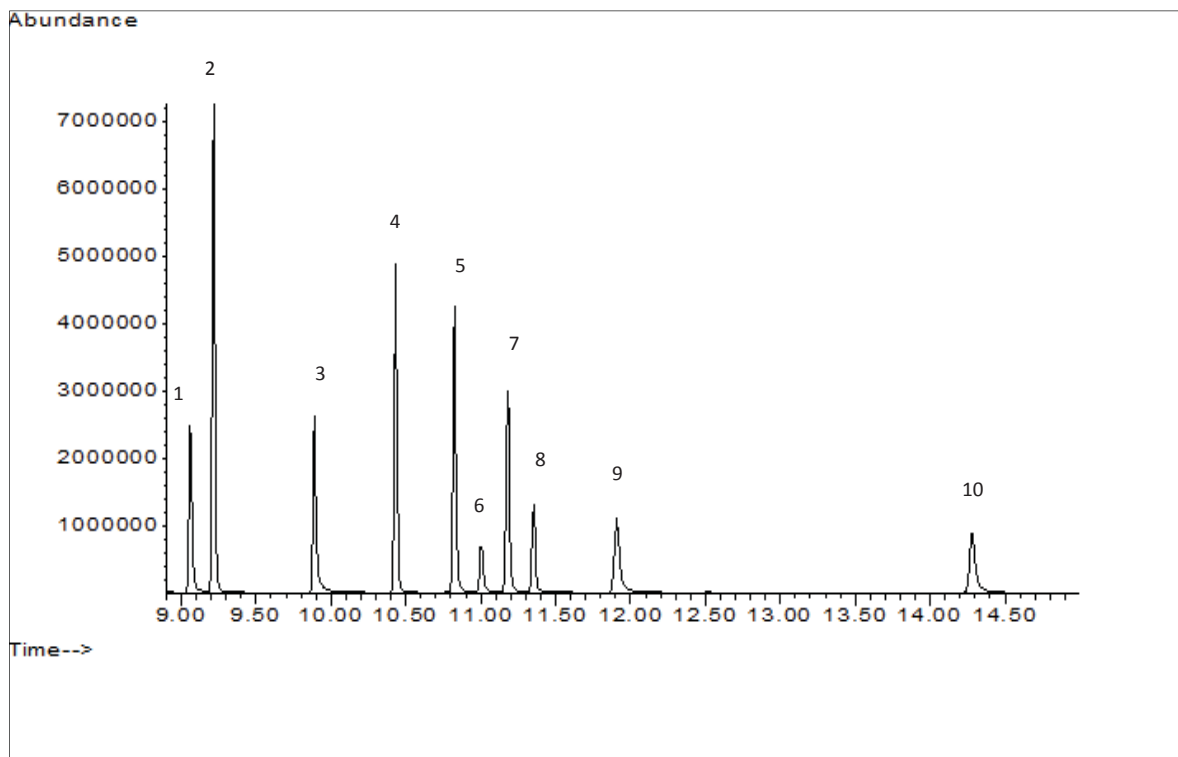
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 μ m

Injector: 1 μ L Splitless 250 $^{\circ}$ C

Oven temperature program: 160 $^{\circ}$ C for 0.5min; 15 $^{\circ}$ C/min to 310 $^{\circ}$ C for 4.50 minutes

Carrier gas: Helium

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 250 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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 μ m

SLPFPP100ID21-5UM - Selectra[®] PFPP HPLC Column, 100 x 2.1 mm, 5 μ m

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.

Add 1-2 mL of blood, plasma/ serum, 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 CH₂Cl₂

Dry column thoroughly under full vacuum or positive pressure for a minimum of 5-10 minutes.

4. ELUTION

1 x 3 mL Ethyl Acetate:NH₄OH (98:2)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUTE

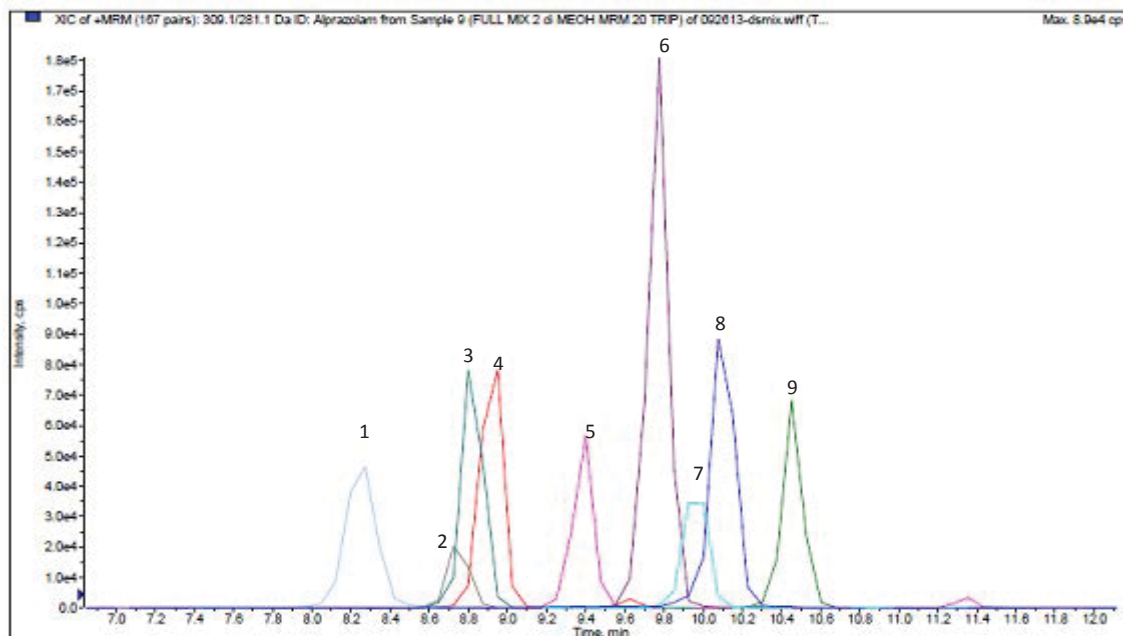
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10-20 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of ACN and 50 μ L MTBSTFA w/ 1%TBDMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1-2 μ L

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. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 20 µL

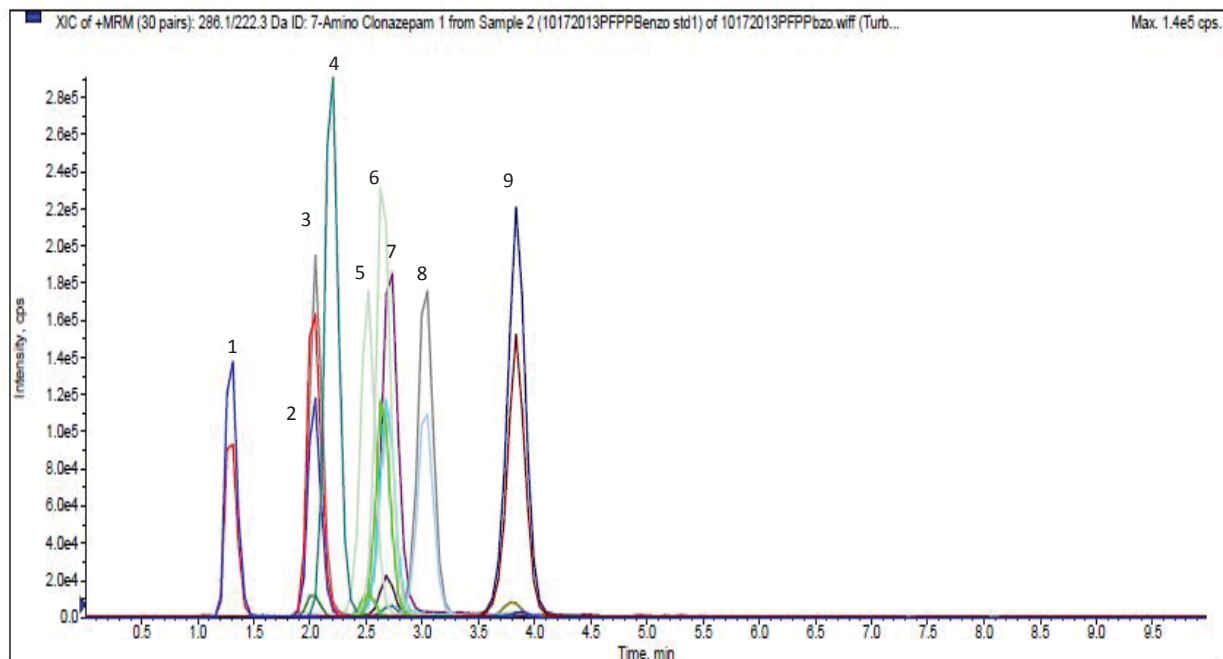
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

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. H₂O

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

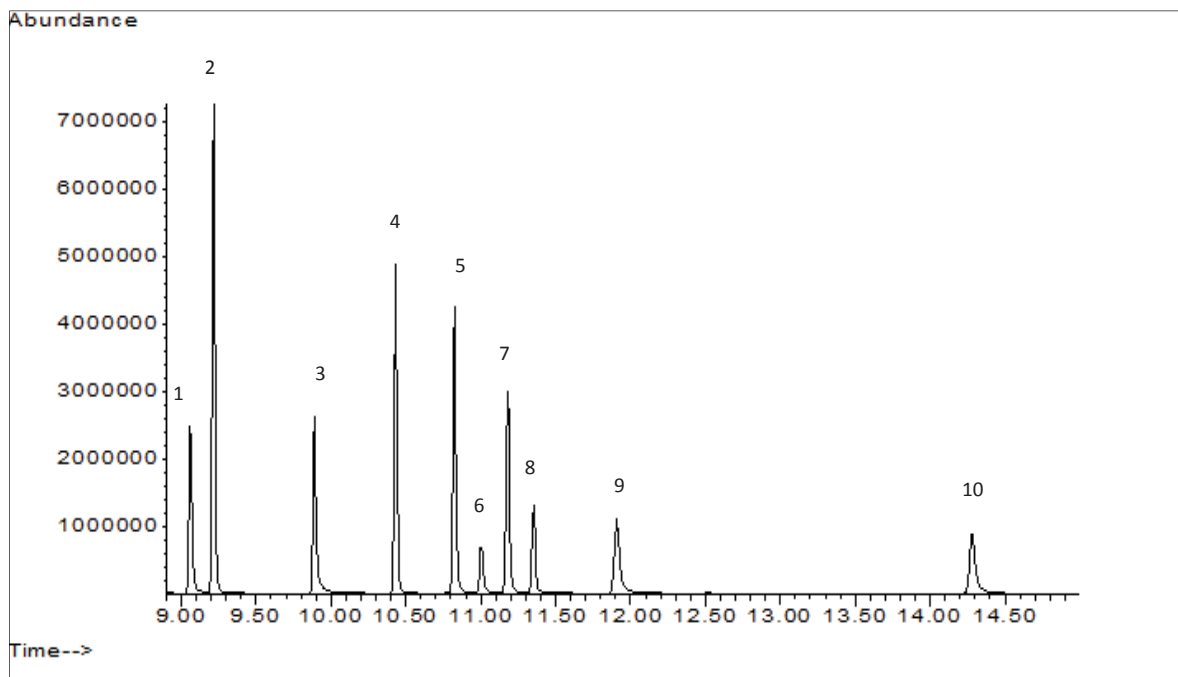
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 μ m

Injector: 1 μ L Splitless 250 $^{\circ}$ C

Oven temperature program: 160 $^{\circ}$ C for 0.5min; 15 $^{\circ}$ C/min to 310 $^{\circ}$ C for 4.50 minutes

Carrier gas: Helium

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 250 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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 μ m

SLPFPP100ID21-5UM - SELECTRA[®] PFP HPLC Column, 100 x 2.1 mm, 5 μ m

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 μ L 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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O.

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% NH₄OH

collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

6. DRY ELUATE:

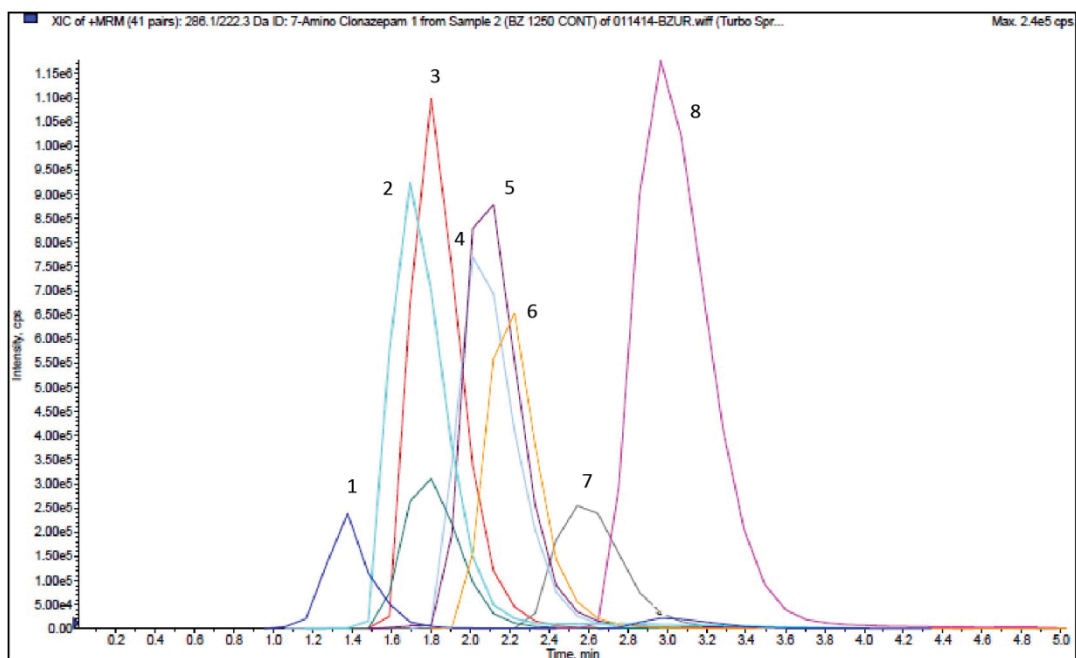
Evaporate to dryness at < 40 °C.

7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of ACN and 50 μ L MTBSTFA w/
1% TBDMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 -2 μ L

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



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: 0.1% Formic Acid in D.I. H₂O

Flow Rate: 0.1 mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

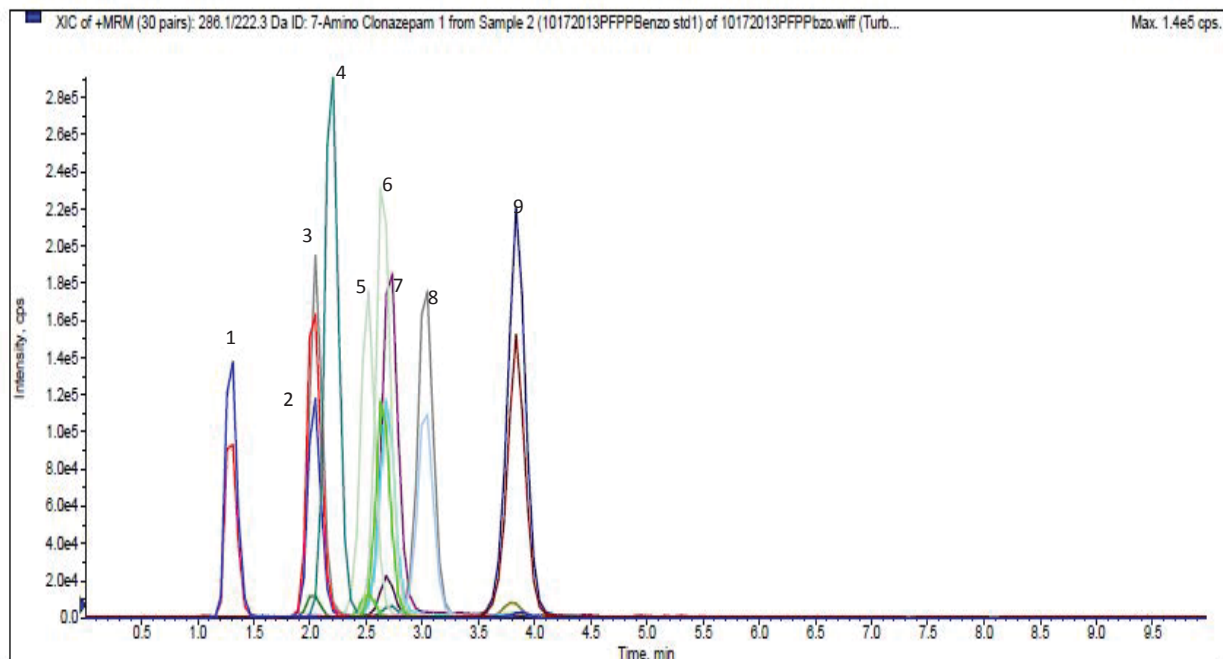
Polarity: Positive

Injection Volume: 10 µL

Isocratic Flow:

Time	%A	%B
0.00	50	50
7.50	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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

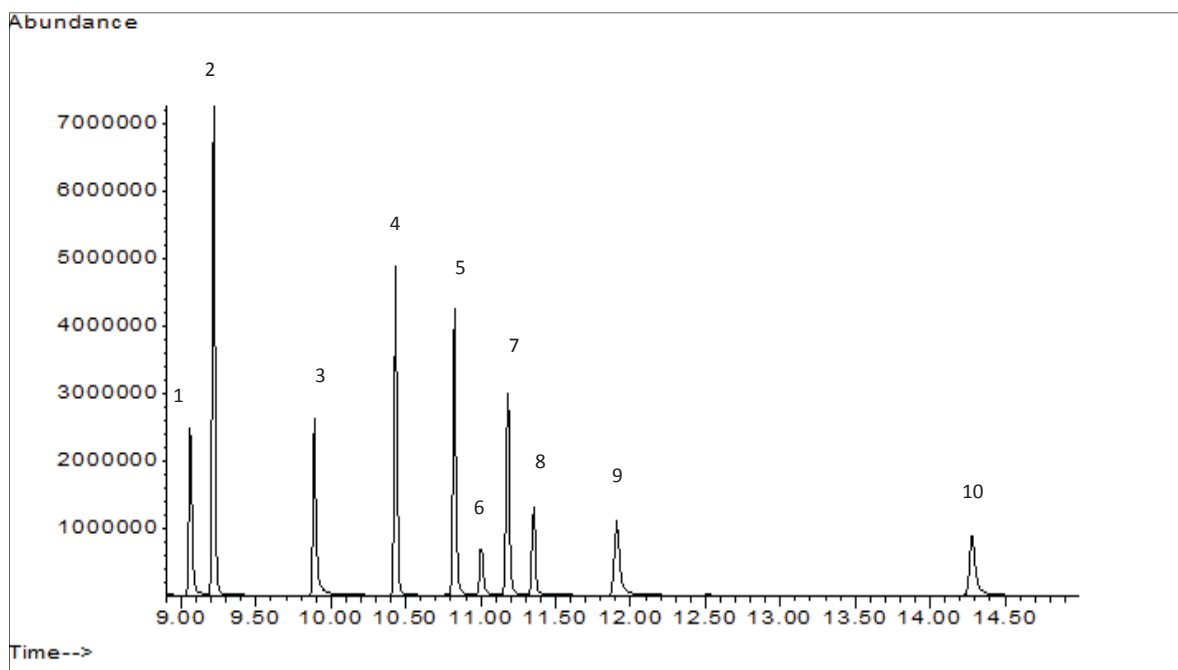
Injection Volume: 10 µL

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. Midazolam	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 μ m

Injector: 1 μ L Splitless 250 $^{\circ}$ C

Oven temperature program: 160 $^{\circ}$ C for 0.5min; 15 $^{\circ}$ C/min to 310 $^{\circ}$ C for 4.50 minutes

Carrier gas: Helium

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 250 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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 μ m

SLPFPP100ID21-5UM - Selectra[®] PFPP HPLC Column, 100 x 2.1 mm, 5 μ m

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 μ L 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 CH₂Cl₂

Dry column thoroughly under full vacuum or positive pressure for a minimum of 5-10 minutes.

4. ELUTION

1 x 3 mL Ethyl Acetate:NH₄OH (98:2)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUTE

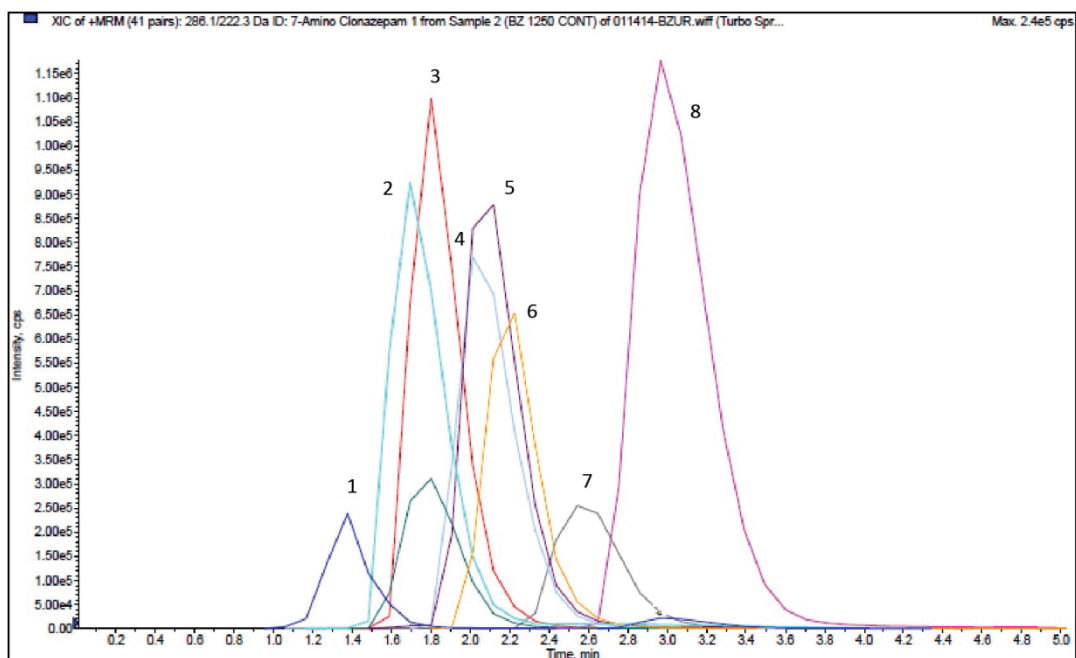
Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of ACN and 50 μ L MTBSTFA w/
1% TBDMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1-2 μ L

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



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: 0.1% Formic Acid in D.I. H₂O

Flow Rate: 0.1 mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

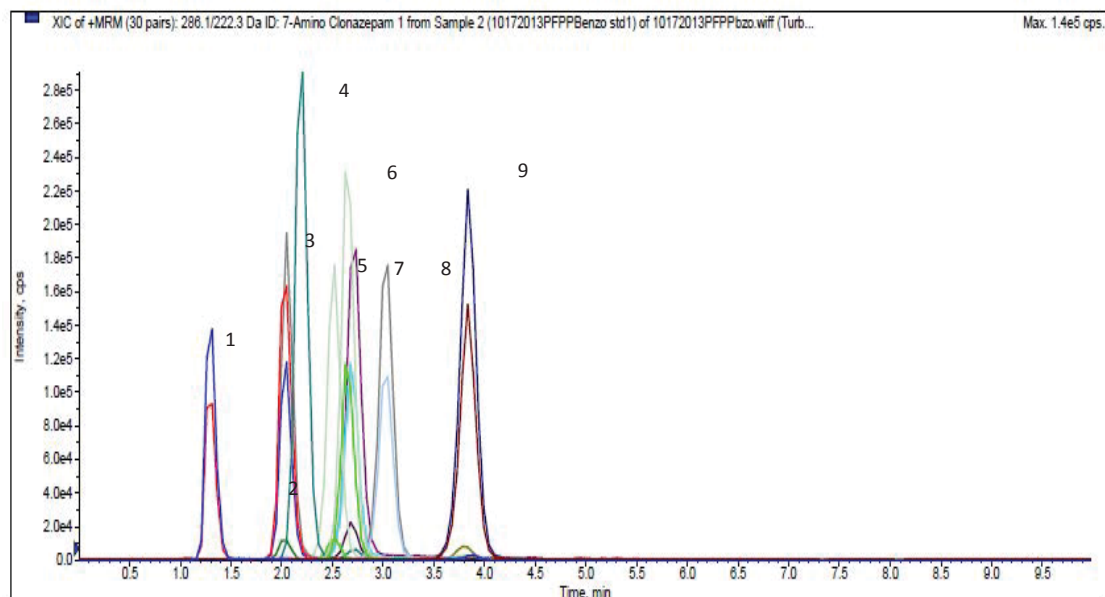
Polarity: Positive

Injection Volume: 10 µL

Isocratic Flow:

Time	%A	%B
0.00	50	50
7.50	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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

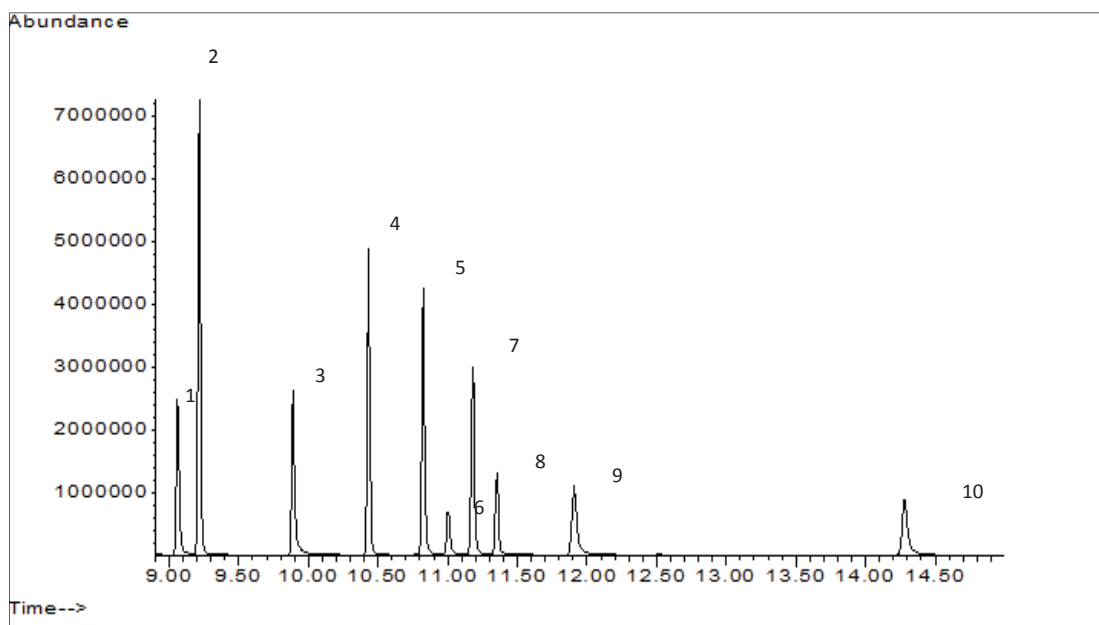
Injection Volume: 10 µL

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. Midazolam	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 μ m

Injector: 1 μ L Splitless 250 $^{\circ}$ C

Oven temperature program: 160 $^{\circ}$ C for 0.5min; 15 $^{\circ}$ C/min to 310 $^{\circ}$ C for 4.50 minutes

Carrier gas: Helium

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 250 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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-glucuronidase

SMTBSTFA-1-1 – SELECTRA- SIL[®] MTBSTFA w/ 1% TBDMCS

SLDA50ID21-5UM – Selectra[®] DA HPLC Column, 50 x 2.1 mm, 5 μ m

or

SLPFPF100ID21-5UM – Selectra[®] PFPP HPLC Column, 100 x 2.1 mm, 5 μ m

1. PREPARE SAMPLE

To 1-2 mL whole blood, plasma/ serum add 500 μ L 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 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 μ L 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 CH₂Cl₂

Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. ELUTION

1 x 1 mL Ethyl Acetate/NH₄OH (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

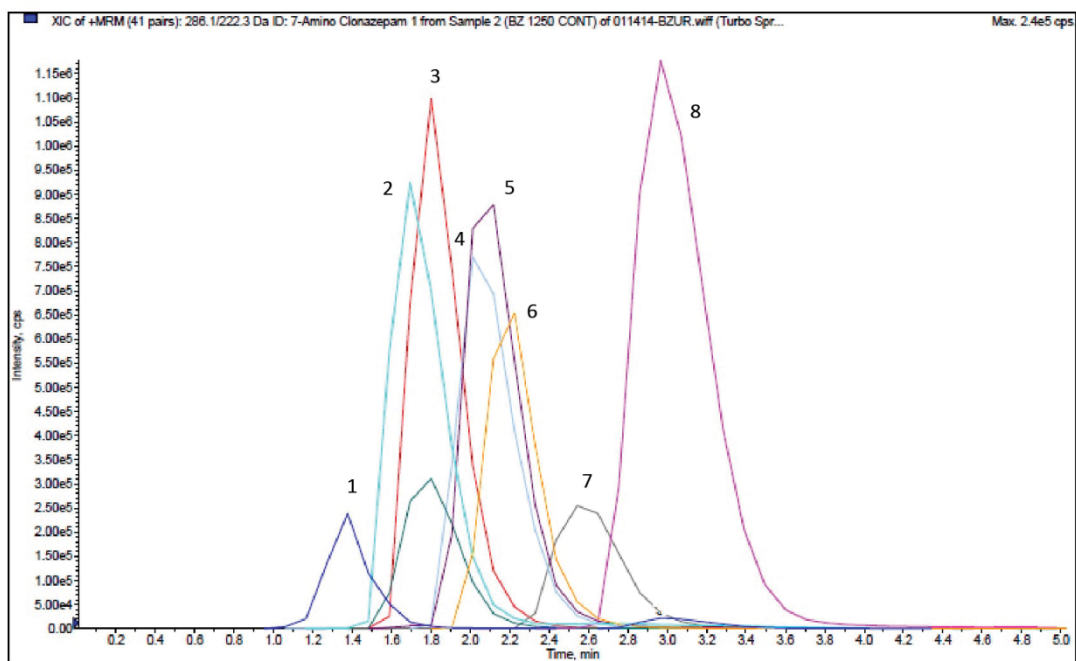
- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of ACN and 50 μ L MTBSTFA w/ 1% TBDMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1-2 μ L

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



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: 0.1% Formic Acid in D.I. H₂O

Flow Rate: 0.1 mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

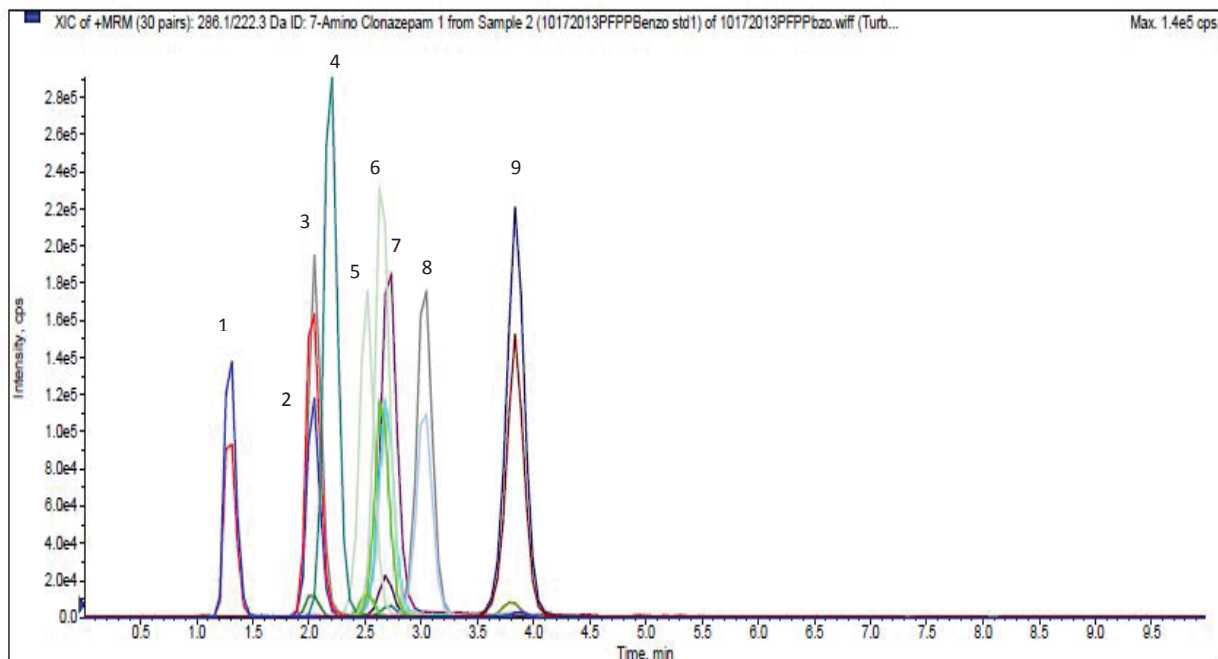
Polarity: Positive

Injection Volume: 10 µL

Isocratic Flow:

Time	%A	%B
0.00	50	50
7.50	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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

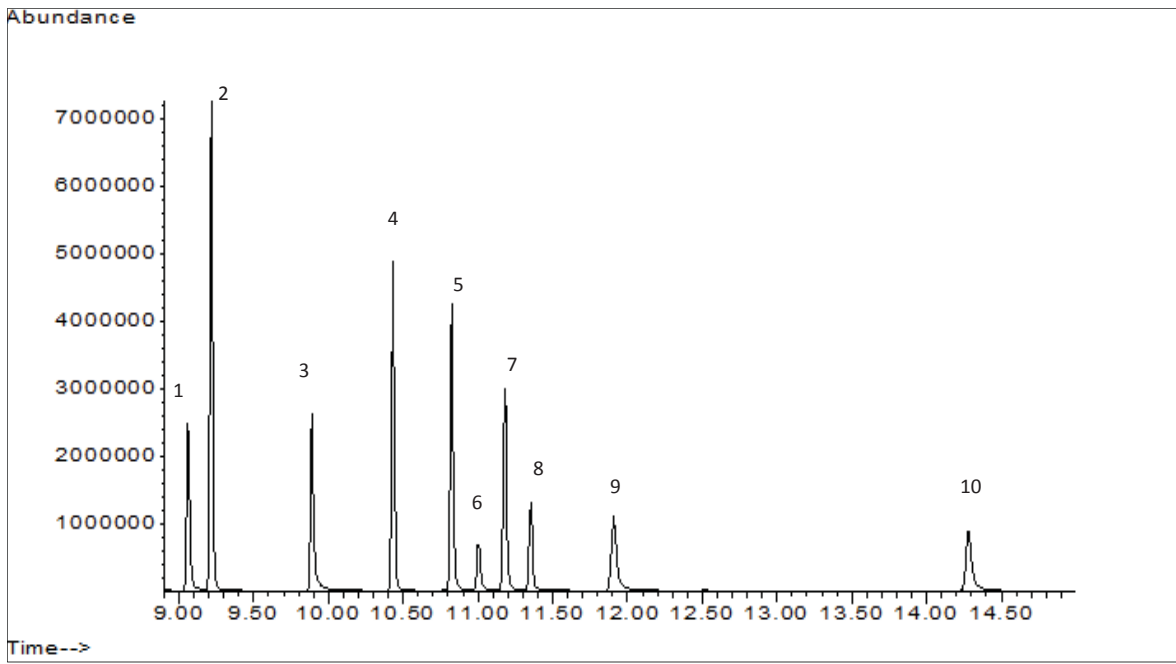
Injection Volume: 10 µL

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. Midazolam	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 °C, MS Source: 250 °C, MS Quad: 150 °C



CLINICAL



FORENSICS



UCT

Cannabinoids





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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O

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:

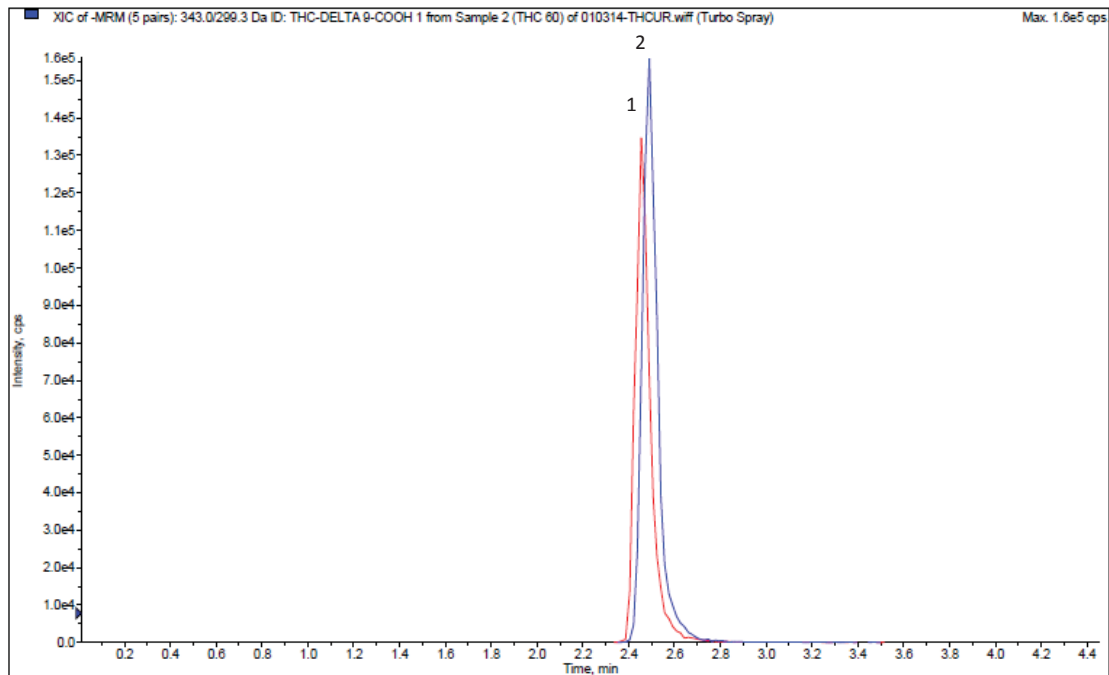
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 20 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and
50 µL MSTFA w/1%TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 µL

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. H₂O

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

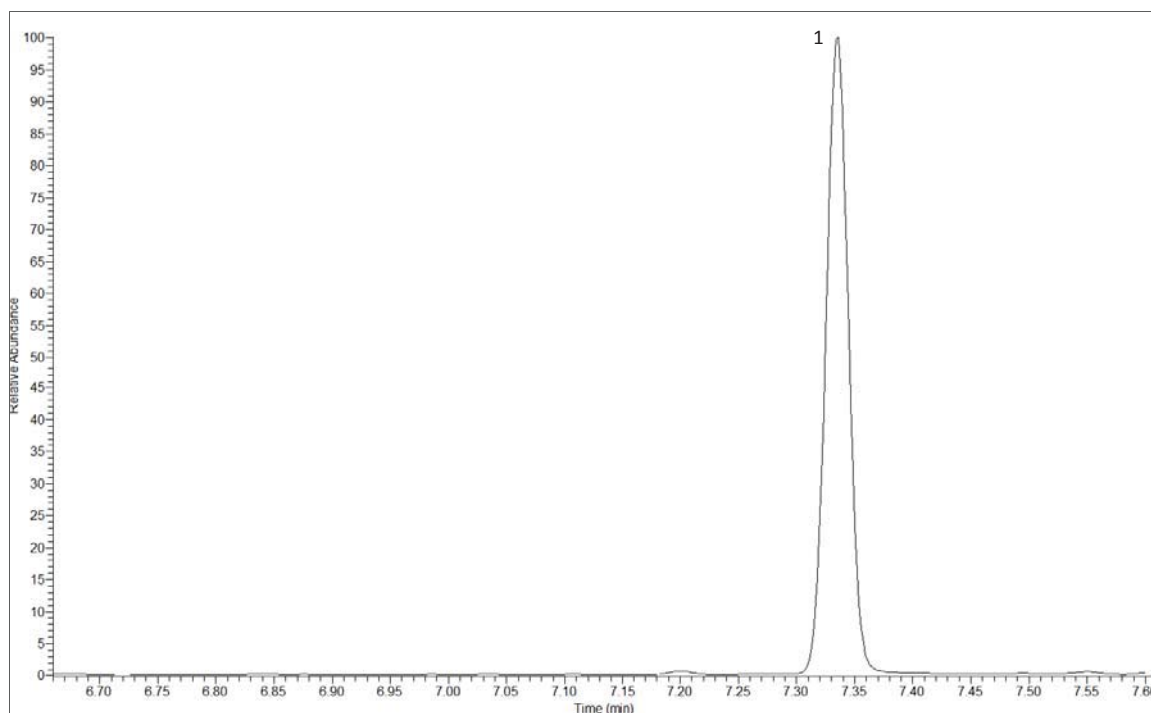
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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 °C, MS Source: 350 °C, MS Quad: 150 °C



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 μ m

1. PREPARE SAMPLE-BASE HYDROLYSIS OF GLUCURONIDES:

To 2 mL of urine add internal standard and 100 μ L 10M NaOH.

Mix/vortex. Hydrolyze for 20 mins at 60 °C. Cool before proceeding.

Adjust sample pH to 3.5 \pm 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. H₂O.

1 x 1 mL 0.1M HCl/Acetonitrile (70/30).

Dry column (3 mins at > 10 inches Hg).

1 x 200 μ L 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:

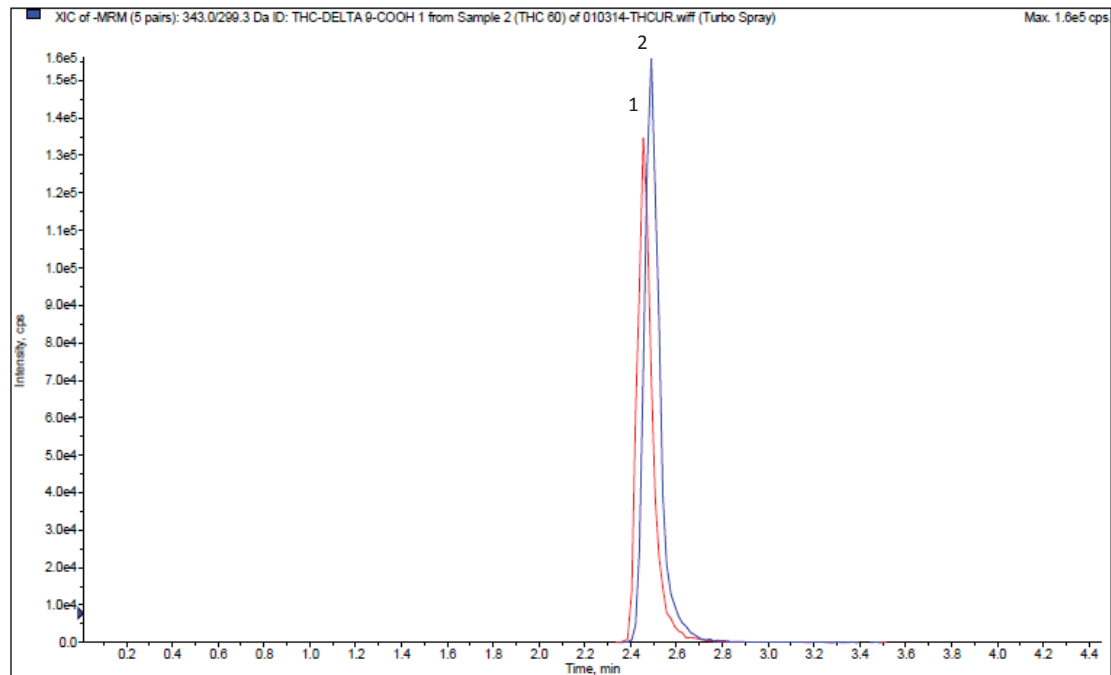
- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 20 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of Ethyl Acetate and 50 μ L MSTFA w/
1%TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 μ L

Alternate Derivatization

1. Form TMS Derivatives by adding 50 μ L BSTFA w/ 1% TMCS and 50 μ L 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. H₂O

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

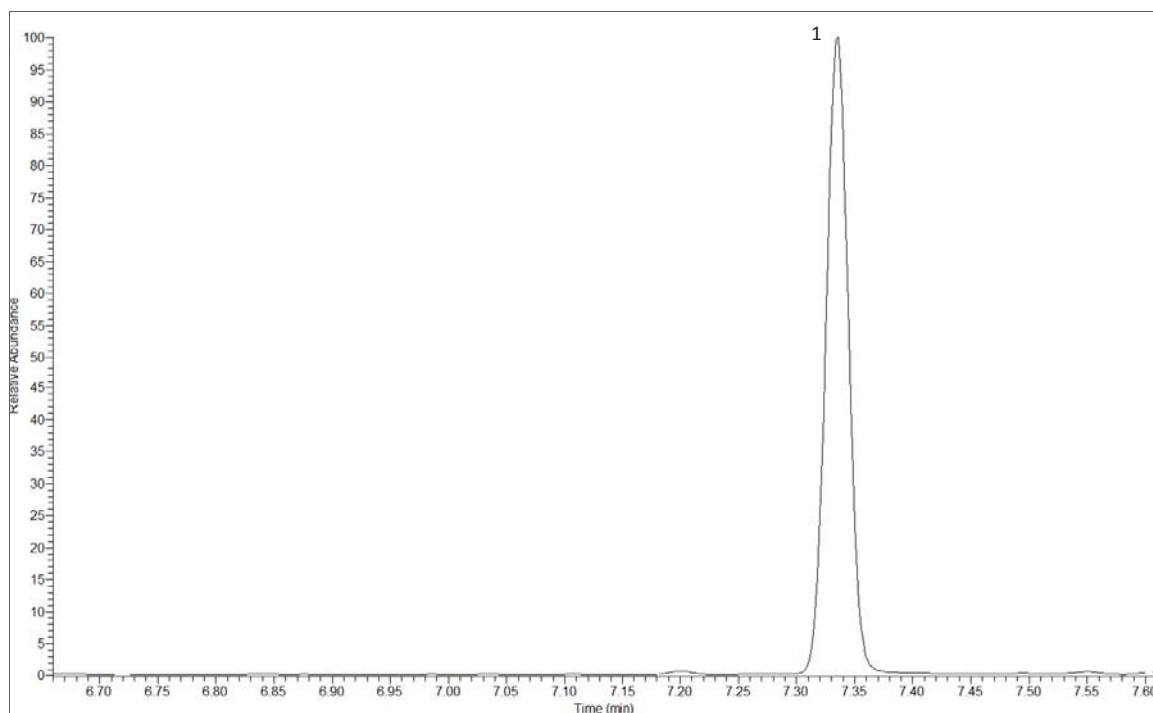
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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 °C, MS Source: 350 °C, MS Quad: 150 °C



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 H₂O: 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:

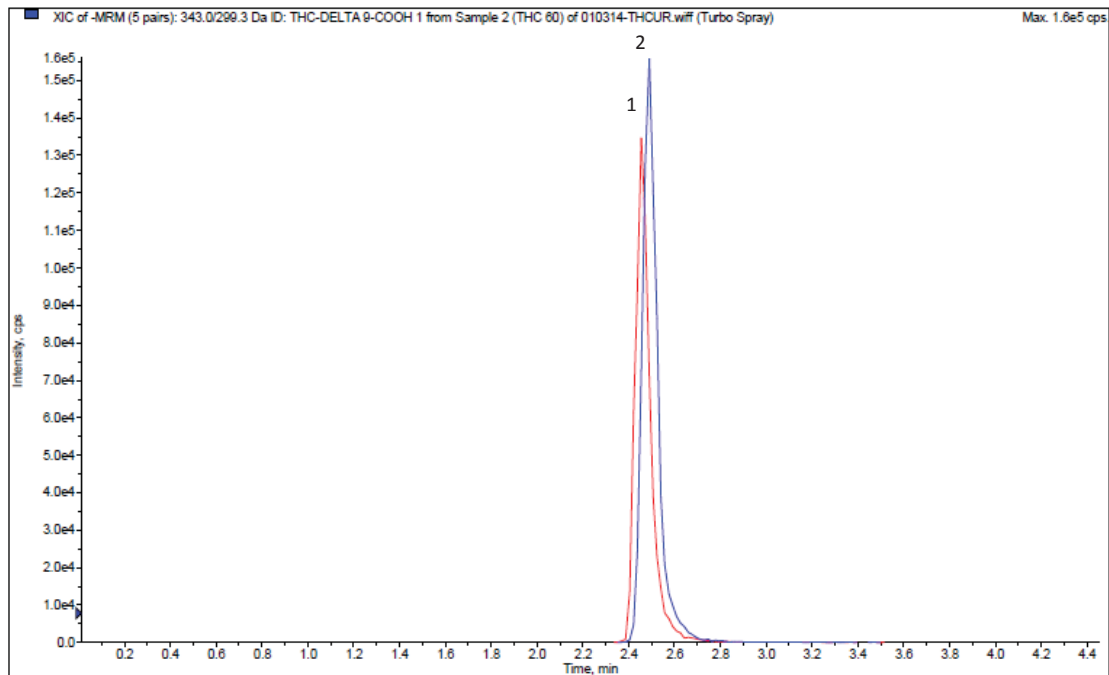
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 20 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and 50 µL MSTFA (with 1%TMCS)
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 µL

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. H₂O

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

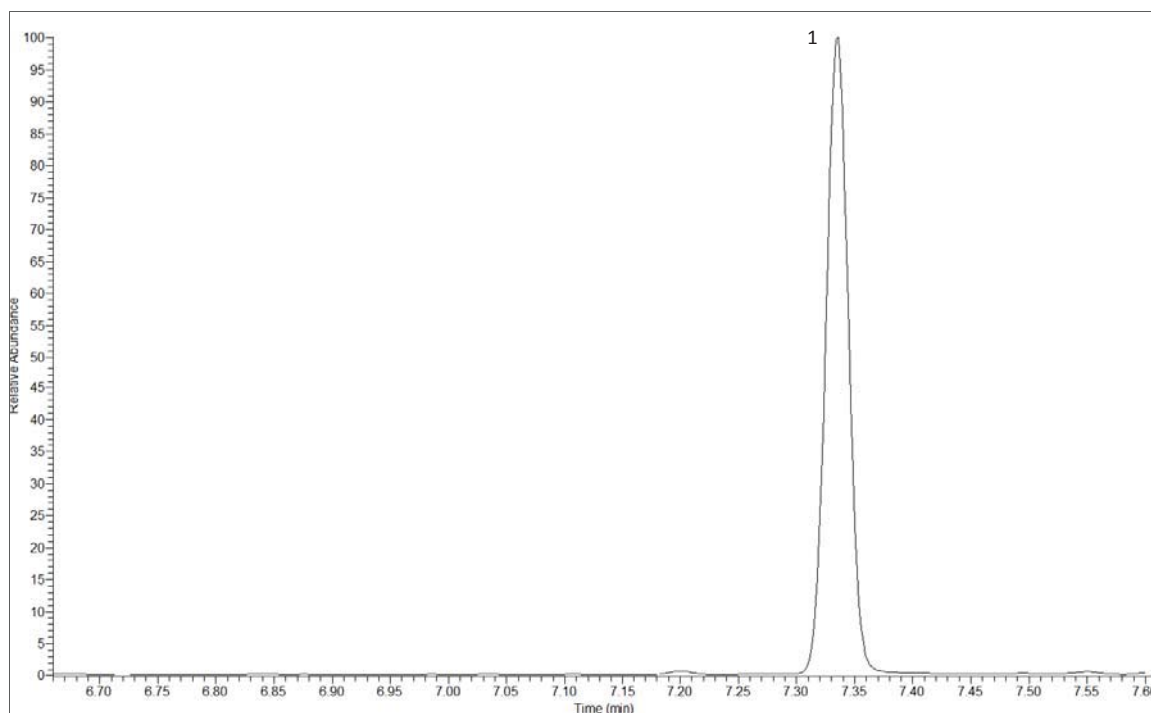
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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 °C, MS Source: 350 °C, MS Quad: 150 °C



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 μ m

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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O

1 x 2 mL of 100 mM HCl/Acetonitrile (95:5)

Aspirate at full vacuum or pressure for 5 minutes

1 x 200 μ L 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 μ L hexane in the wash/dry step. The 200 μ L 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 N₂ and cap. Mix/vortex
React 30 minutes at 70°C; Cool and inject 2 µL

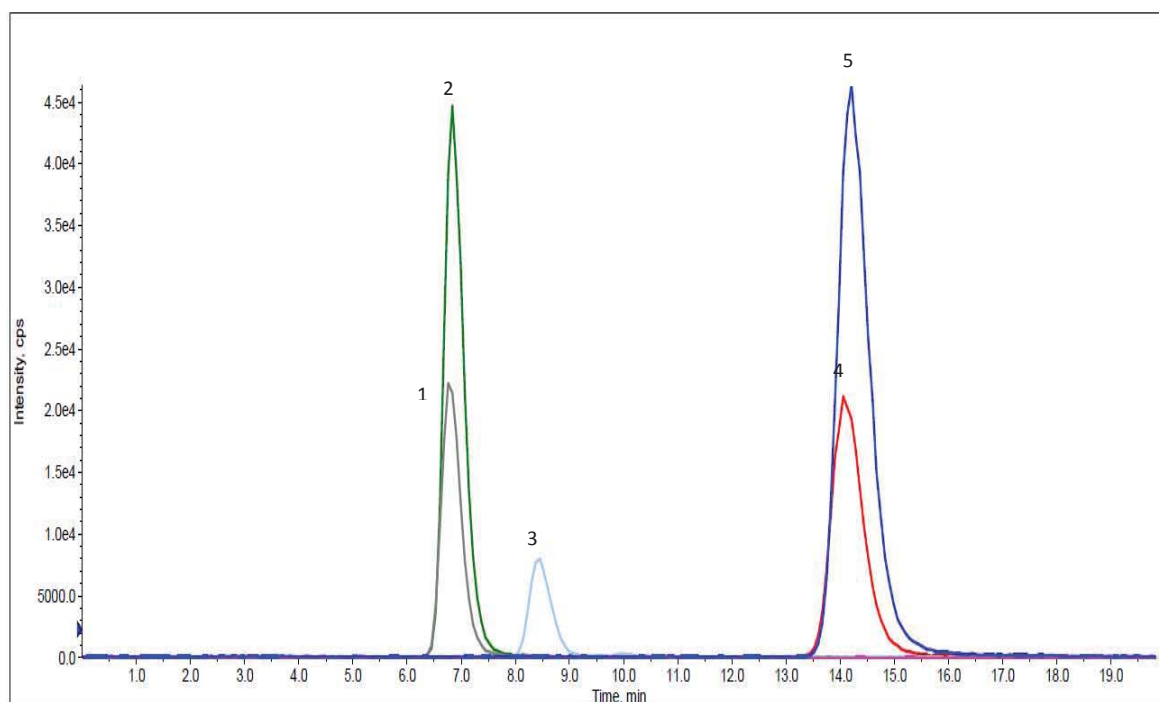
Alternate Derivatization

1. Derivatize with MTBSTFA (with 1% TBDMCS):

Dissolve residue in 50 µL of pyridine and 50 µL MTBSTFA w/ 1%TBDMCS
Overlay with N₂ 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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Negative/Positive

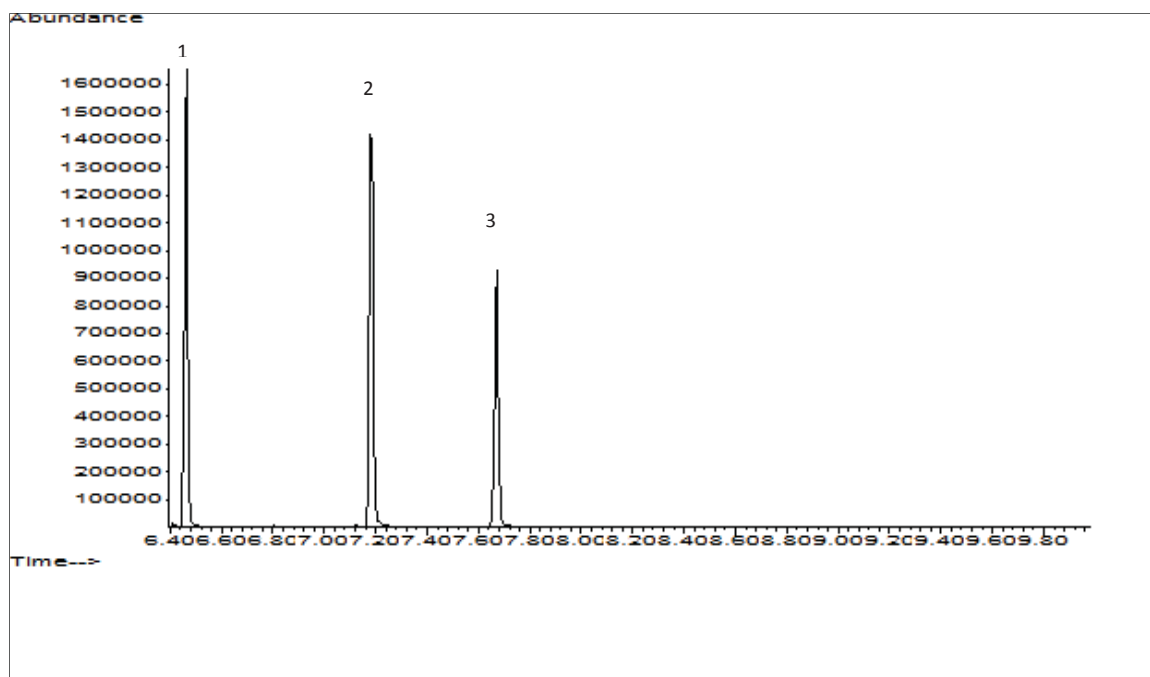
Injection Volume: 5 µL

Isocratic:

Time	%A	%B
0.00	25	75
20.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

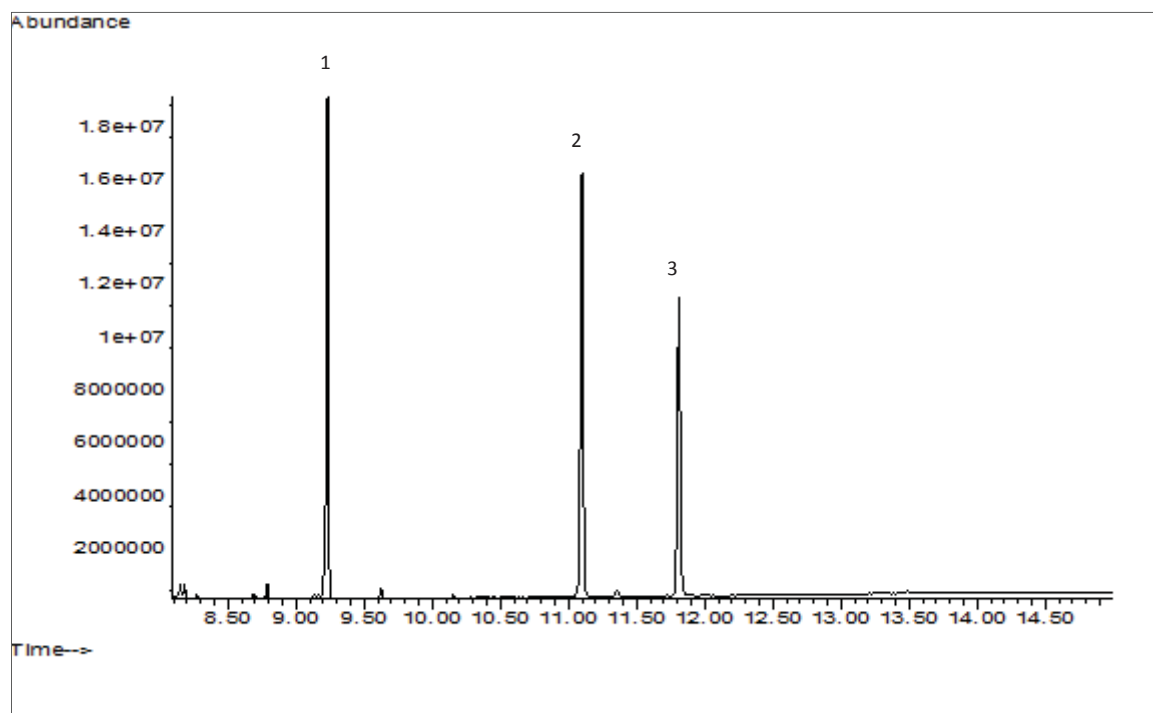
CHROMATOGRAM



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 °C, MS Source: 250 °C, MS Quad: 150 °C



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 of air or nitrogen to ~ 200 µL

Add 2 mL D.I. H₂O (pH of H₂O 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. H₂O: Acetonitrile: NH₄OH (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 N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 2 µL

Alternate Derivatization

1. Derivatize with MTBSTFA w/ 1% TBDMCS:

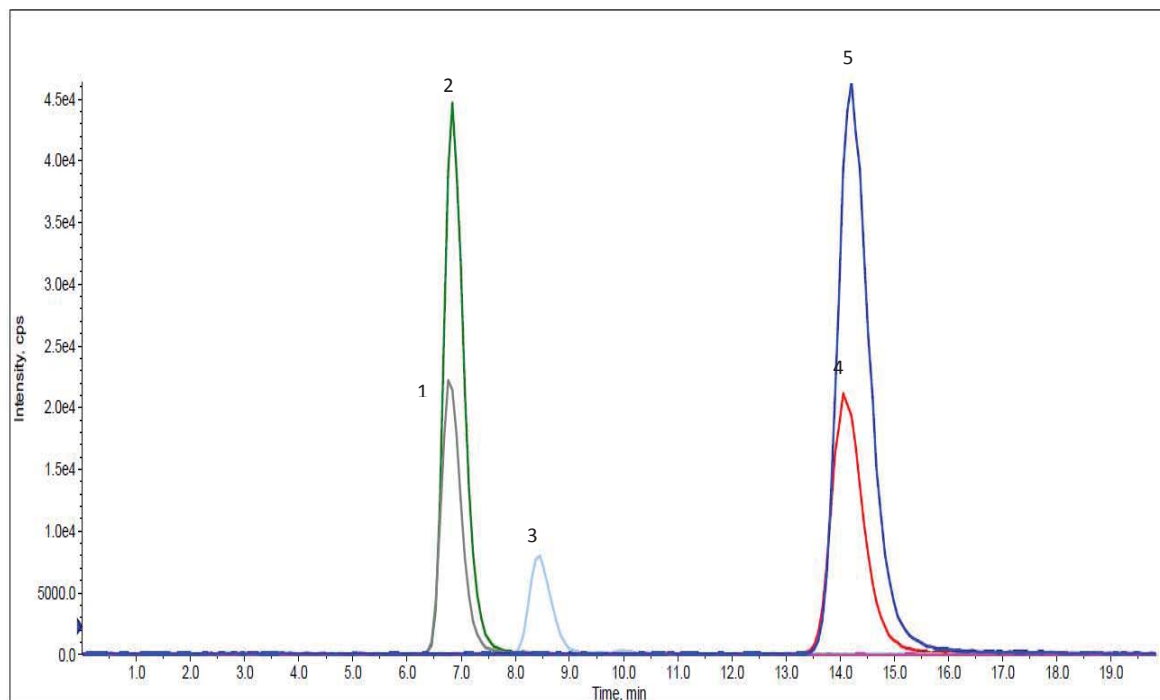
Dissolve residue in 50 µL of pyridine and 50 µL MTBSTFA w/ 1%TBDMCS

Overlay with N₂ 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. H₂O

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

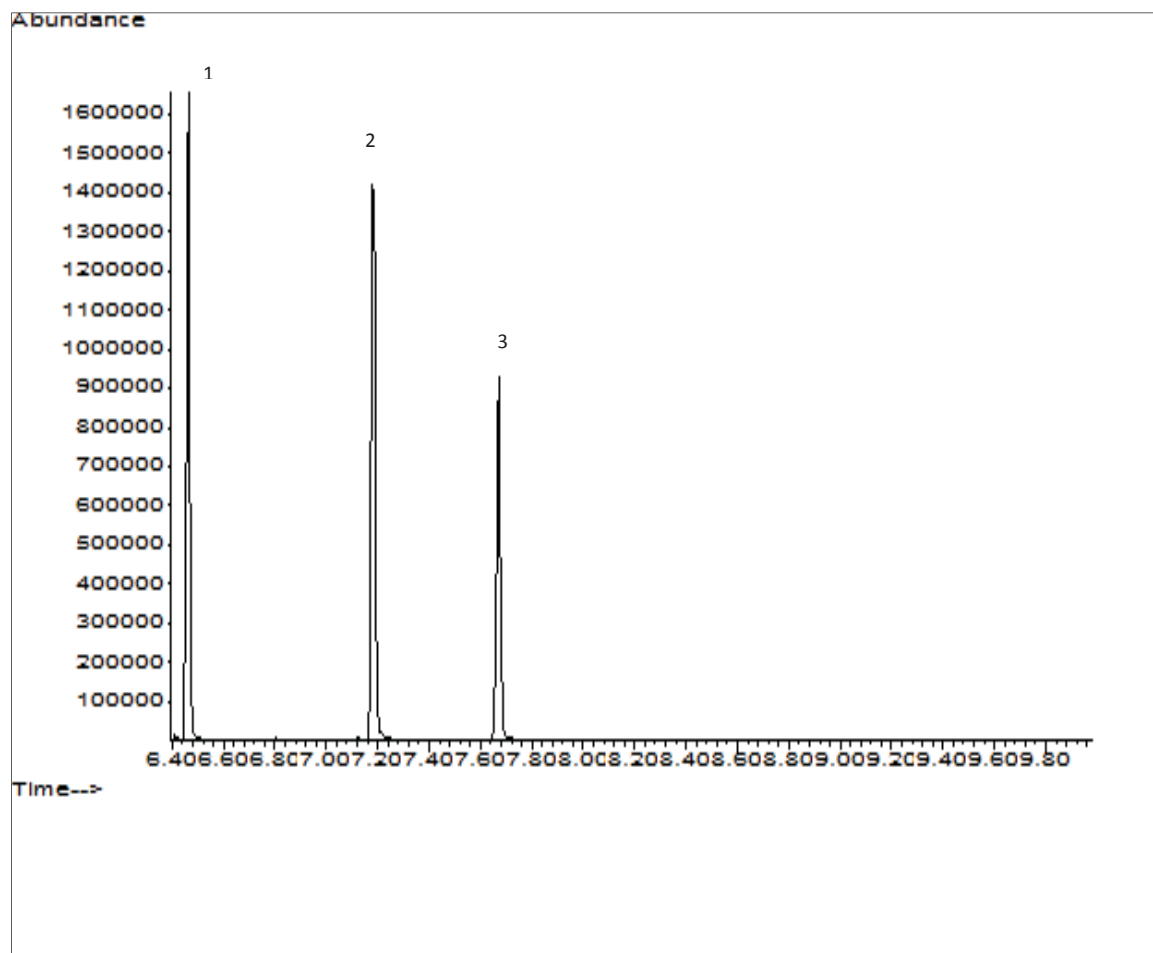
LC Column: Selectra[®] DA HPLC Column 100 x 2.1 mm 5 µm

Isocratic:

Time	%A	%B
0.00	25	75
20.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

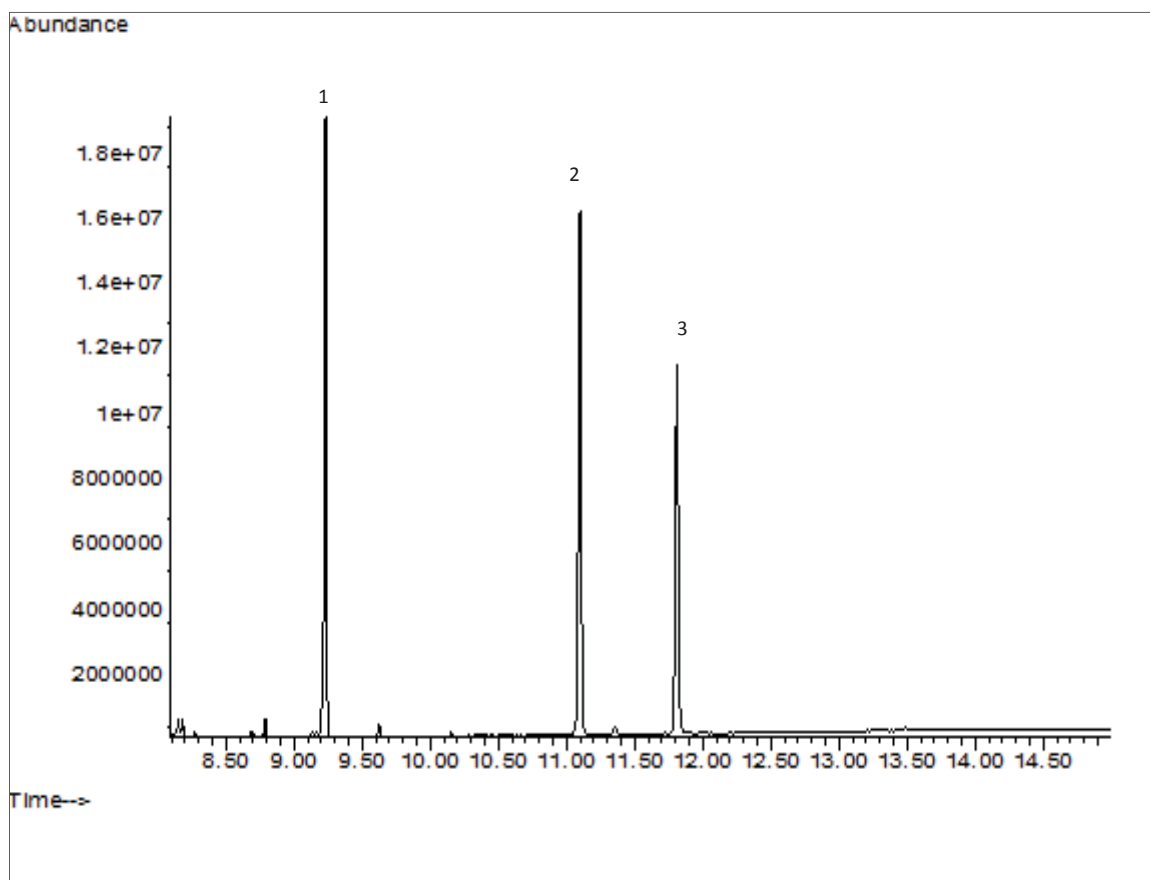
CHROMATOGRAM



TMS IONS

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
THC-D ₃	389.25	374.3		
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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 °C, MS Source: 250 °C, MS Quad: 150 °C



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 μ m

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 \pm 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 μ L of concentrated β -glucuronidase.
Vortex and heat for 1-2 hours at 65 $^{\circ}$ 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 CH₃OH.
1 x 3 mL D.I. H₂O.
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. H₂O
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% CH₃OH
Collect eluate at 1-2 mL /minute

6. DRY ELUATE:

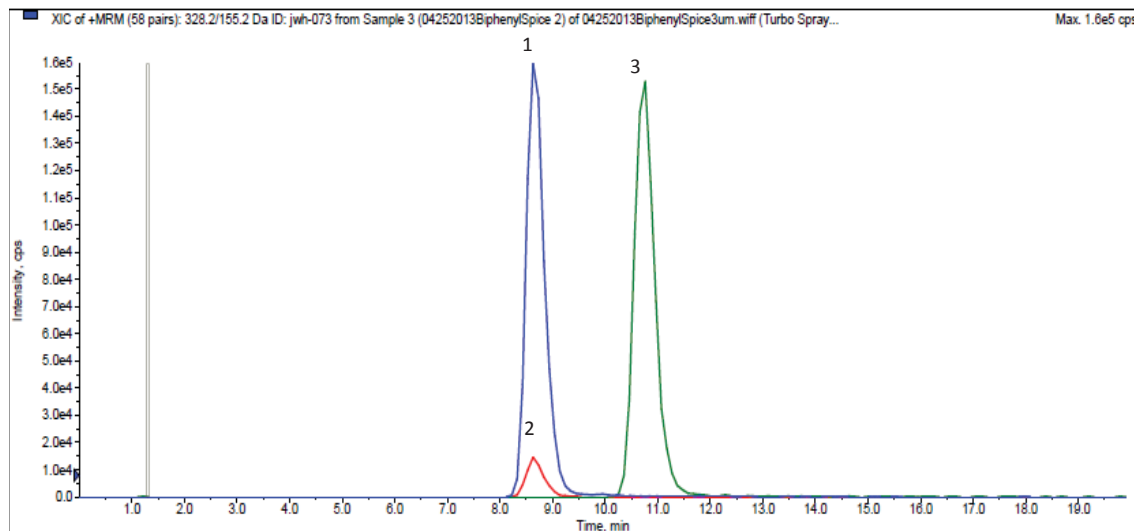
Evaporate to dryness at < 40 $^{\circ}$ C.

7. . RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L.
- **GC-MS:** Dissolve residue in 50 μ L of Ethyl Acetate and
50 μ L MSTFA w/1% TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 μ L

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. H₂O

Flow Rate: 0.7 mL/minute

Reconstitute: 100 μ L

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

Injection Volume: 3 μ L

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 - Sselectrazyme[®] 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 Sselectrazyme[®] 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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O

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 % CH₃OH

Collect eluate at 1-2 mL /minute

6. DRY ELUATE:

Evaporate to dryness at < 40 °C.

7. . RECONSTITUTE / DERIVATIZE:

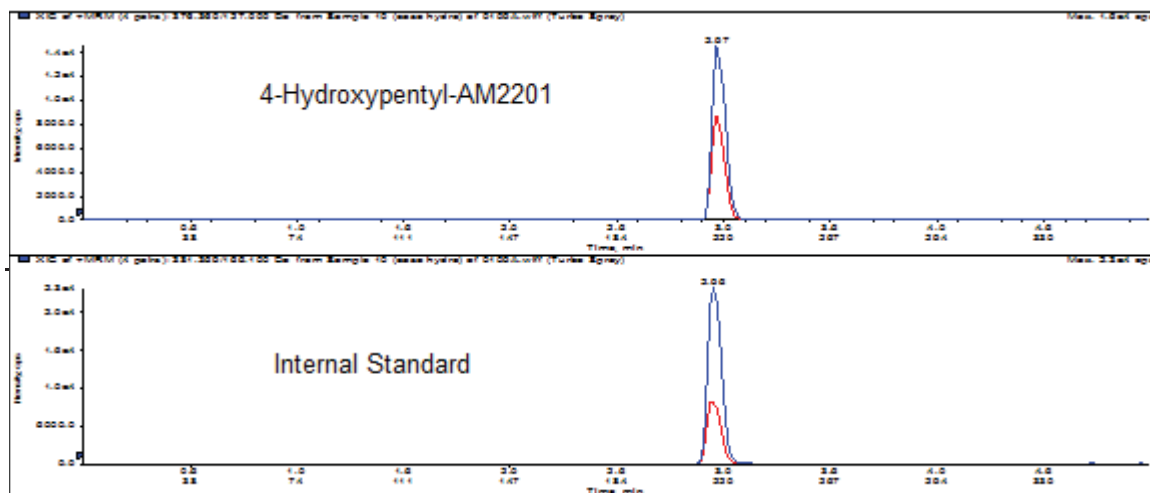
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 10 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and 50 µL MSTFA w/
1%TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 mL

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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 µL

LC Column: Selectra[®] C18 HPLC Column 50 x 2.1 mm 3 µm

Instrument: API 3200 QTrap MS/MS Compound MRM Transition

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Polarity: Positive

Injection Volume: 10 µL

Gradient:

Time	%A	%B
0	65	35
0.5	65	35
3.5	10	10
4.0	65	35
5.0	65	35



CLINICAL



FORENSICS



UCT

Opiates





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 μ m

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 \pm 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 μ L of concentrated β -glucuronidase.
Vortex and heat for 1-2 hours at 65 $^{\circ}$ 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. H₂O
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 CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

5. DRY ELUTE

Evaporate fraction to complete dryness under stream of dry air or nitrogen at \sim 35 $^{\circ}$ C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L

- GC-MS: Derivatize with propionic anhydride: pyridine**
 Add 200 μ L of a 1:1 solution of propionic anhydride: pyridine
 Make this solution fresh daily.
 Mix/vortex.
 React for 60 minutes at 60 $^{\circ}$ C in a heater block.
 Remove from heat source to cool.
 Evaporate to dryness at < 40 $^{\circ}$ C.
 Reconstitute the residue with 50 μ L of Ethyl Acetate / Methanol (70:30)

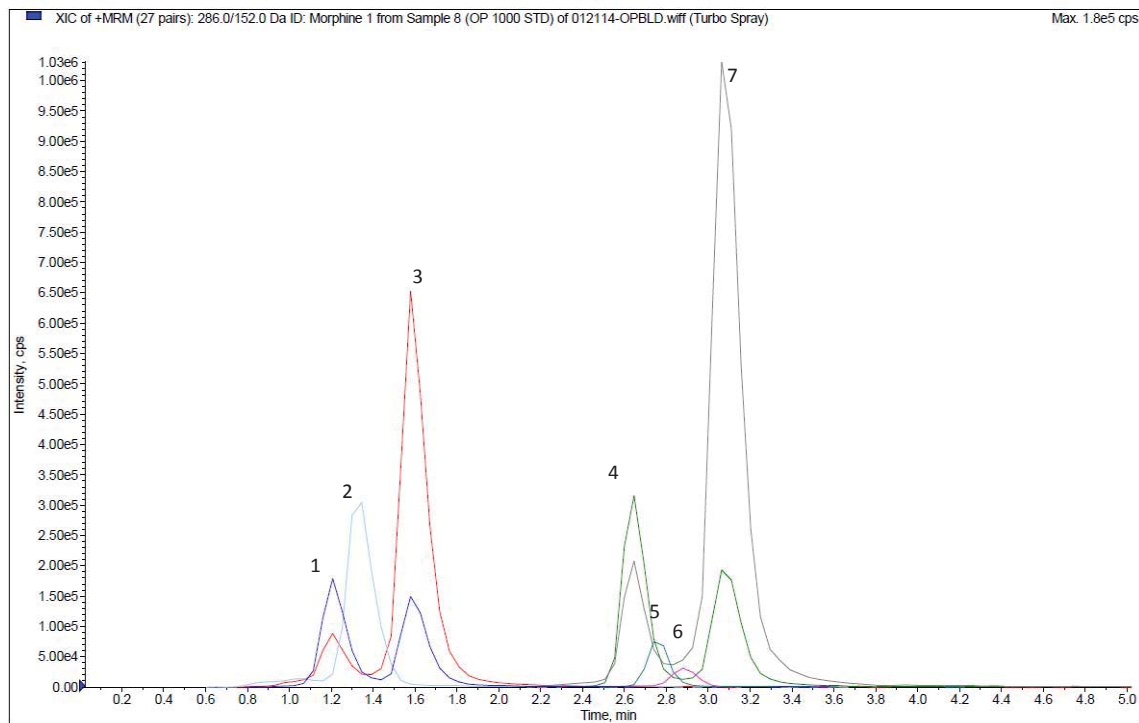
Alternate Derivatization

1. DERIVATIZE with TMS

- Add 50 μ L Ethyl Acetate and 50 μ L BSTFA w/ 1% TMCS
- Overlay with N₂ and cap. Mix/vortex.
- React 30 minutes at 70 $^{\circ}$ 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 μ L 10% Hydroxylamine solution.
 Heat to 60 $^{\circ}$ 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. Oxycodone	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. H₂O

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

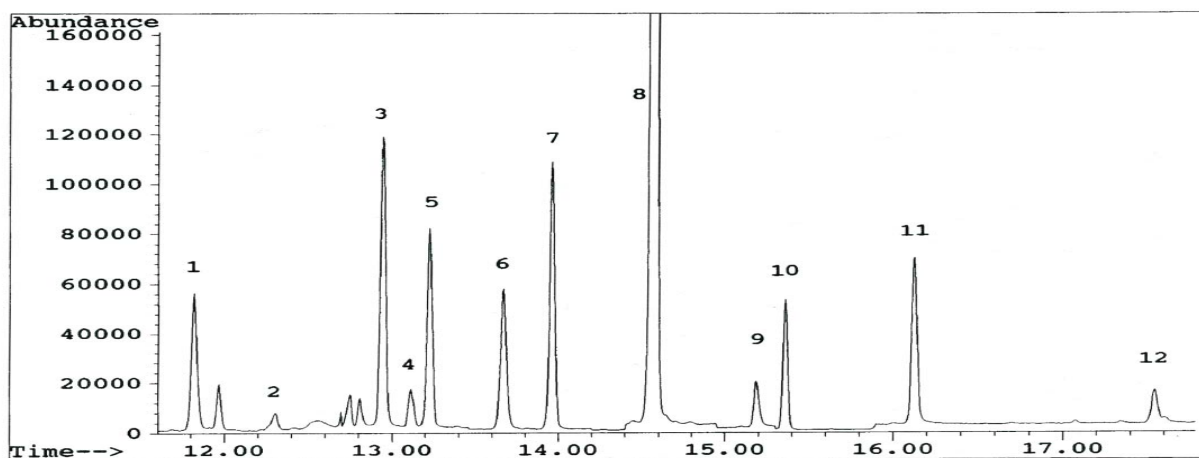
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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 μ m

Injector: 2 μ L Splitless, 250 $^{\circ}$ C

Oven temperature program: 100 $^{\circ}$ C (1 minute) to 250 $^{\circ}$ C (25 $^{\circ}$ C/minute): hold (2 minutes) to 290 $^{\circ}$ C (10 $^{\circ}$ C/minute): hold (0.5 minutes) to 325 $^{\circ}$ C (25 $^{\circ}$ C/minute): hold (3.1 minutes).

Carrier gas: Helium (1.2 mL/minute)

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 350 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C

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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O.

1 x 3 mL 100 mM acetate buffer (pH 4.5).

1 x 3 mL CH₃OH.

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

5. ELUTE OPIATES:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (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 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 $^{\circ}\text{C}$ in a heater block.
Remove from heat source to cool.
Evaporate to dryness at < 40 $^{\circ}\text{C}$.
Reconstitute the residue with 50 μL of Ethyl Acetate / Methanol (70:30)

Alternate Derivatization

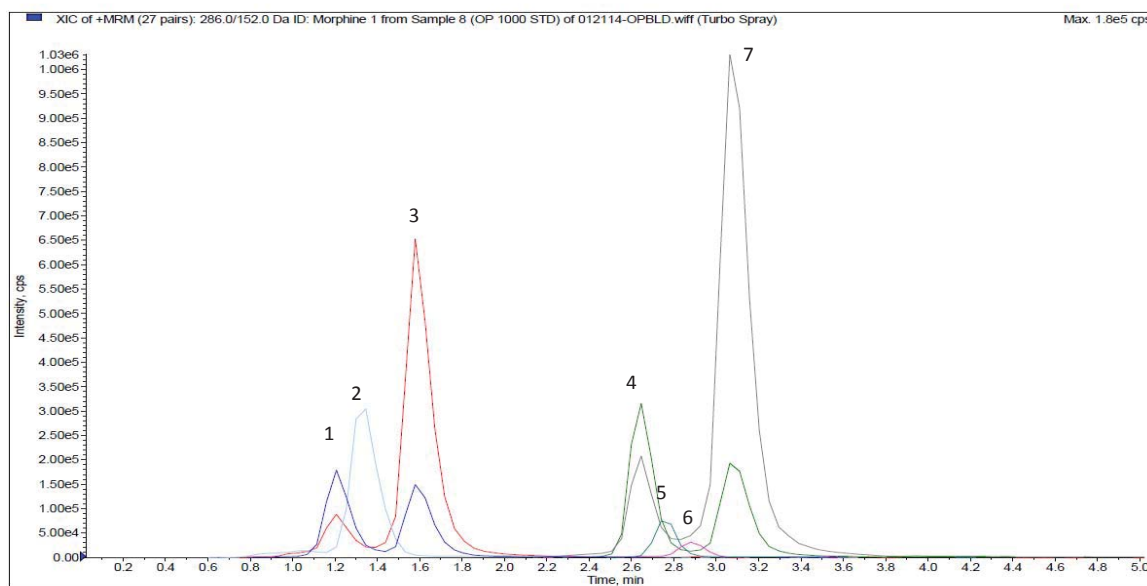
1. DERIVATIZE with TMS

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 $^{\circ}\text{C}$. 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 $^{\circ}\text{C}$ 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. H₂O

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

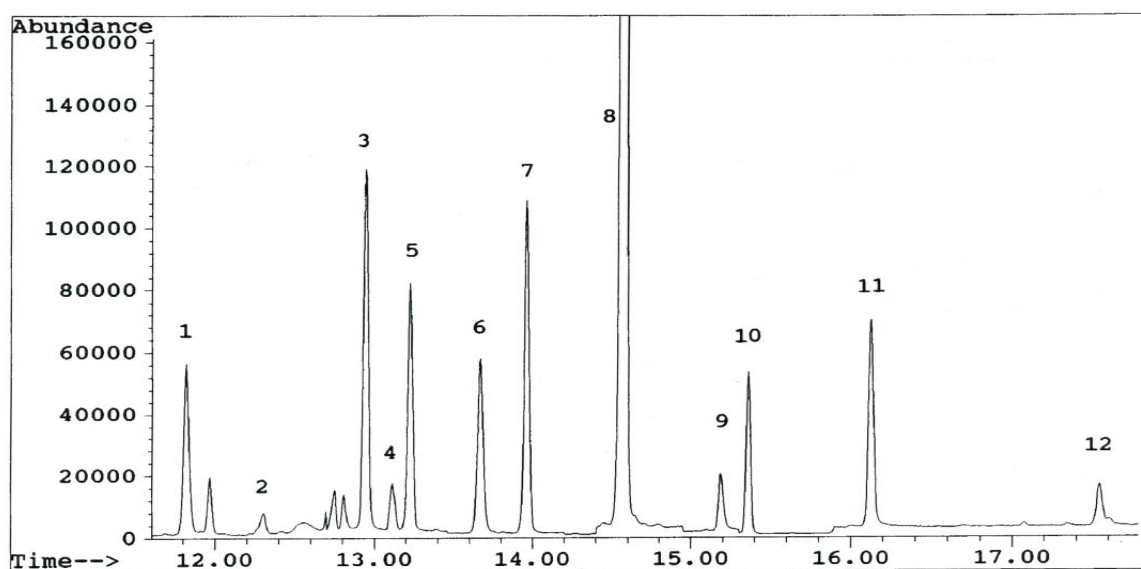
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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
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 μ m

Injector: 2 μ L Splitless, 250 $^{\circ}$ C

Oven temperature program: 100 $^{\circ}$ C (1 minute) to 250 $^{\circ}$ C (25 $^{\circ}$ C/minute): hold (2 minutes) to 290 $^{\circ}$ C (10 $^{\circ}$ C/minute): hold (0.5 minutes) to 325 $^{\circ}$ C (25 $^{\circ}$ C/minute): hold (3.1 minutes).

Carrier gas: Helium (1.2 mL/minute)

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 350 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C

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 μ m

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 μ L 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. H₂O.
1 x 1 mL 100 mM acetate buffer (pH 4.5).
1 x 1 mL CH₃OH.
Dry column (5 minutes at full vacuum or pressure).

4. ELUTE OPIATES:

2 x 0.5 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily. Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

5. DRY ELUATE:

Evaporate to dryness at < 40 °C.

6. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 μ L of mobile phase
Inject 10 μ L
- **GC-MS: Derivatize with propionic anhydride: pyridine**
Add 200 μ L 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 μ L of Ethyl Acetate / Methanol (70:30)

Alternate Derivatization

1. DERIVATIZE with TMS

Add 50 μ L Ethyl Acetate and 50 μ L BSTFA w/ 1% TMCS

Overlay with N₂ 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 μ L 10% Hydroxylamine solution.

Heat to 60 °C for 30 min in a heating block.

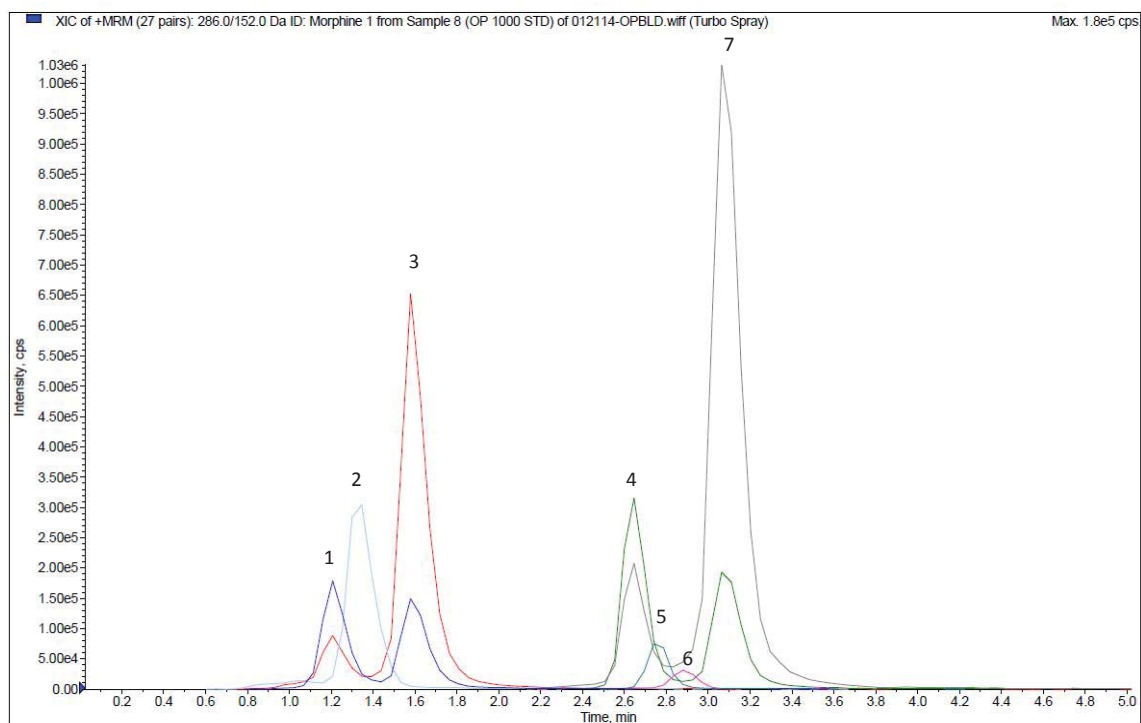
Allow sample to cool then adjust pH back to 5 with 1.0 M NaOH.

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. H₂O

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

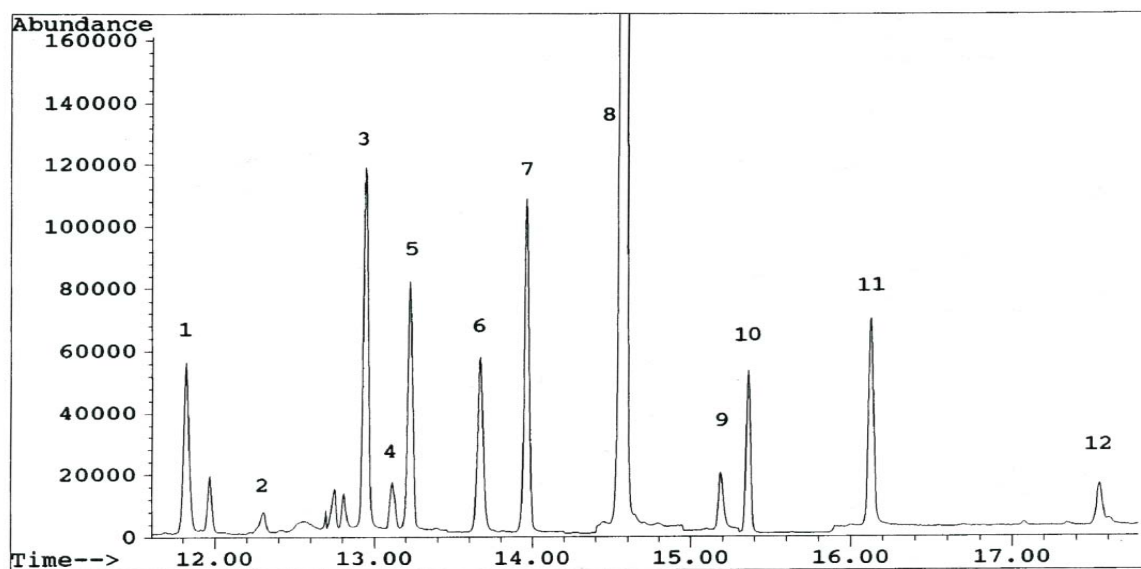
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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-D₄	251.0	222.0	250.0
Meperidine	247.0	218.0	246.0
Normeperidine-D₄ 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 °C (1 minute) to 250 °C (25 °C/minute): hold (2 minutes) to 290 °C (10°C/minute): hold (0.5 minutes) to 325°C (25°C/minute): hold (3.1 minutes).

Carrier gas: Helium (1.2mL/minute)

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

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





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. H₂O

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

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) CH₃OH in D.I. H₂O

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

5. ELUTE ANABOLIC STEROIDS (Choose a or b):

a. 1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2); Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily. Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

b. 1 x 3 mL CH₂Cl₂/IPA (80:20).

6. DRY ELUATE:

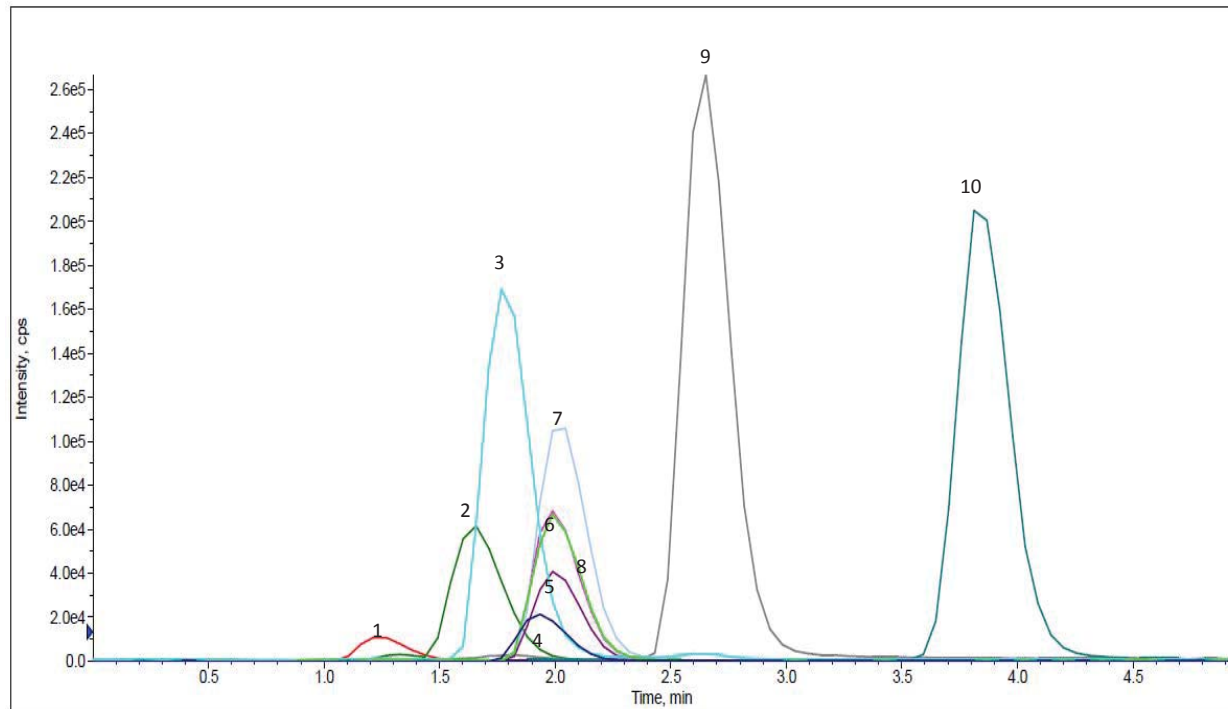
Evaporate to dryness at < 40°C.

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 N₂ 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. H₂O

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
Dehydroepiandrosterone-2TMS	432	327	297
10-Nortestosterone-2TMS	418	287	194
Oxymethalone Metabolite #1	640	52	462
Oxymethalone Metabolite #2	625	462	370
11- β -Hydroxyandosterone	522	417	158
Methandienone	409	313	281
19-Norandosterone-2TMS	405	315	225
Alpha-Hydroxyetiocholanone	504	417	-
17- α -Epi testosterone-TMS	432	341	327
Stanozolol	472	381	342



CLINICAL

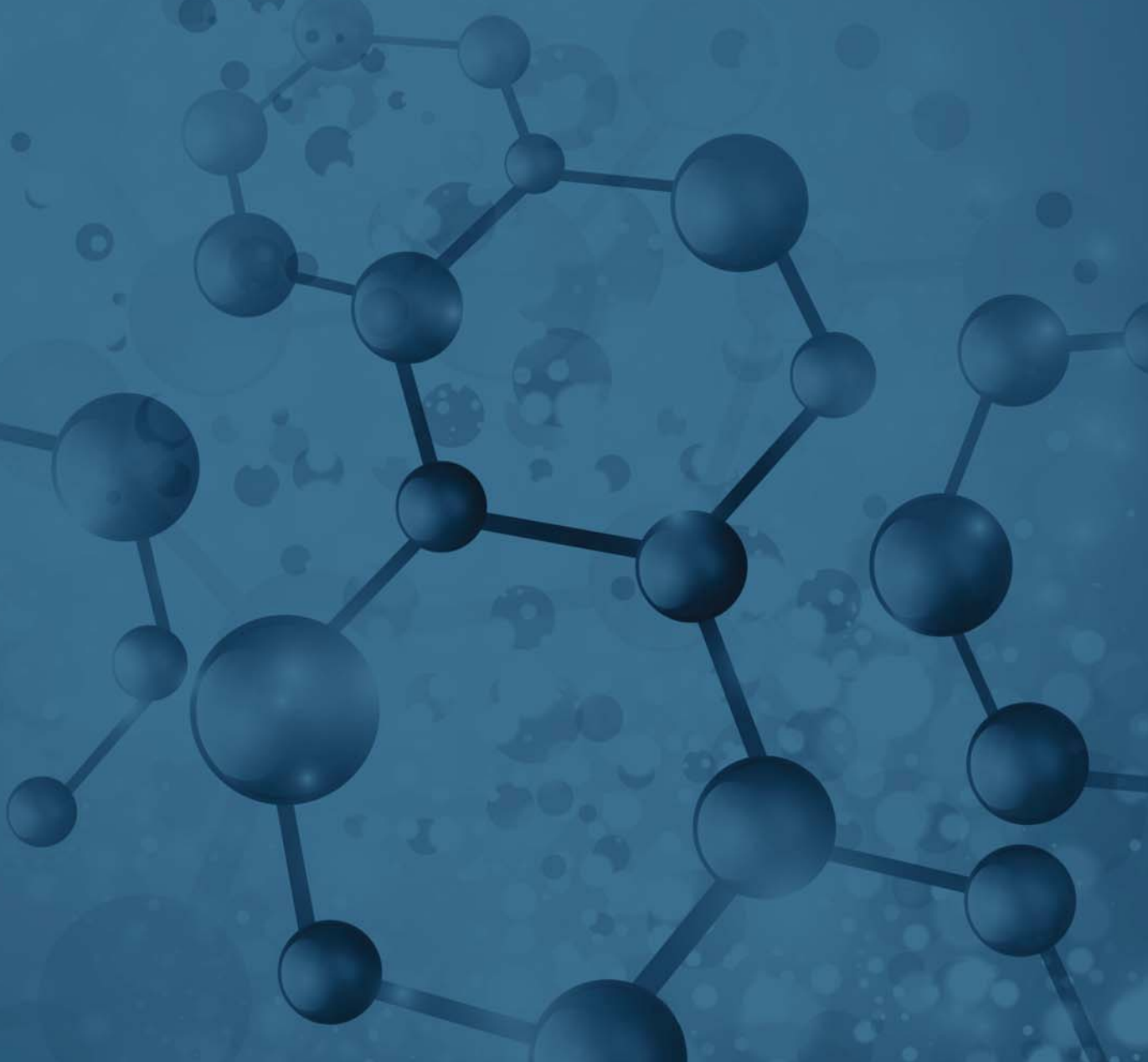


FORENSICS



UCT

Hair





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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O.

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% NH₄OH

collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

6. DRY ELUATE:

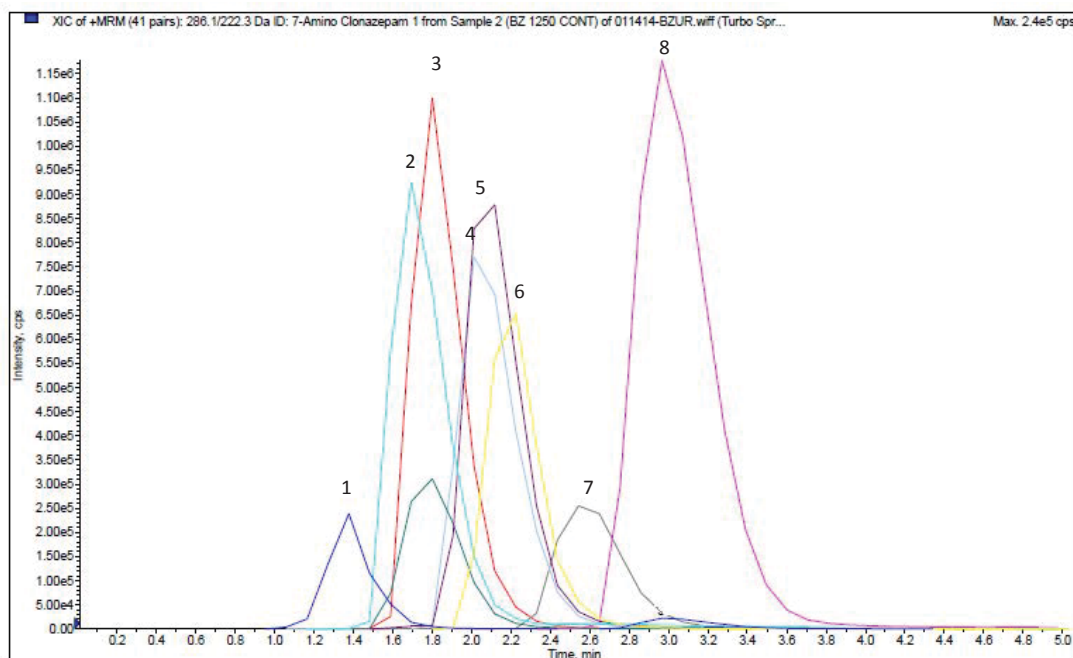
Evaporate to dryness at < 40 °C.

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 N₂ 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. H₂O

Flow Rate: 0.1 mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

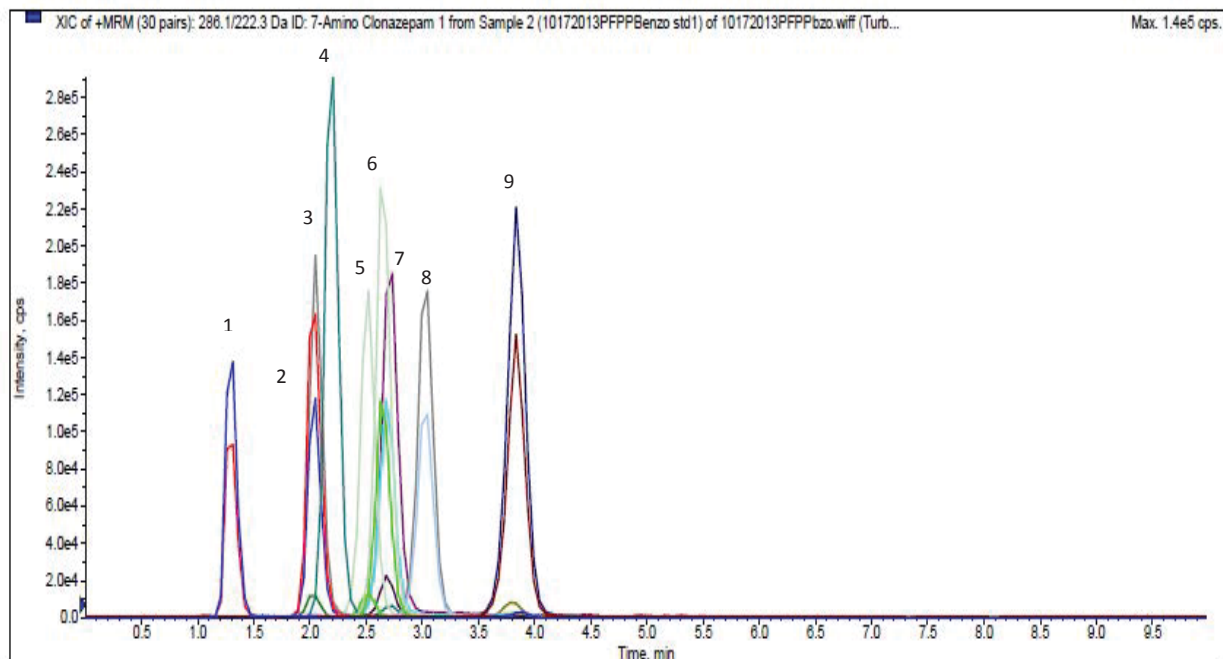
Polarity: Positive

Injection Volume: 10 µL

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. H₂O

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

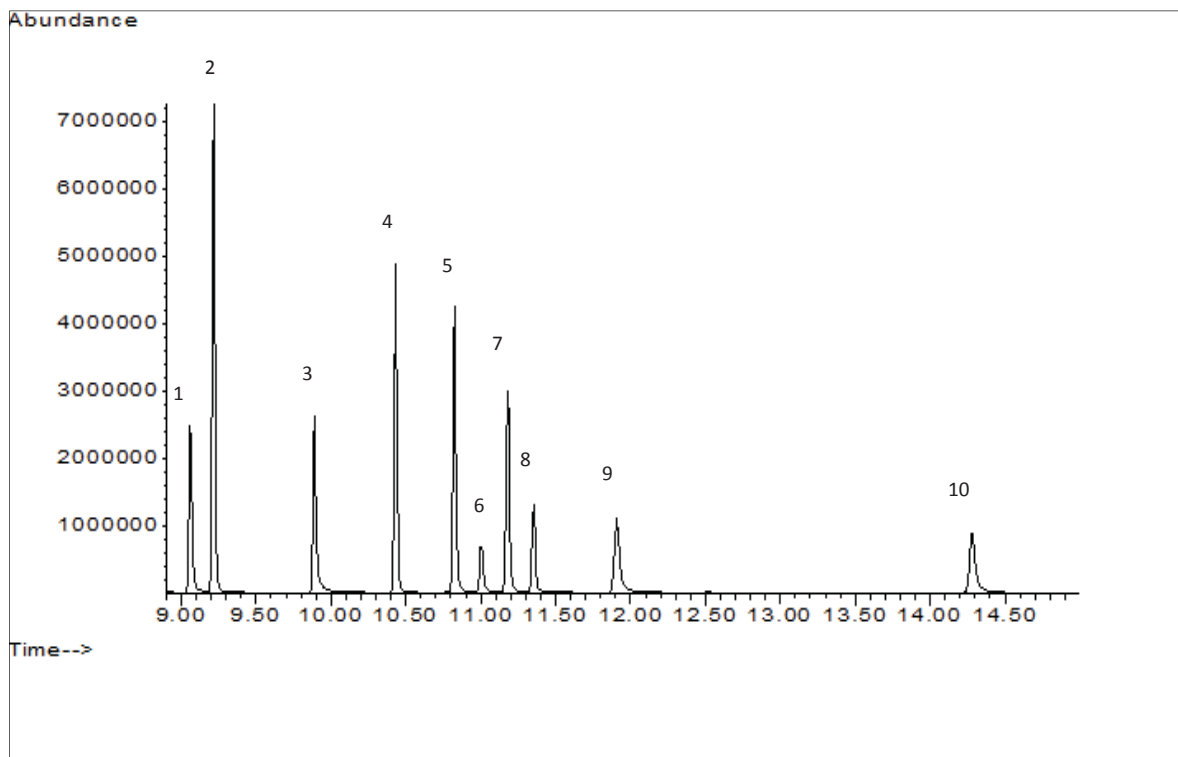
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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. Midazolam	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 μ m

Injector: 1 μ L Splitless 250 $^{\circ}$ C

Oven temperature program: 160 $^{\circ}$ C for 0.5 min; 15 $^{\circ}$ C/min to 310 $^{\circ}$ C for 4.50 minutes

Carrier gas: Helium

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 250 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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 μ m

1. PREPARE SAMPLE:

Into a clean glass tube add approx 100 mg of decontaminated hair
Add 1 mL of D.I. H₂O, add internal standard* and 100 μ L of 10 M NaOH
Digest at 70^oC for 12 hours
Cool and adjust to pH 3

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH.
1 x 3 mL D.I. H₂O.
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. H₂O
1 x 3 mL CH₃CN /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:

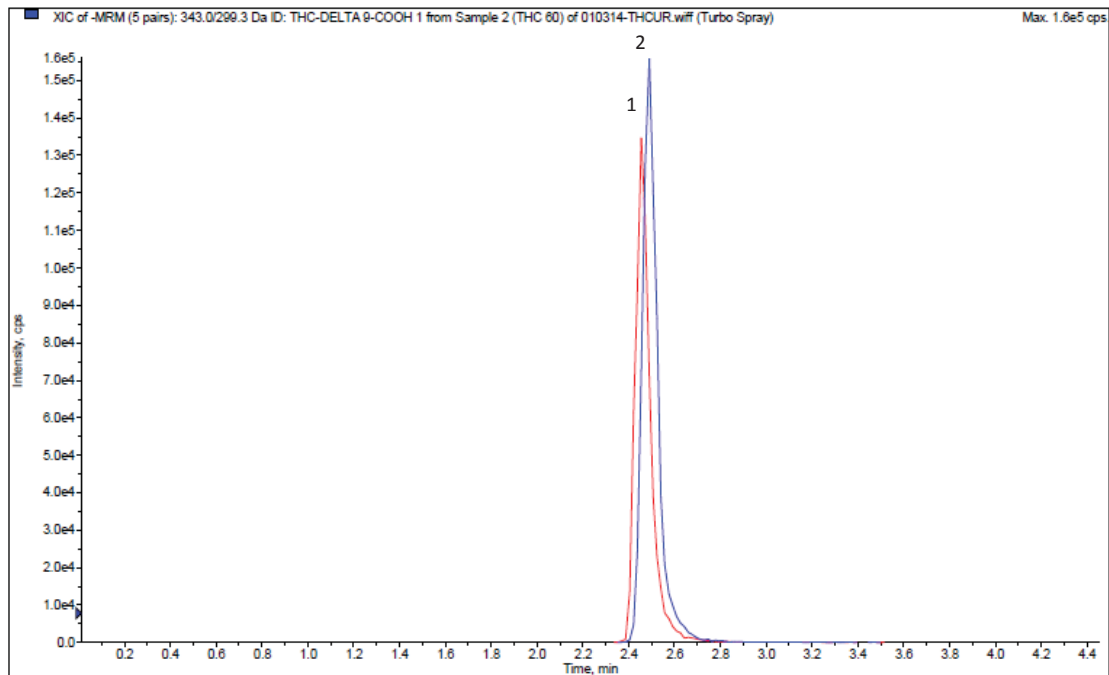
Evaporate to dryness at < 40 $^{\circ}$ C.

7. RECONSTITUTE:

Reconstitute sample in 100 μ L of mobile phase
Inject 20 μ L.

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. H₂O

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 EXTRACTION 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 CH₃OH 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. H₂O (pH 7)

Vortex Mix

2. CONDITION CLEAN-UP[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

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 CH₃OH.

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)

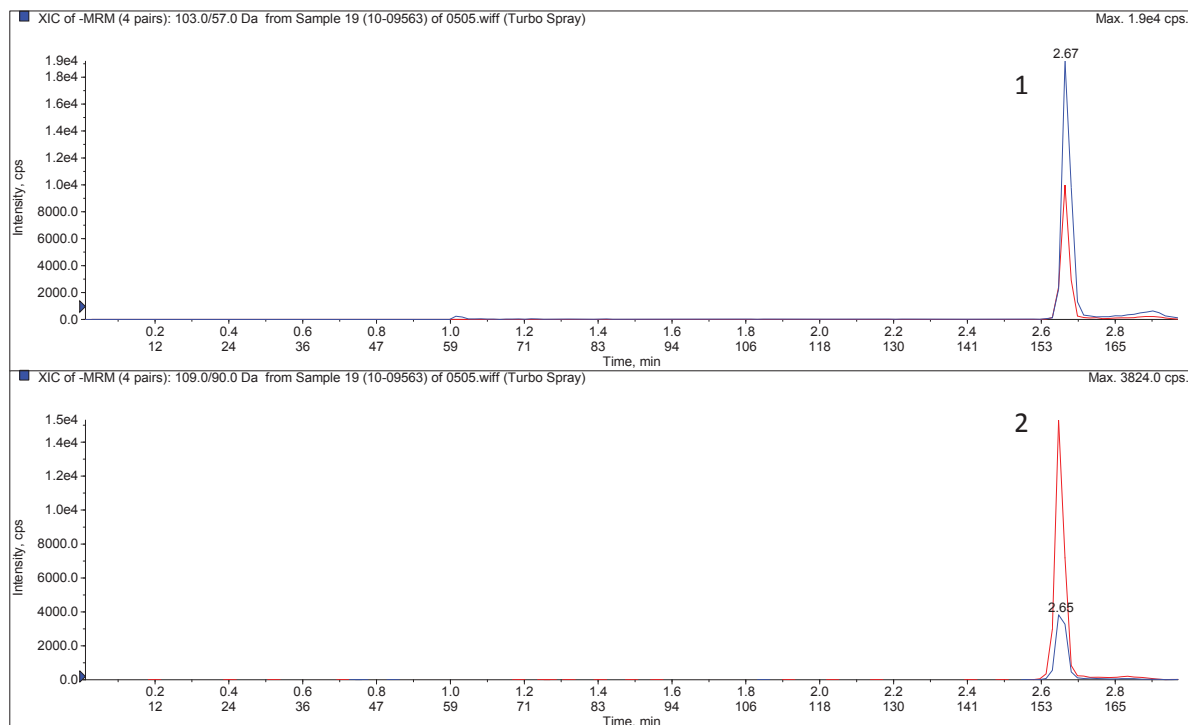
Overlay with N₂ and cap. Mix/vortex.

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. H₂O

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

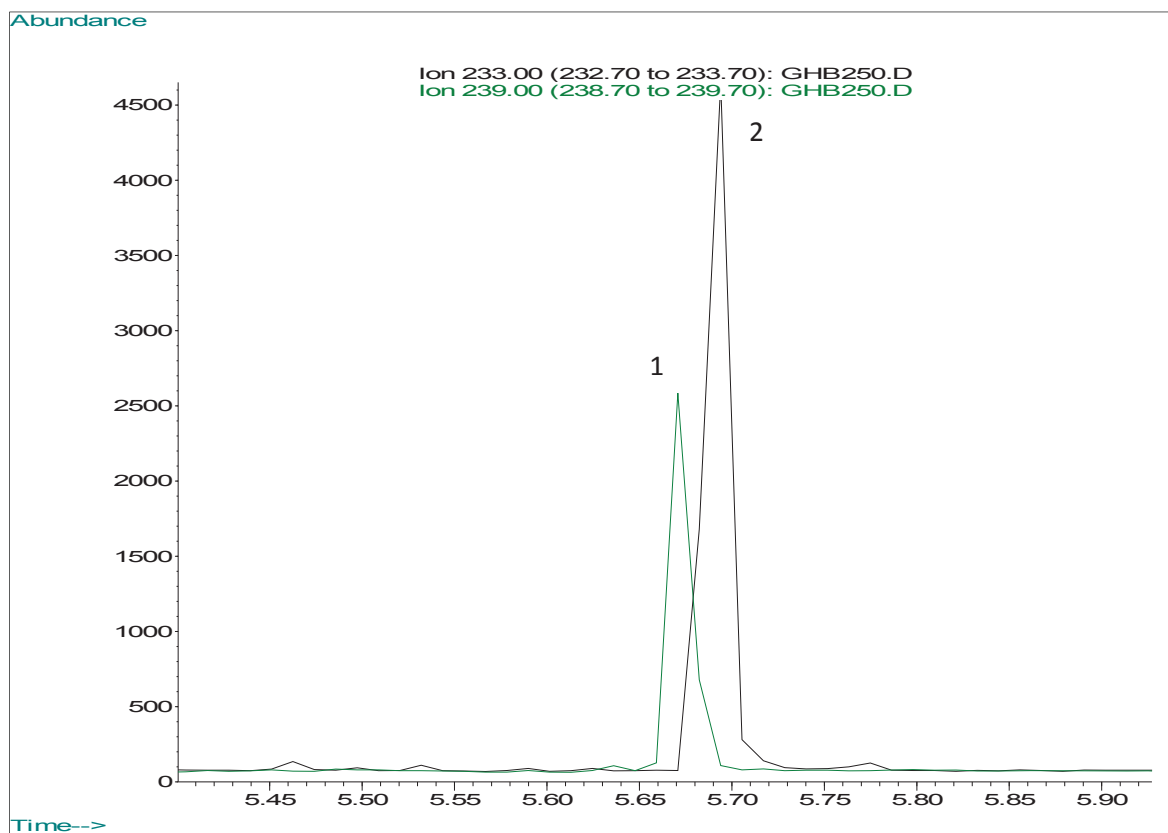
Instrument: API 3200 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 °C, MS Source: 250 °C, MS Quad: 150 °C



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. H₂O, add internal standards*

Vortex mix

Incubate at 40 °C for 12 hours

Centrifuge as appropriate

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN:

1 x 3 mL CH₃OH containing 1% Formic Acid

1 x 3 mL D.I. H₂O 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. H₂O:

Dry column (**10 minutes** at full vacuum or pressure)

5. ELUTE EtG/EtS ANALYTES:

2 x 3 mL CH₃OH containing 1% Formic Acid

Collect eluate at 1 to 2 mL/minute

6. DRY ELUATE:

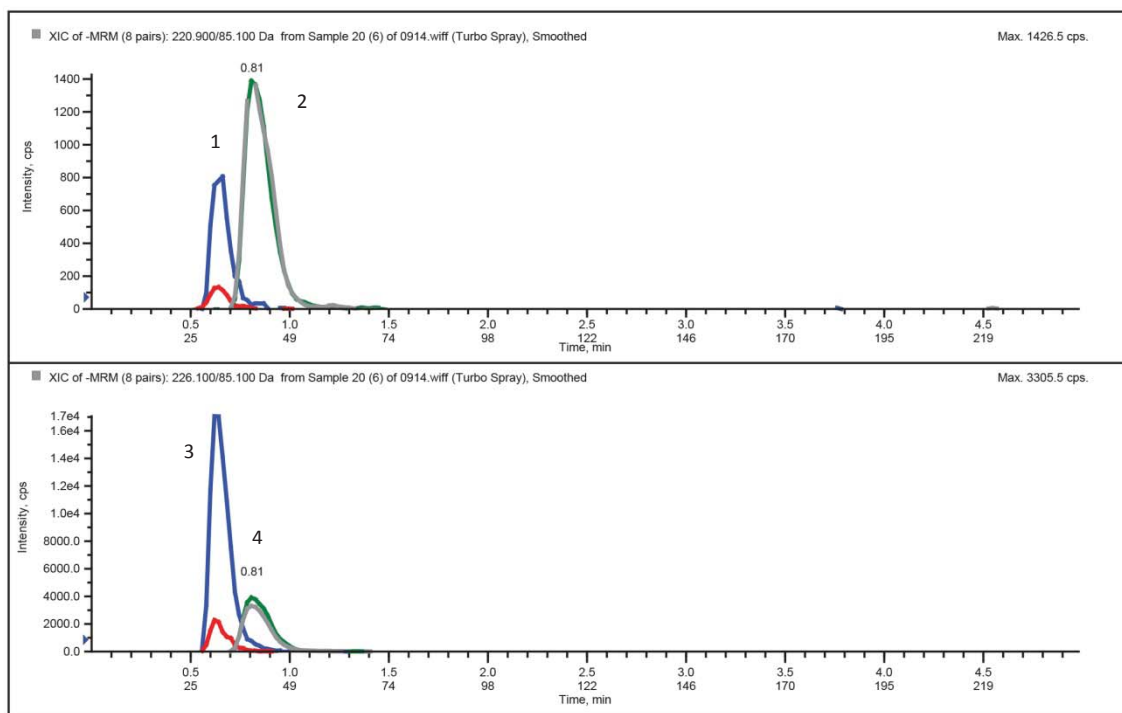
Evaporate to dryness at < 40 °C

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. H₂O

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



CLINICAL



FORENSICS



UCT

Oral Fluids



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 μ m

or

SLPFPP50ID21-5UM – Selectra[®] PFPP HPLC Column, 50 x 2.1 mm, 5 μ m

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 CH₃OH
1 x 3 mL D.I. H₂O
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. H₂O
1 x 3 mL 100 mM Acetic Acid
1 x 3 mL CH₃OH
Dry column (5 minutes at full vacuum or pressure)

5. ELUTE SMA's:

1 x 3 mL CH₂Cl₂/ IPA/ NH₄OH (78:20:2)
Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily
Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

Add 100 μ L 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 μ L of mobile phase
Inject 20 μ L.

- **GC-MS:** Fluoroaclyate with PFPA (PFAA)
Add 50 µL PFPA. Over lay with N₂ 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

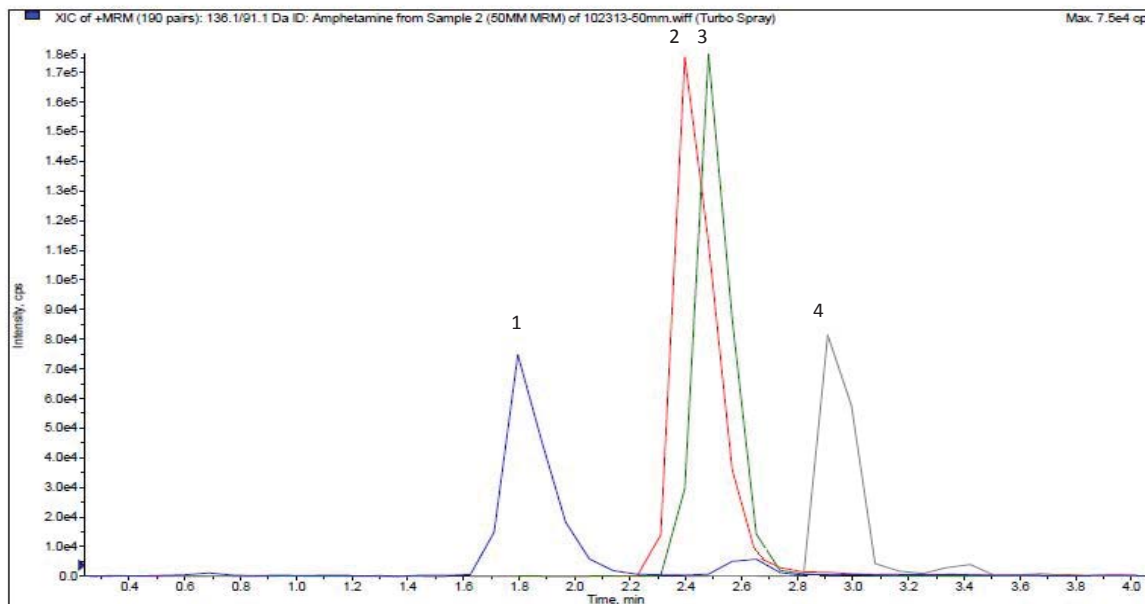
Alternate Derivatization

1. Fluoroaclyate with HFPA (HFAA)
Add 50 µL HFPA. Over lay with N₂ 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. H₂O

Flow Rate: 0.5 mL/minute

Reconstitute: 100 µL

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

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

Mobile Phase B: 0.1% Formic Acid in Methanol

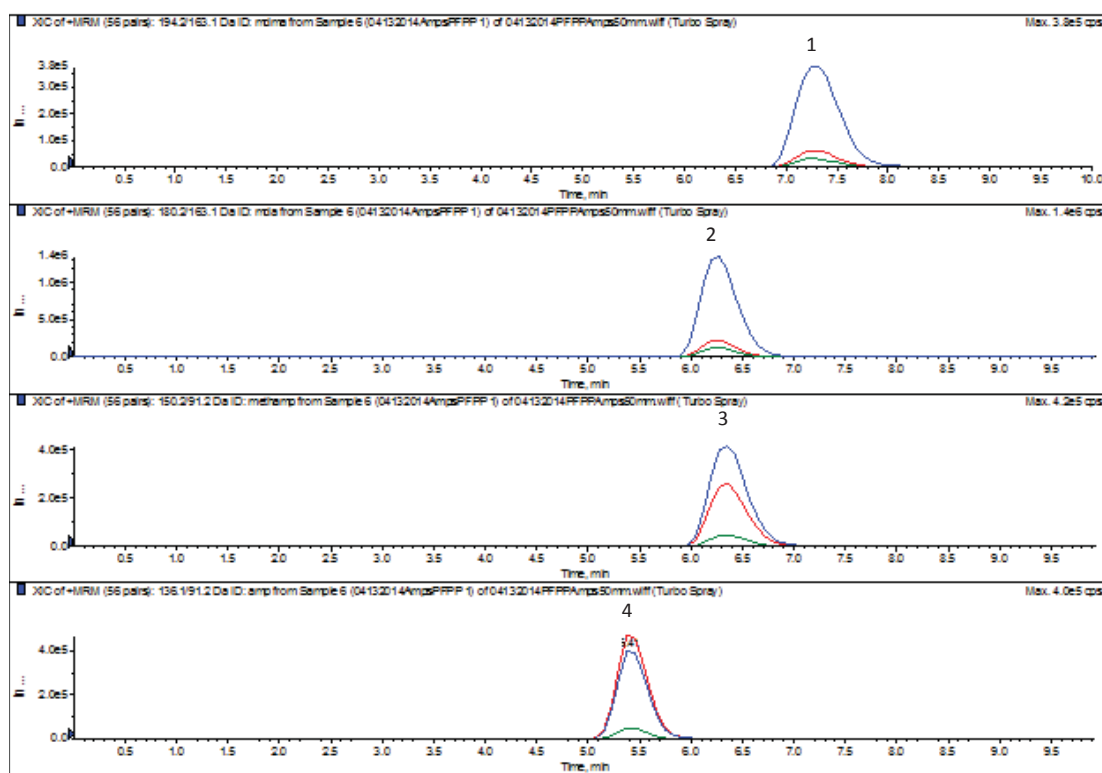
Polarity: Positive

Injection Volume: 20 µL

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. H₂O

Flow Rate: 0.3 mL/minute

Reconstitute: 100 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

Mobile Phase B: 0.1% Formic Acid in Methanol

Polarity: Positive

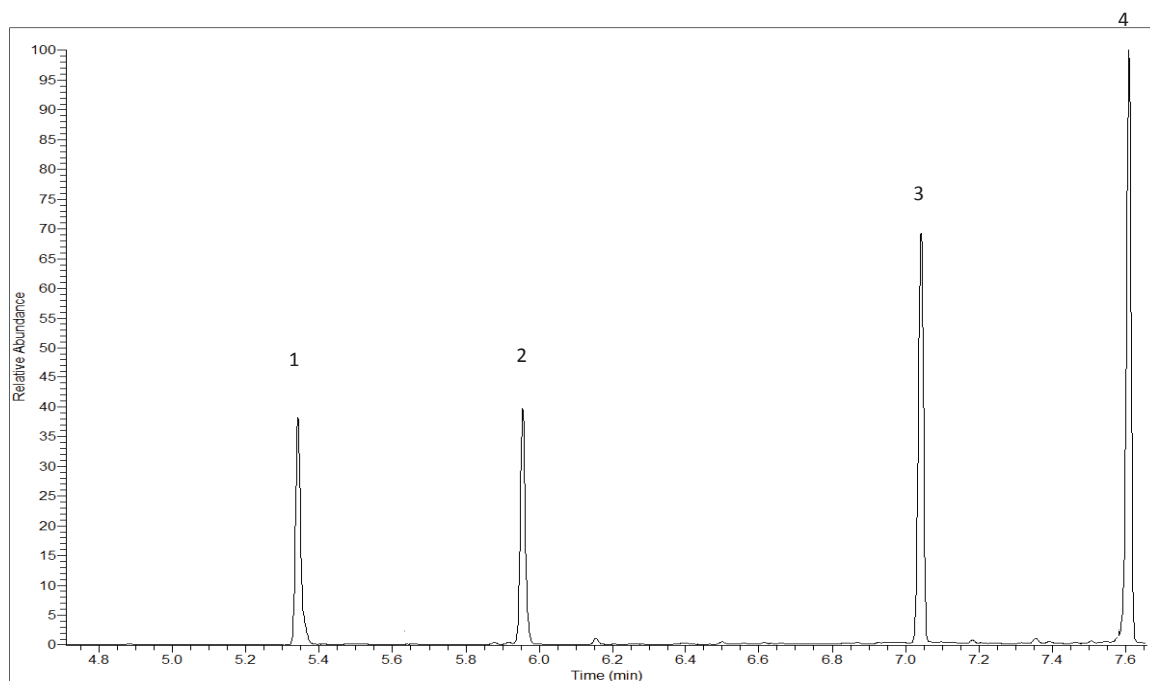
Injection Volume: 10 µL

Isocratic:

Time	%A	%B
0.00	30	70
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

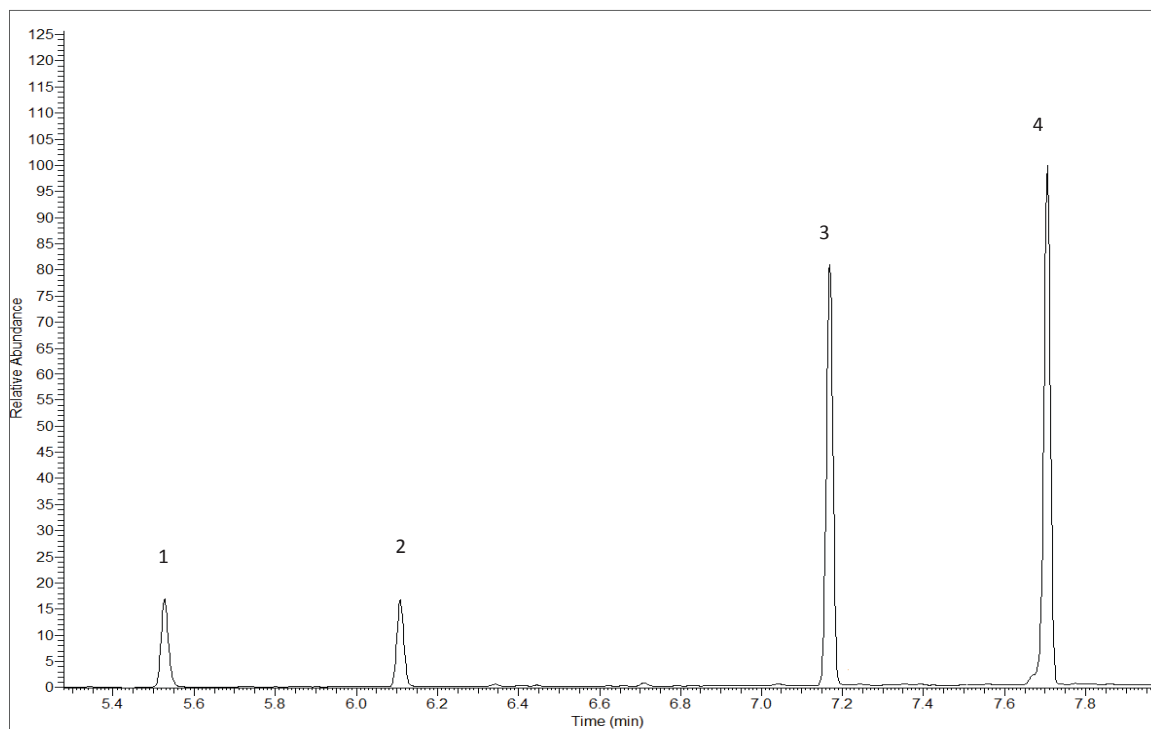
CHROMATOGRAM



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 μ m) TG-1MS

Injector: 1 μ L Splitless, 250 $^{\circ}$ C

Oven temperature program: 70 $^{\circ}$ C (0.5) to 320 $^{\circ}$ C (25 $^{\circ}$ C/ minute): hold (2 minutes)

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

MSD condition: Aux temperature: 280 $^{\circ}$ C, MS Source: 350 $^{\circ}$ C, MS Quad: 150 $^{\circ}$ C



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 CH₃OH.

1 x 200 µL D.I. H₂O.

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. H₂O.

1 x 500 µL 100 mM HCl

1 x 500 µL CH₃OH/D.I.H₂O (50:50)

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

5. ELUTE COCAINE/METABOLITES:

1 x 800 µL CH₂Cl₂/ IPA/ NH₄OH (70:26:4)

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

6. DRY ELUATE:

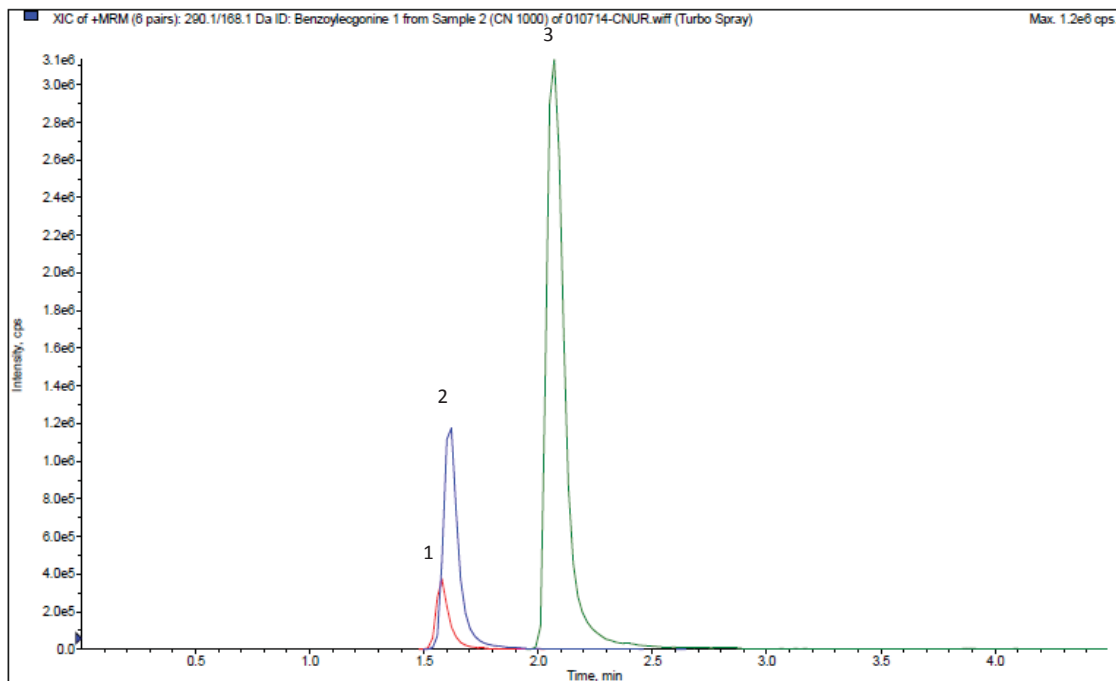
Evaporate to dryness at < 40 °C.

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 N₂ 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. Benzoylcegonine D ₈	298.1	171.1	1.58
2. Benzoylcegonine	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. H₂O

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

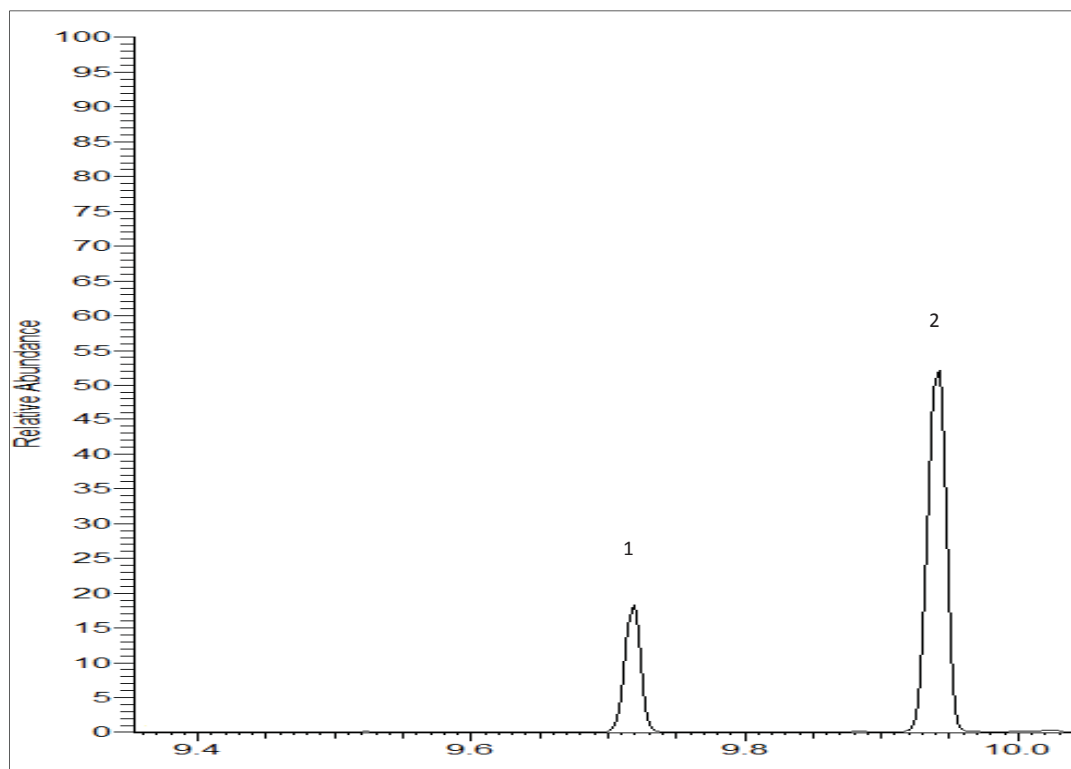
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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.Benzoyecgonine TMS	240	256	361	9.94
Benzoyecgonine 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 °C, MS Source: 350 °C, MS Quad: 150 °C



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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

1 x 3 mL 1% acetic acid

1 x 3 mL CH₃OH

Dry column (5 minutes at full vacuum or pressure)

5. ELUTE FENTANYL/NORFENTANYL:

1 x 3 mL Ethyl Acetate/ Acetonitrile / NH₄OH (78:20:2v/v)

Collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily

Add IPA/ NH₄OH, mix, then add CH₂Cl₂ (pH 11-12)

6. DRY ELUATE:

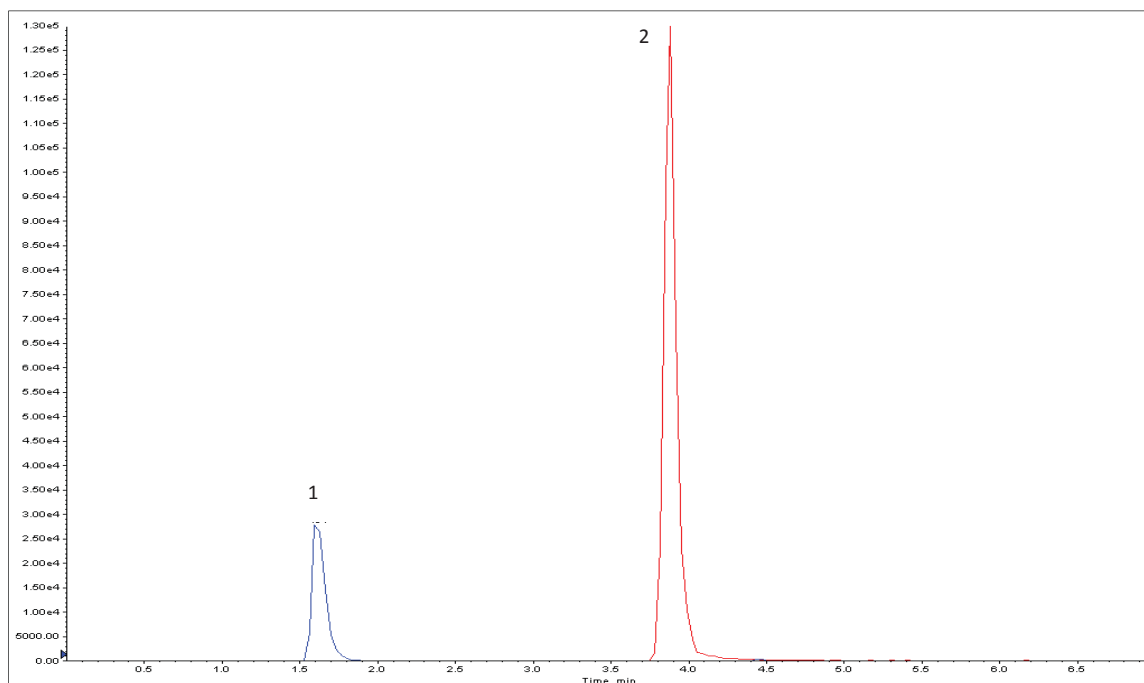
Evaporate to dryness at < 40 °C

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. H₂O

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

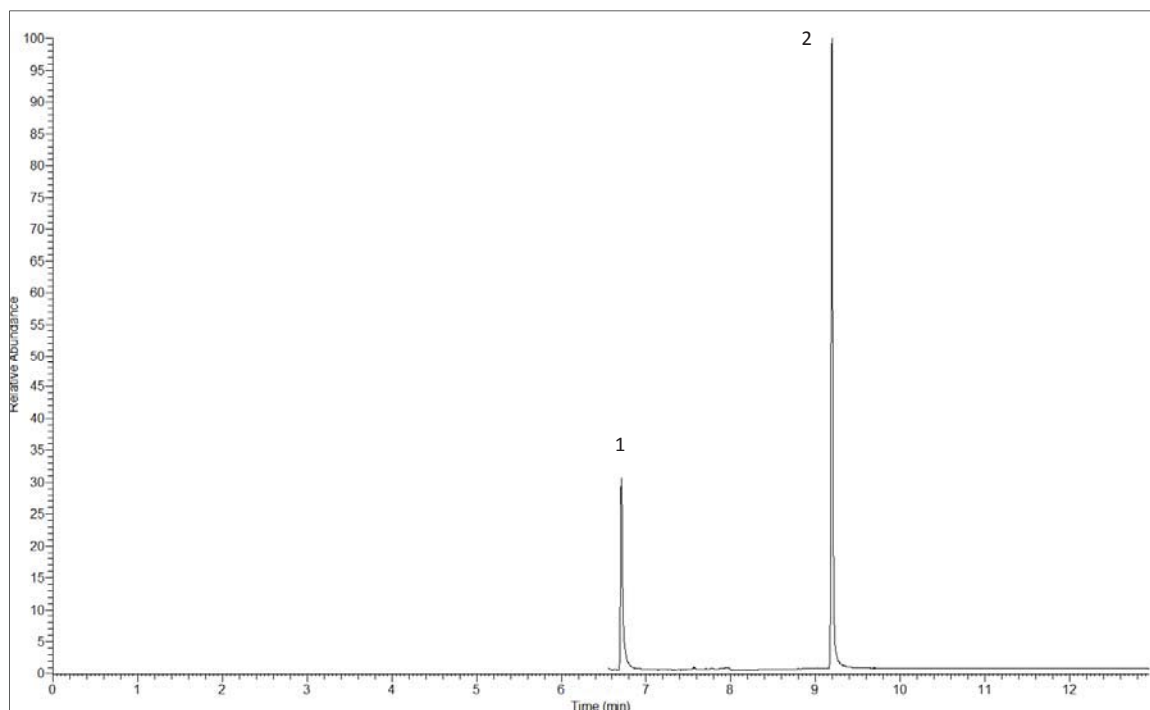
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

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 °C, MS Source: 350 °C, MS Quad: 150 °C



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 750 µL of 85:15:1 D.I. H₂O: ACN: NH₄OH

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:

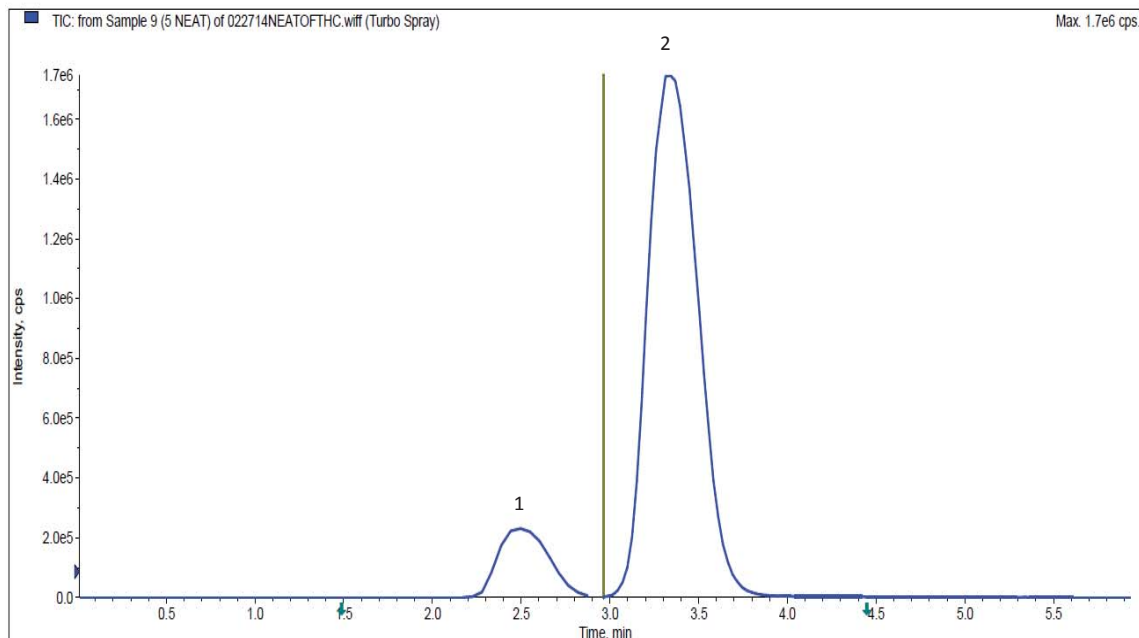
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 20 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and
50 µL BSTFA w/ 1% TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70 °C; Cool and inject 1 µL

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. H₂O

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

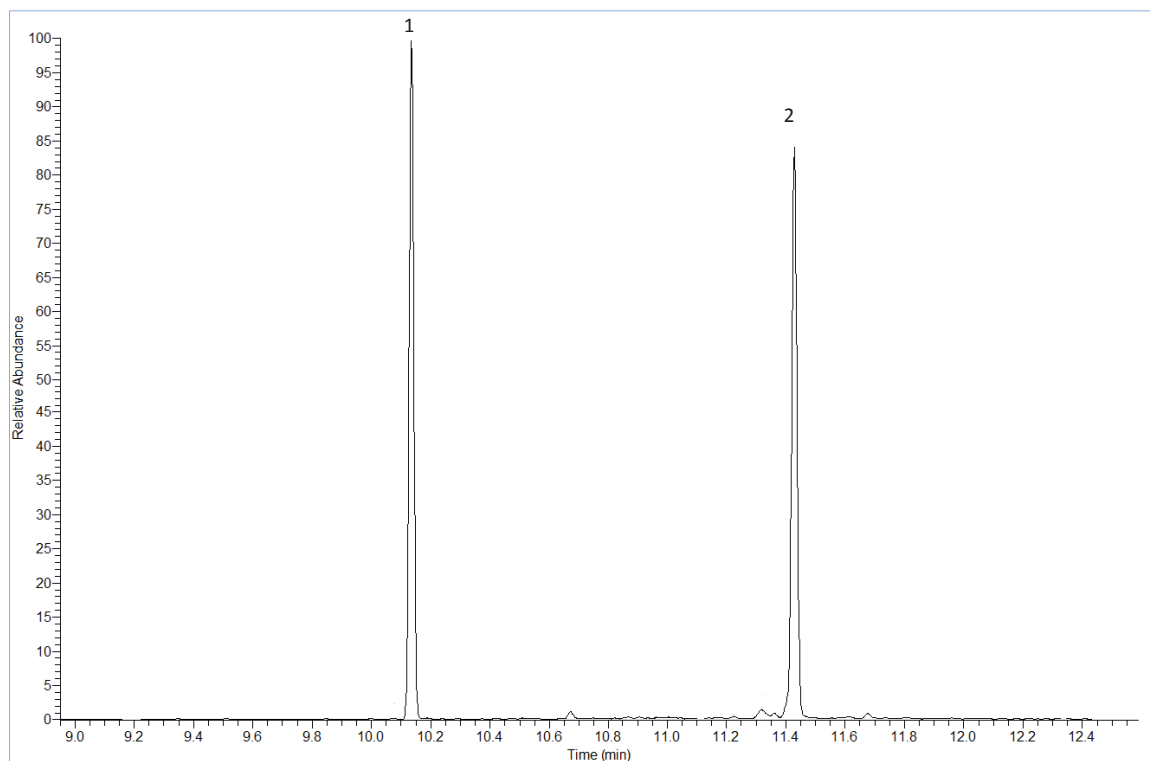
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 °C, MS Source: 350 °C, MS Quad: 150 °C



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 H₂O: Acetonitrile: NH₄OH

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:

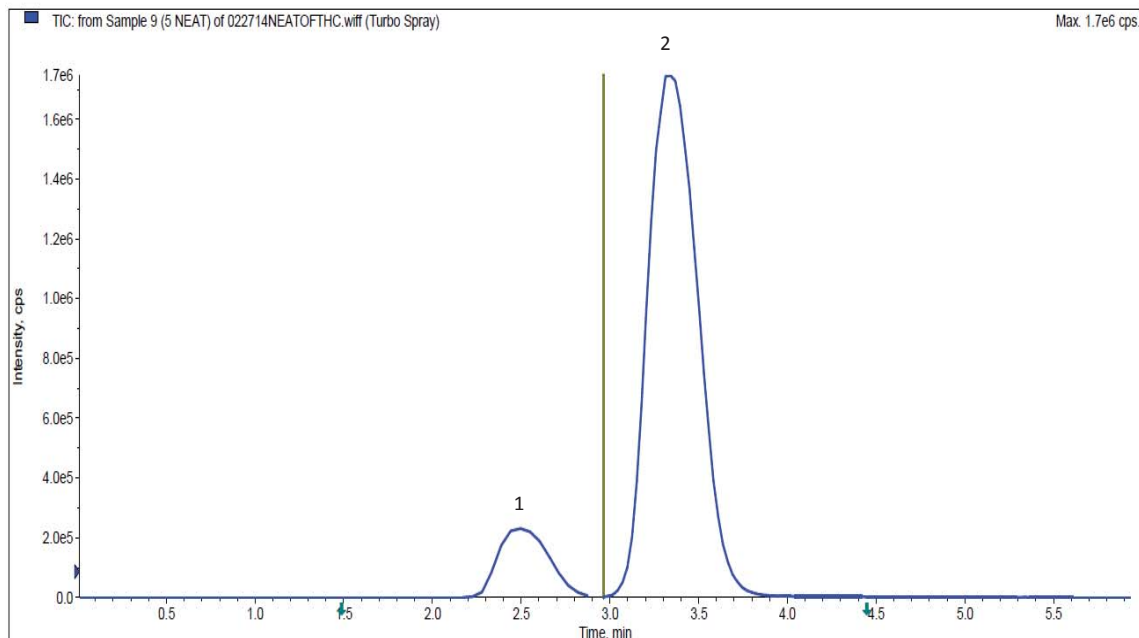
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
Inject 20 µL.
- **GC-MS:** Dissolve residue in 50 µL of Ethyl Acetate and 50 µL BSTFA w/ 1% TMCS
Overlay with N₂ and cap. Mix/vortex
React 30 minutes at 70°C; Cool and inject 1 µL

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. H₂O

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

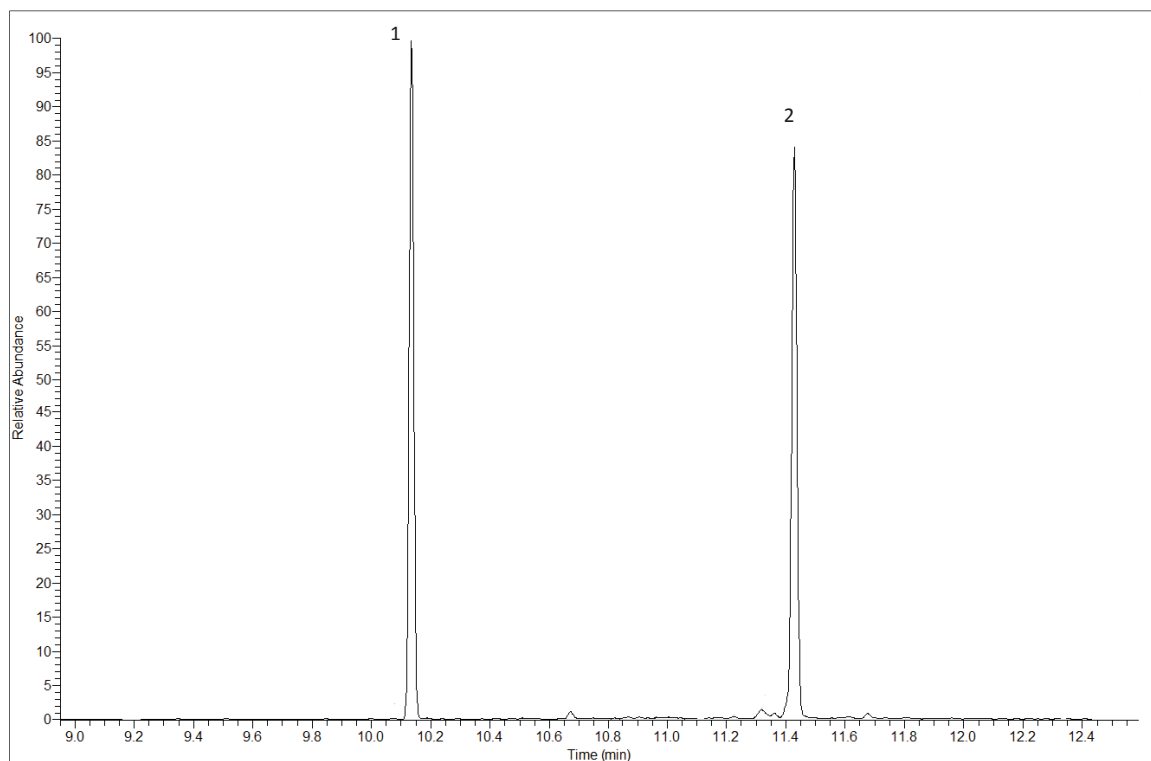
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

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 °C, MS Source: 350 °C, MS Quad: 150 °C

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 CH₃OH.

1 x 3 mL D.I. H₂O.

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. H₂O

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 N₂ and cap. Mix/vortex
React 30 minutes at 70°C; Cool and inject 1 µL

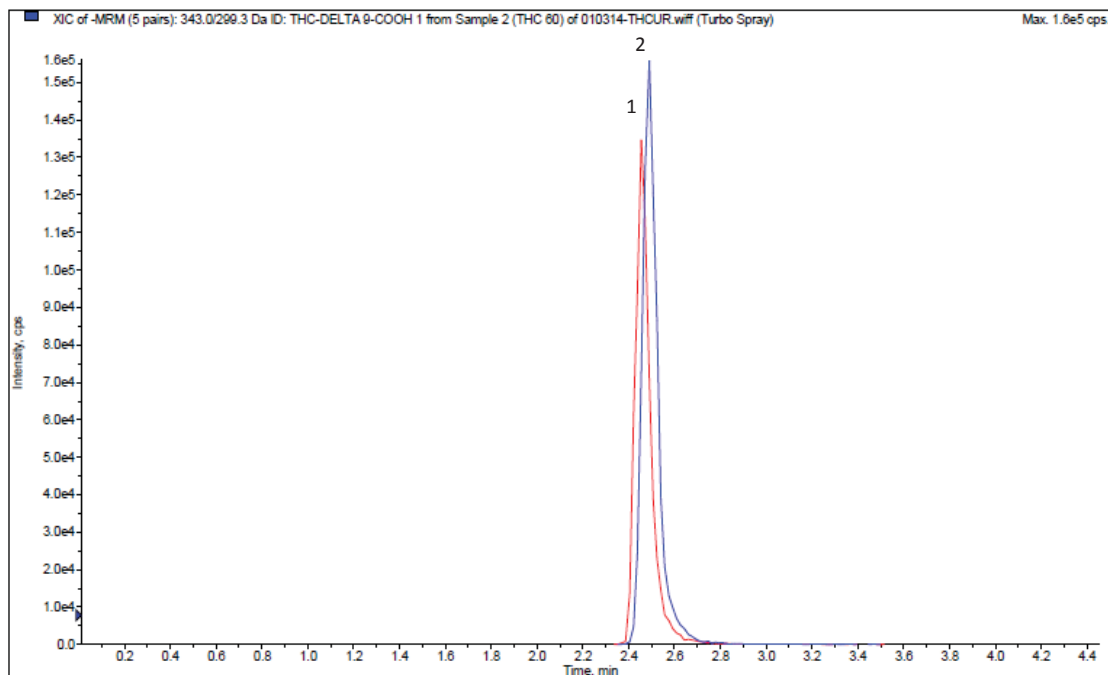
Alternate Derivatization

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. H₂O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Negative

Reconstitute: 100 µL

Injection Volume: 20 µL

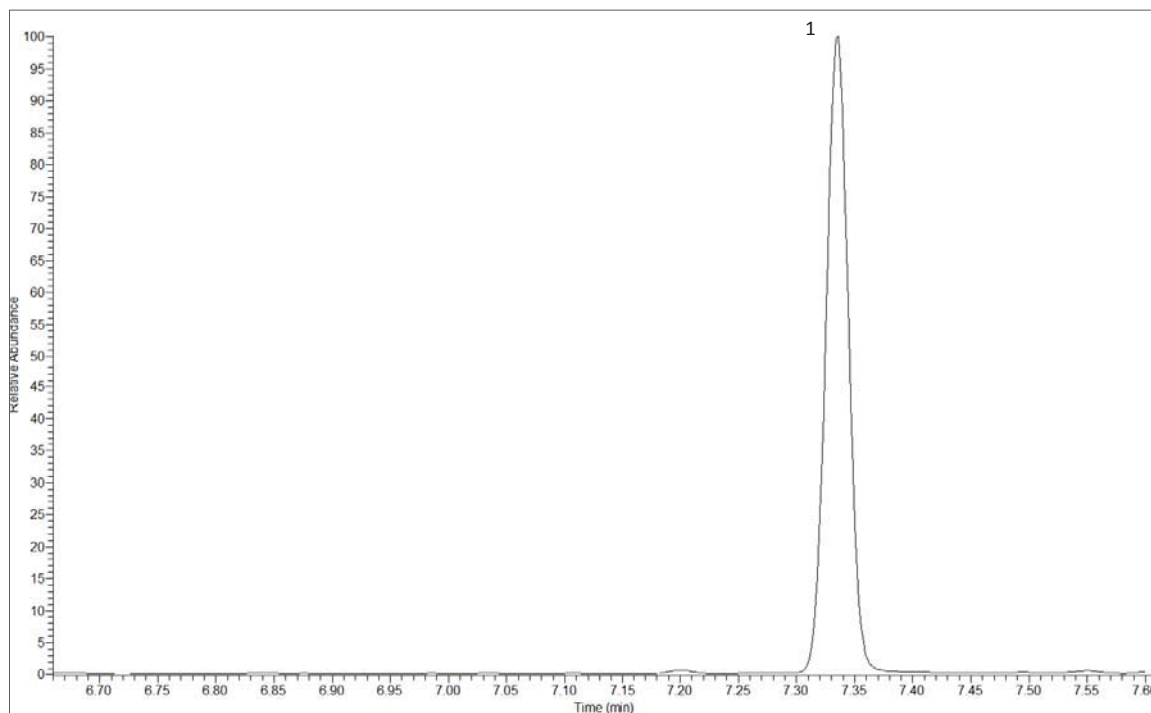
LC Column: Selectra[®] DA HPLC Column 50 x 2.1 mm 5 µm

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):



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 °C, MS Source: 350 °C, MS Quad: 150 °C



CLINICAL

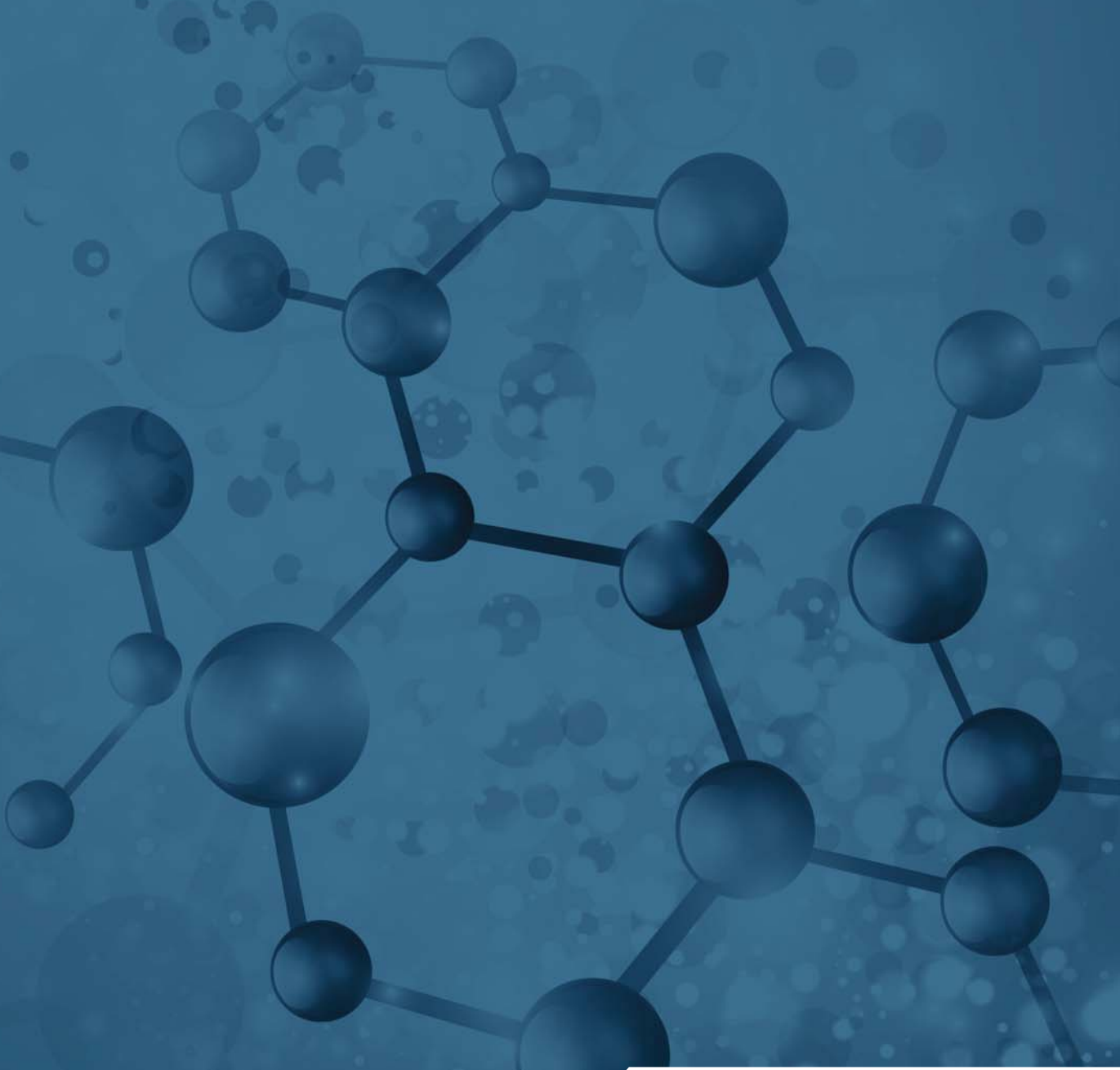


FORENSICS



UCT

FASt





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. H₂O)

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.

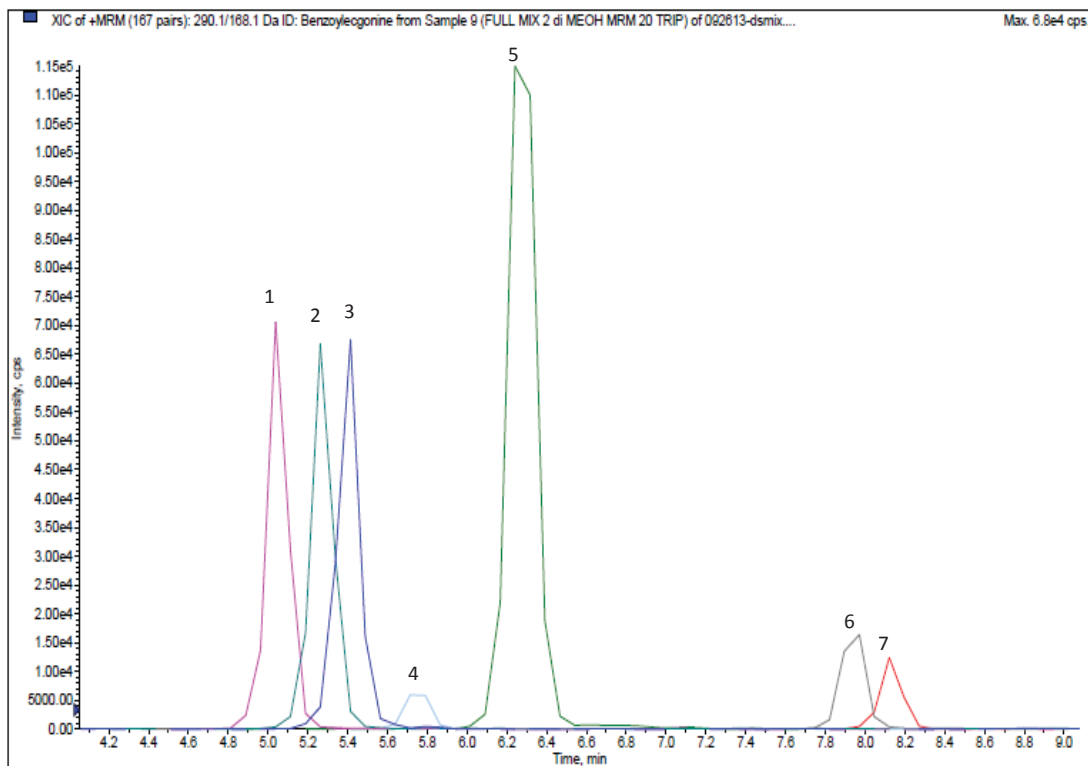
4. ANALYSIS:

Cap vials and put directly onto LC/MS for analysis.

INSTRUMENT CONDITIONS (LC-MS/MS):

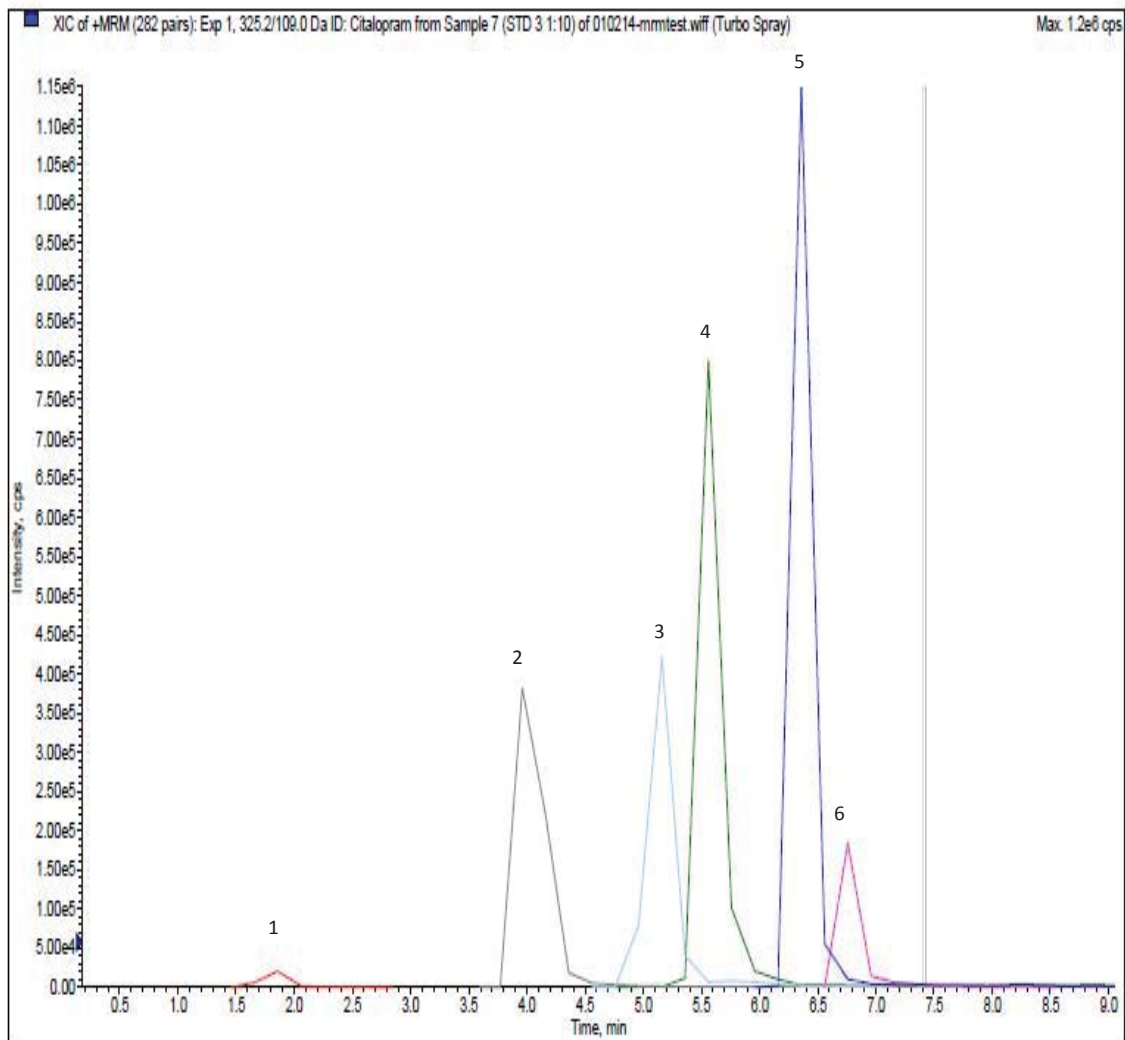
CHROMATOGRAMS

Basic Panel 1



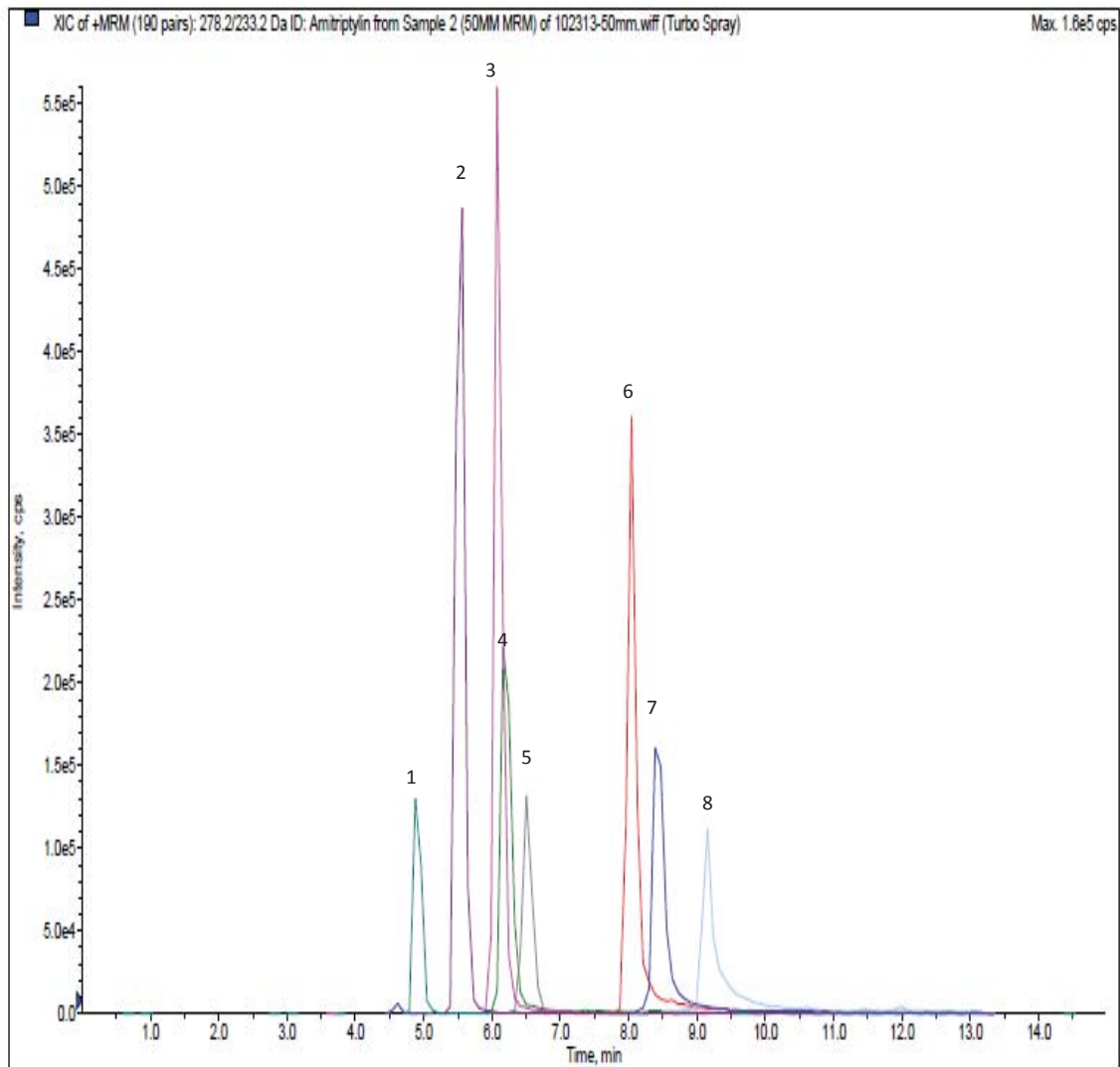
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylcegonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



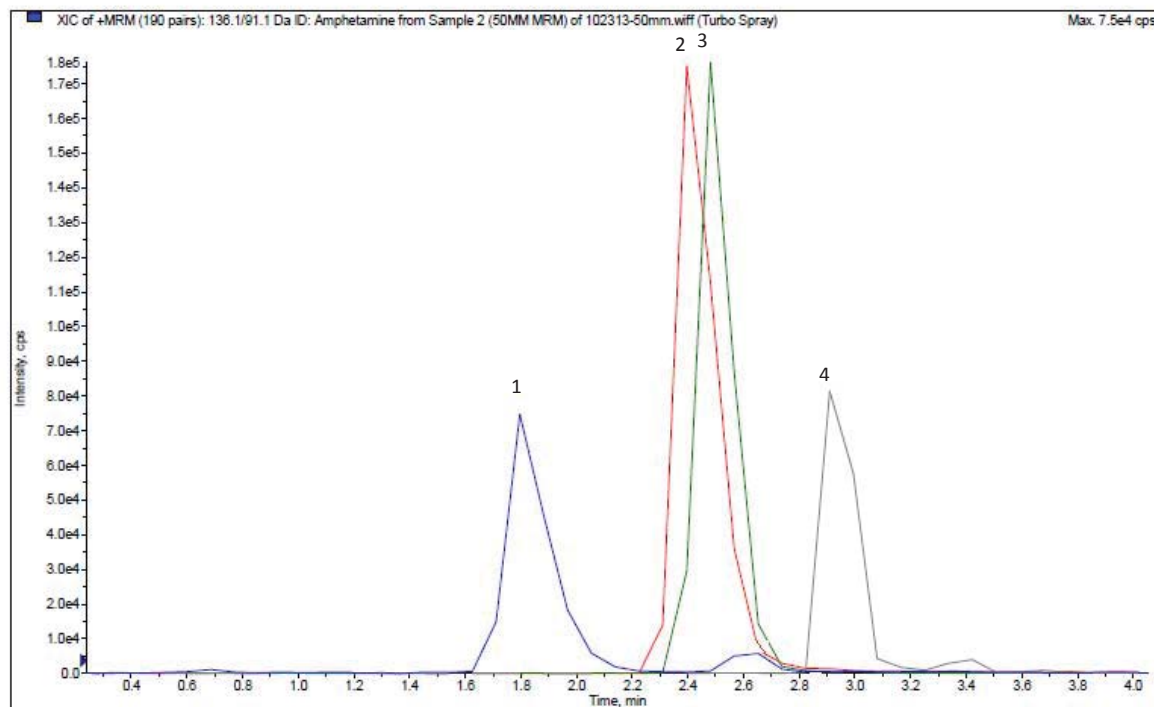
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxine	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. Quetiapine	384.2	253.1	6.50
6. Imipramine	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. H₂O

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 μ m

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 μ L 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 μ L	500 μ L
1:4	200 μ L	800 μ L
1:9	100 μ L	900 μ L

** Diluent is 50:50 (Acetonitrile: D.I. H₂O)

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 μ L.

3. EXTRACTION and COLLECTION:

Set up extraction manifold with FAST cartridges and auto-sampler collection vials.

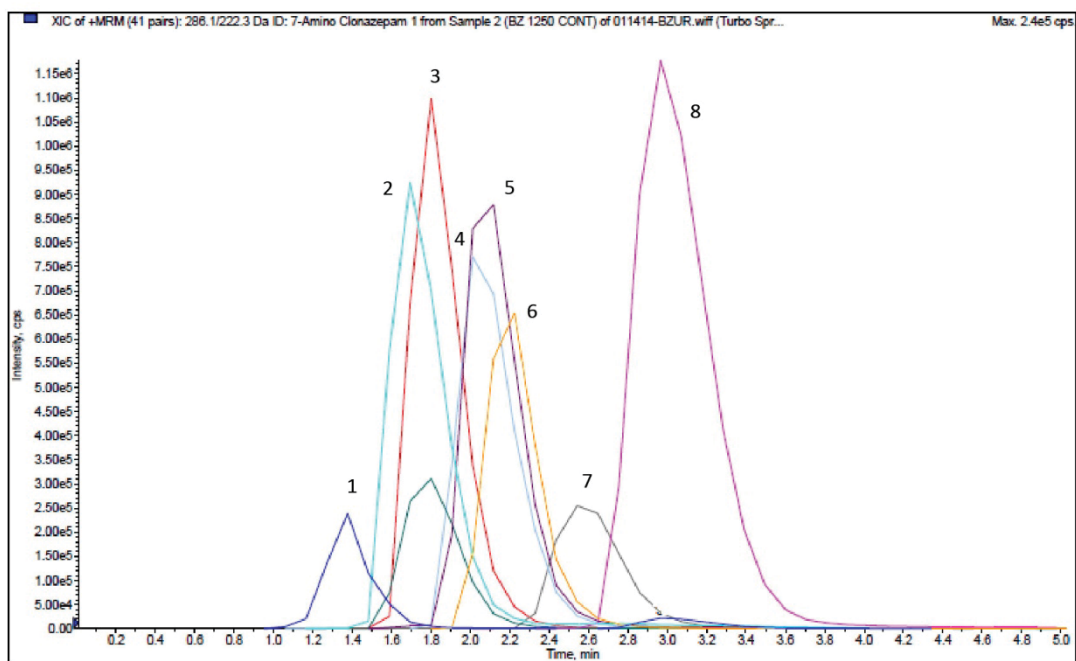
Pour sample into FAST cartridge and elute sample directly into auto-sampler vials.

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. H₂O

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. H₂O)

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.

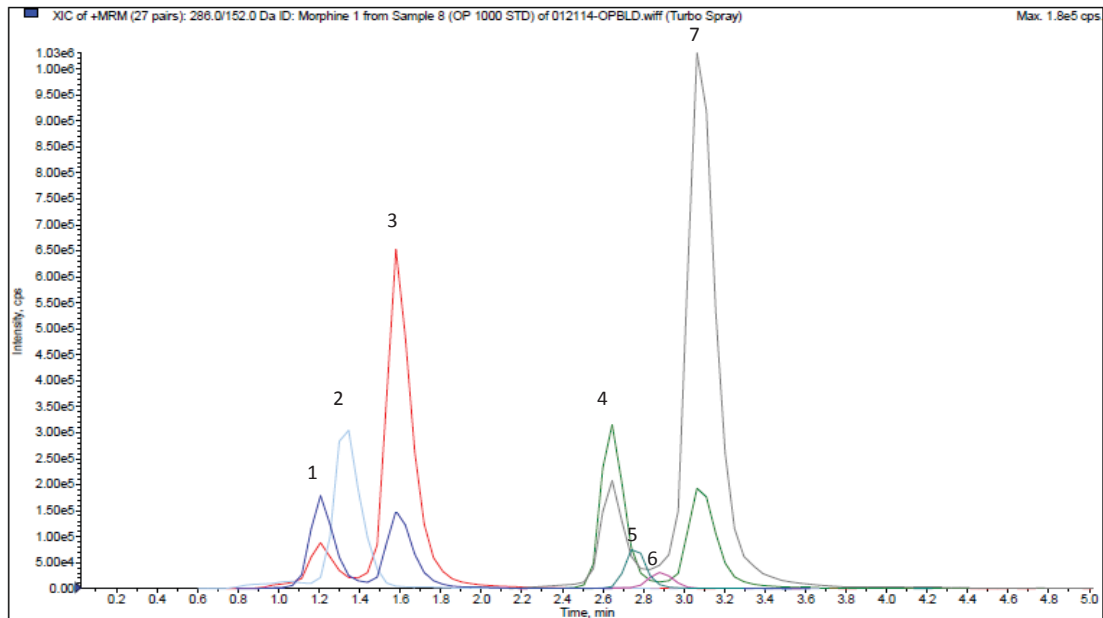
Pour sample into FAST cartridge and elute sample directly into auto-sampler vials.

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. H₂O

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

SLDA501D21-5UM – 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 H₂O: 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. H₂O)

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.

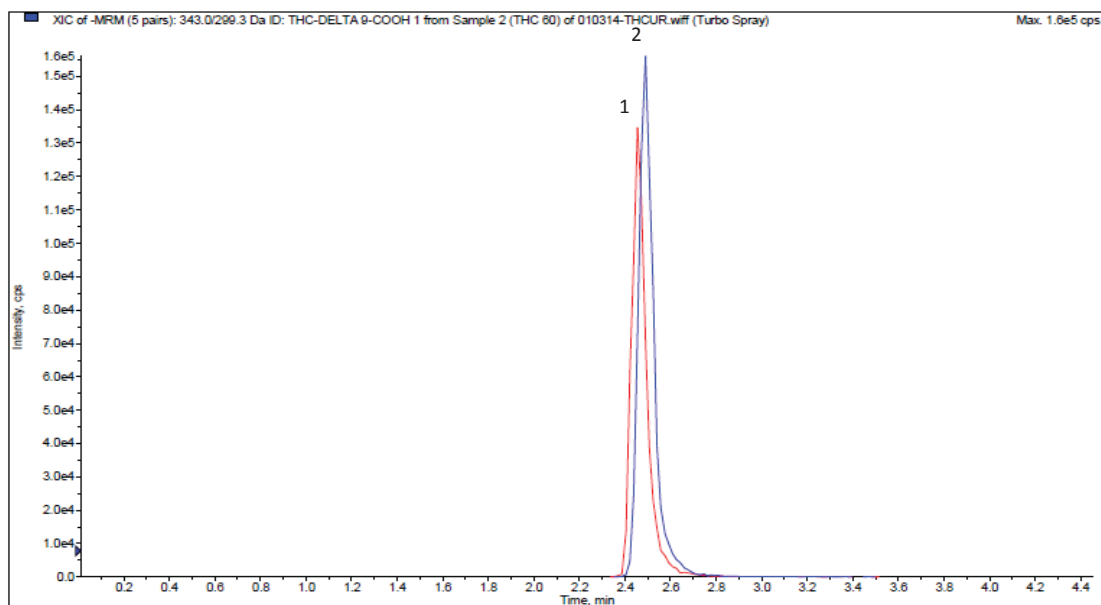
Pour sample into FAST cartridge and elute sample directly into auto-sampler vials.

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. H₂O

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	



CLINICAL



FORENSICS



UCT

Veterinary and Racing



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

SPPH06001-10 - Select pH Buffer Pouches 100mM Phosphate pH 6.0

1. PREPARE SAMPLE:

To 1 mL of 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 x 3 mL CH₃OH

1 x 3 mL D.I. H₂O

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. 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 CH₂Cl₂/ IPA/ NH₄OH (78: 20: 2 v/v)

Collect the eluate at 1-2 mL minute (or gravity)

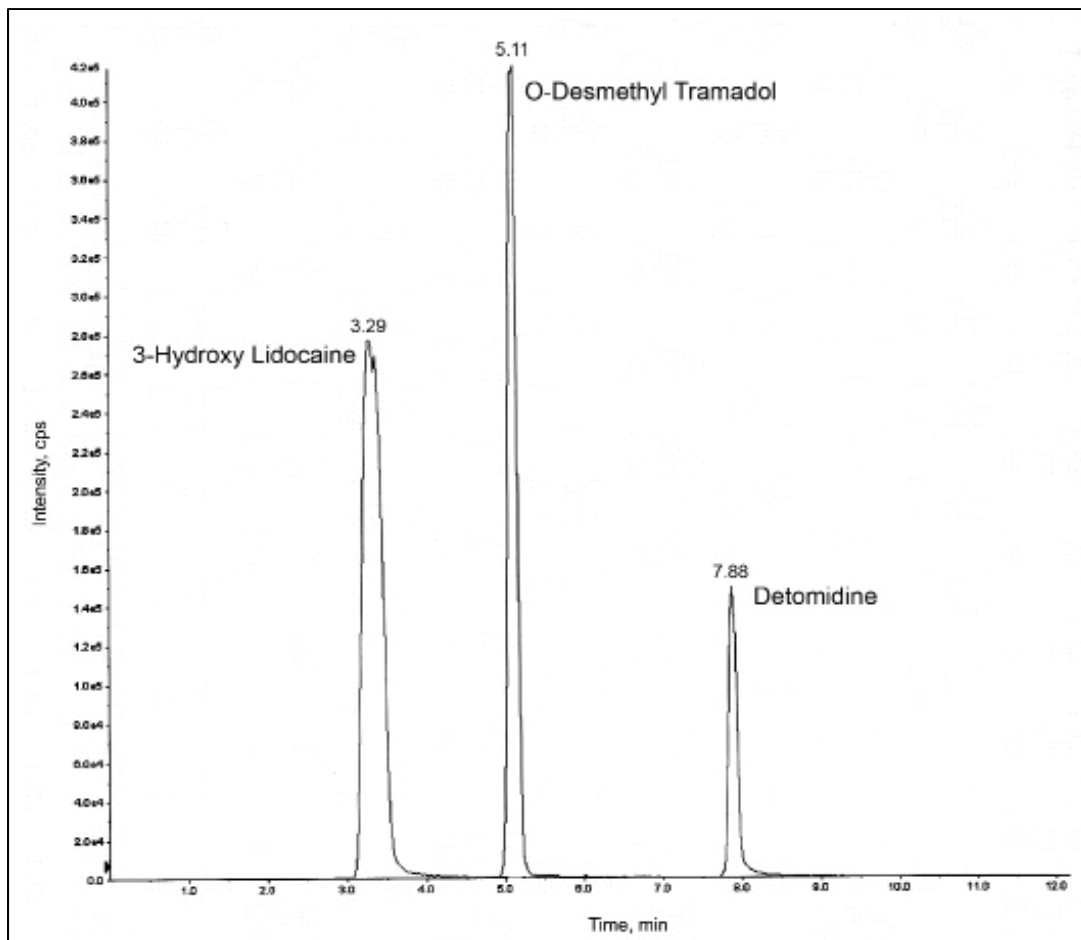
NOTE: Prepare elution solvent daily

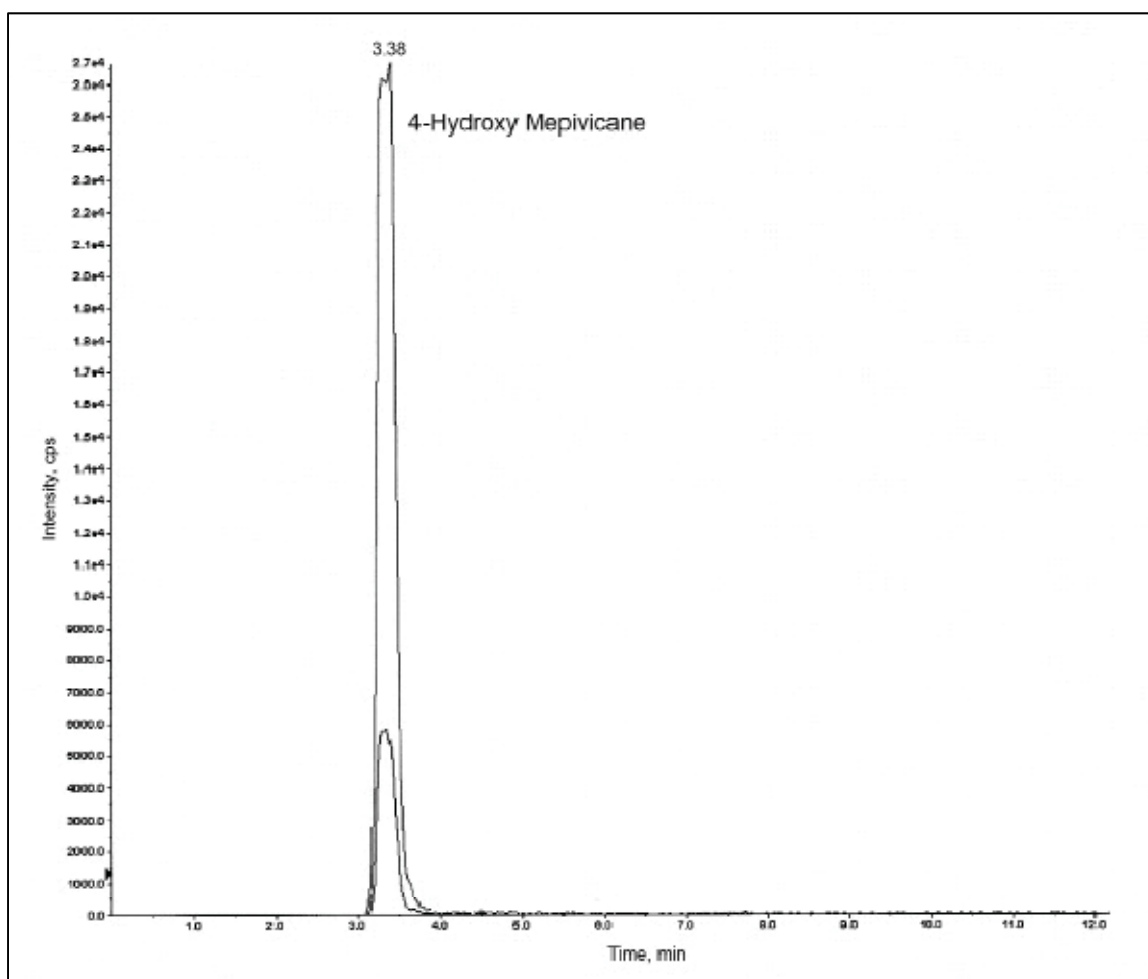
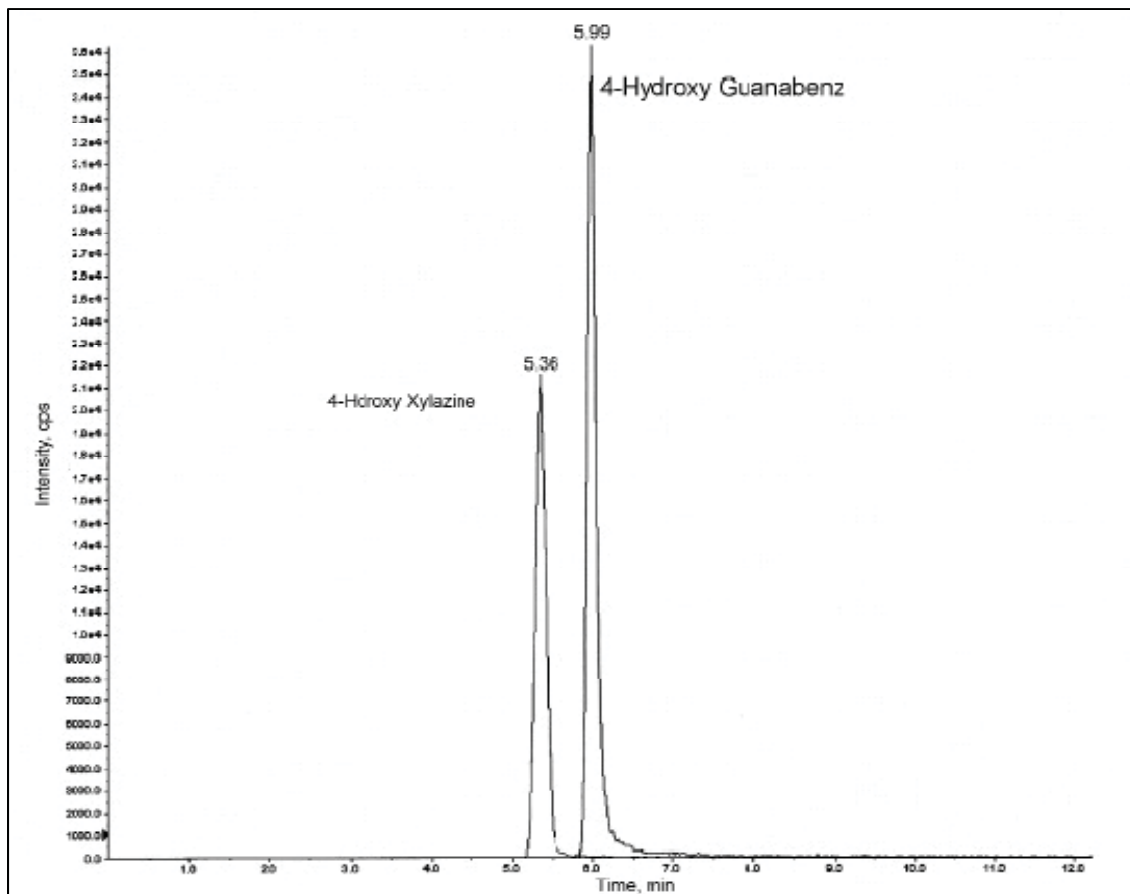
6. DRY ELUATE:

Evaporate to dryness at < 40°C

7. ANALYSIS:

Inject 10 µL sample





Compound	RT	Precursor Ion	Product Ion
3-Hydroxy Lidocaine	3.29	251.5	86.0
4-Hydroxy Mepivacaine	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 XTRACT[®] EXTRACTION COLUMN

Part #

XRDAH515 - Xtract[®] SPE Cartridge, 500 mg DAU, 15 mL Tube

BETA-GLUC-10 - Selectrazyme[®] Beta-glucuronidase

SLDA50ID21-5UM - Selectra[®] DA HPLC Column 50 x 2.1 mm, 5 μ m

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 μ L 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 μ L 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 \pm 0.5 with 0.5 M Phosphoric acid.

2. CONDITION Xtract[®] EXTRACTION COLUMN

1 x 5 mL CH₃OH

1 x 5 mL D.I. H₂O

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 CH₂Cl₂; 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 CH₂Cl₂ / IPA / NH₄OH (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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

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

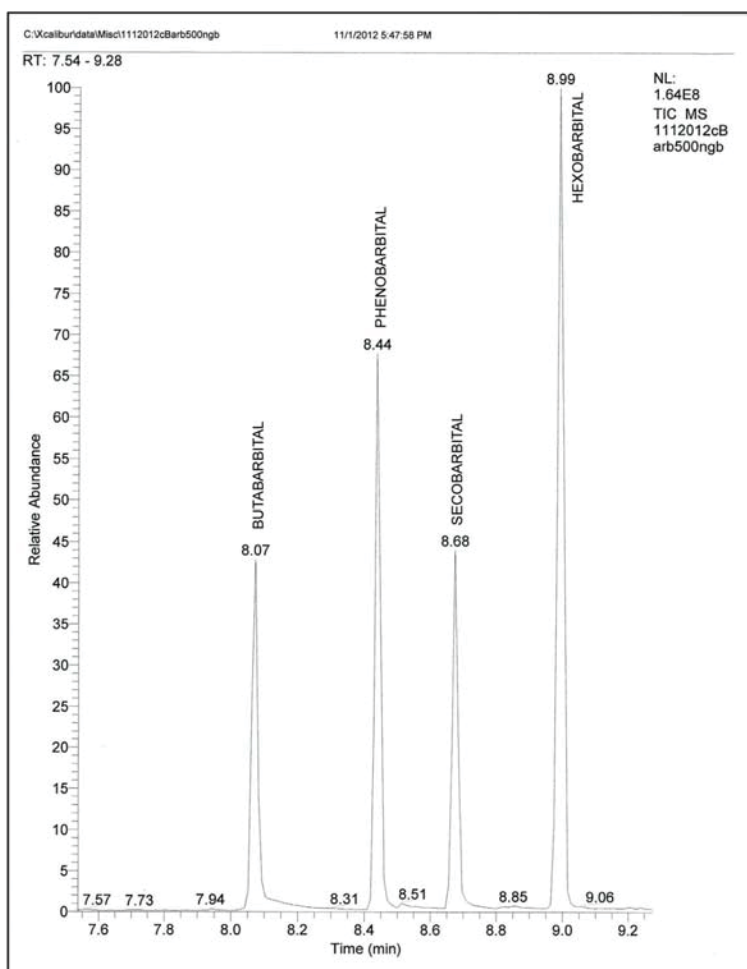
Add 25-50 µL of 0.2 M TMPAH

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 barbituates

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

SPPHO6001-10 - 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 - β -GLUCURONIDASE HYDROLYSIS:

To 2 mL of urine add internal standard(s) and 1 mL of β -glucuronidase solution.

β -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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

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% NH₄OH

collect eluate at 1 to 2 mL/minute

6. DRY ELUATE:

Evaporate to dryness at < 40°C

7. DERIVATIZE:

Add 50 μ L Ethyl Acetate and 50 μ L BSTFA w/1% TMCS

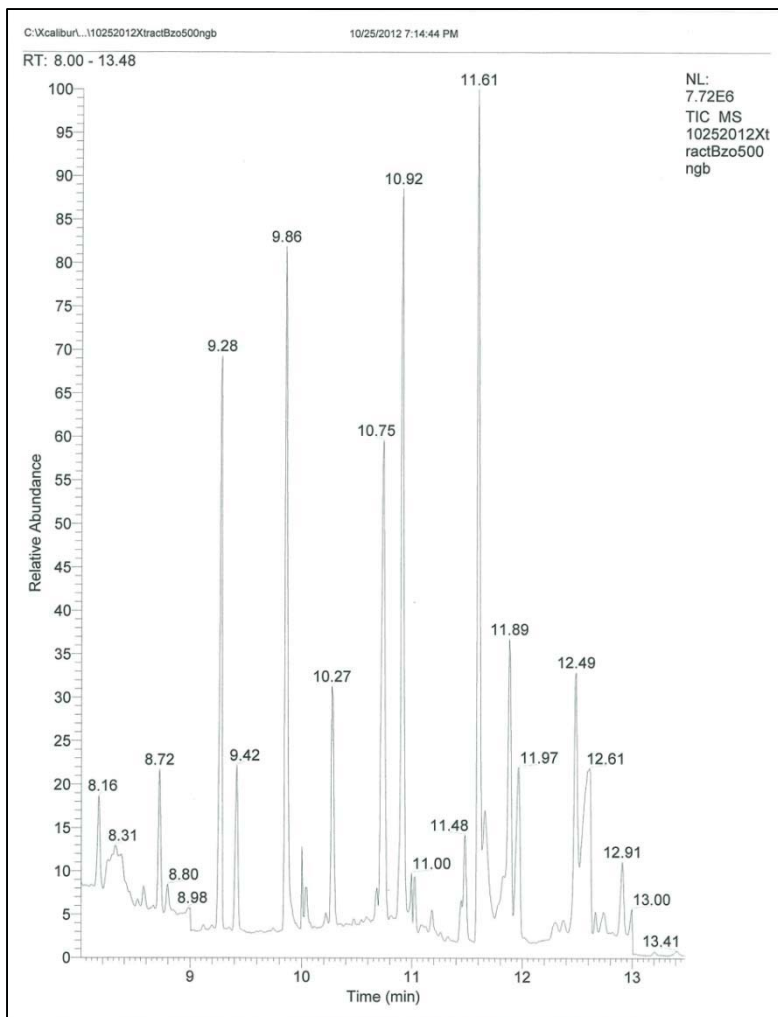
Overlay with Nitrogen and cap. Mix/vortex

React 20 minutes at 70°C. Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution

8. ANALYZE:

Inject 1 to 2 μ L 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 XTRACTT[®] DAU EXTRACTION COLUMN:

1 x 3 mL CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

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 MeCl₂ / IPA / NH₄OH (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:

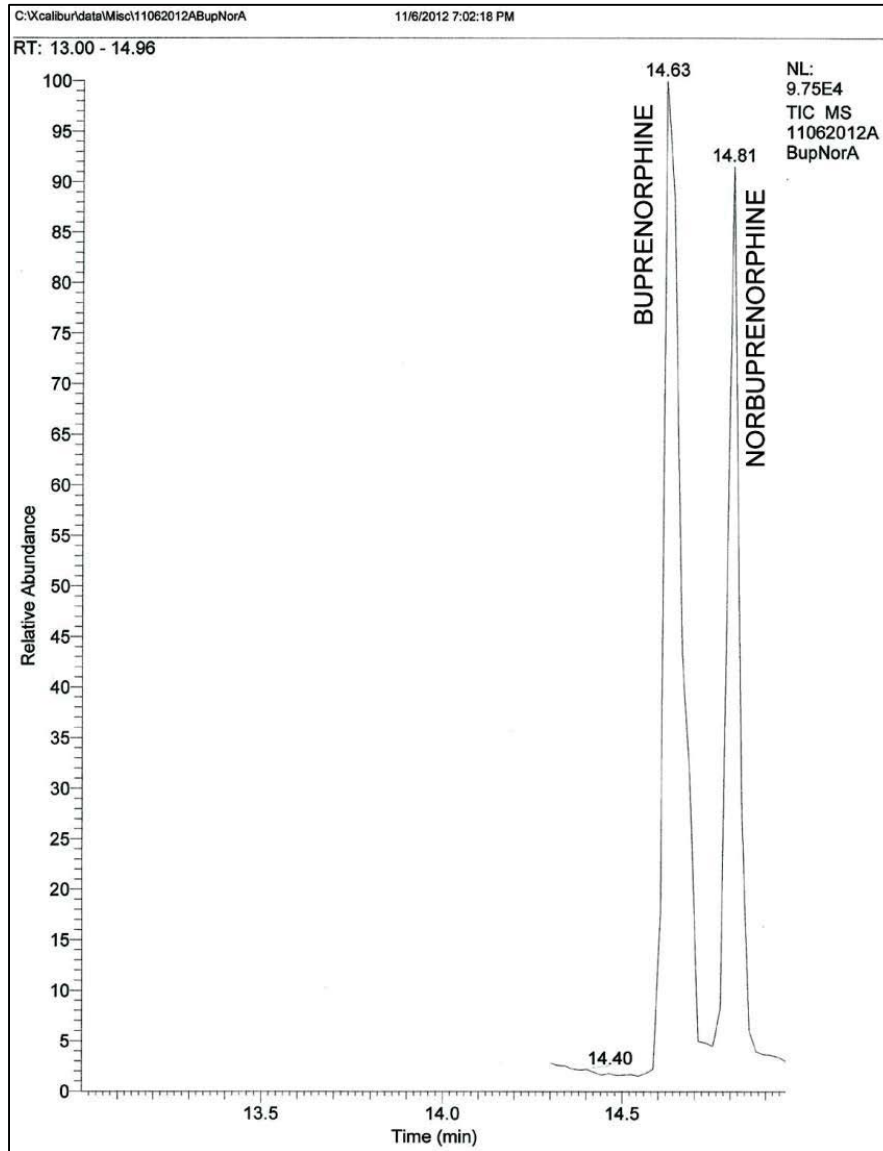
Add 50 μ L Ethyl Acetate and 50 μ L BSTFA w/1 % TMCS

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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

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 $< 40^{\circ}\text{C}$

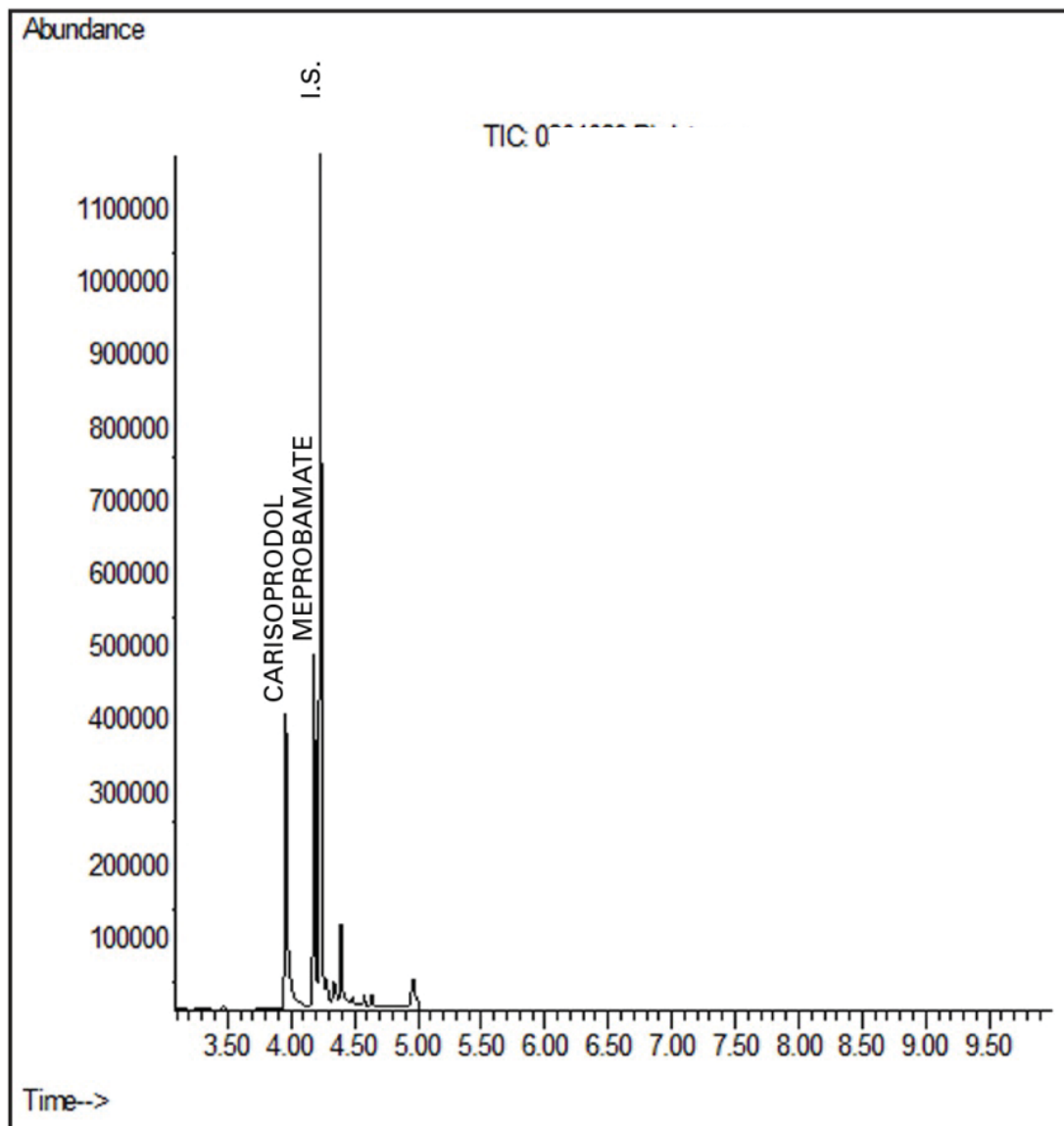
Reconstitute with 100 μL Ethyl Acetate

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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

1 x 3 mL CH₃OH

Dry column (5 minutes at full vacuum or pressure)

5. ELUTE CLENBUTEROL / SALBUTAMOL:

1 x 3 mL CH₃OH containing 4% NH₄OH

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

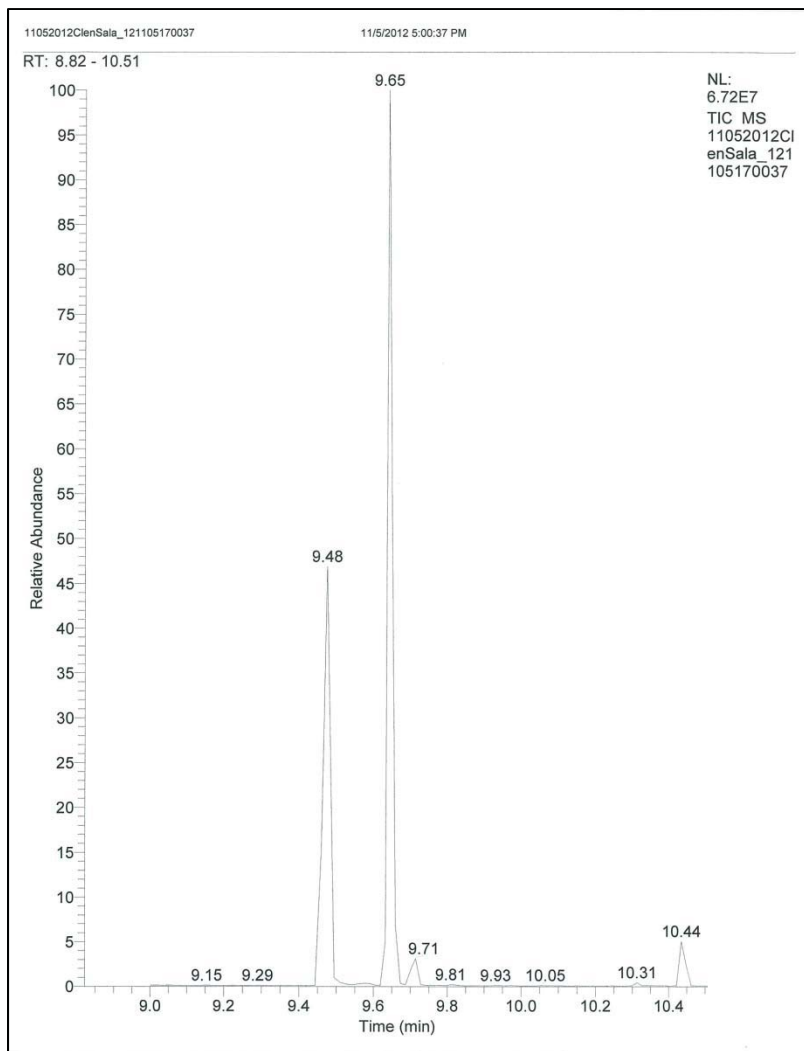
Heat at 70 °C for 30 minutes

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 μ L 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 CH₃OH

1 x 3 mL D.I. H₂O

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. H₂O

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

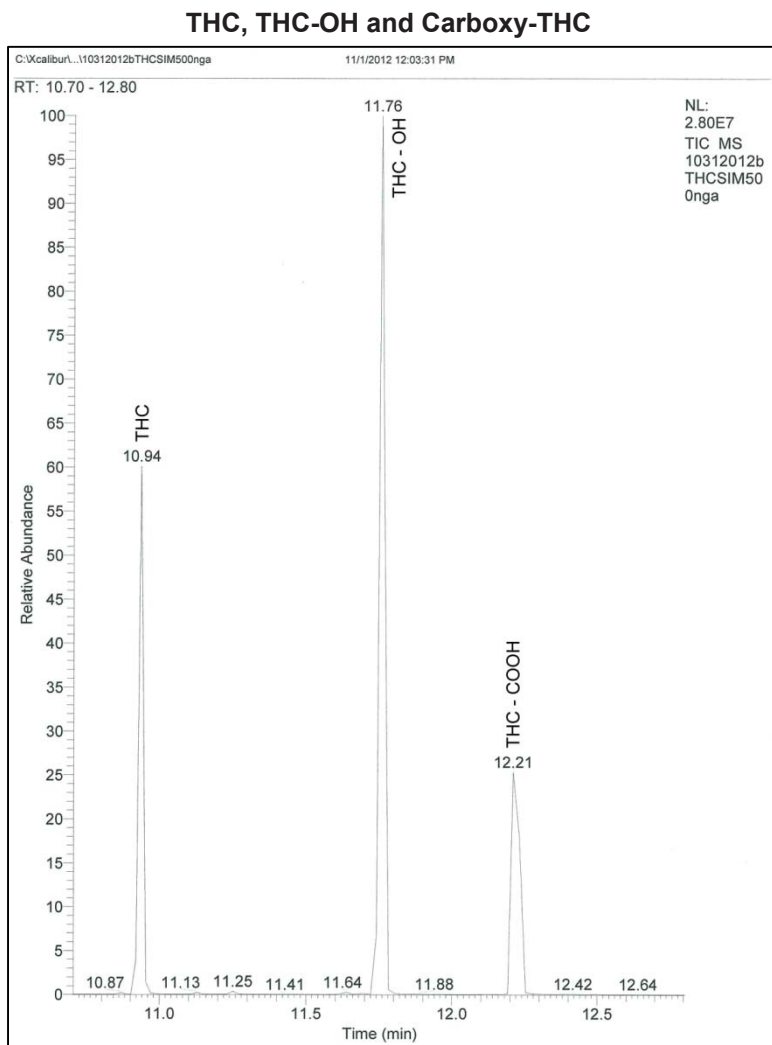
React 20 minutes at 70°C

Remove from heat source to cool

NOTE: Do not evaporate BSTFA

Inject 1 to 2 µL onto gas chromatograph

CHROMATOGRAM



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 H₂O to the sample.

Vortex or shake thoroughly.

Centrifuge for 5 min at 800 rpm.

2. CONDITION CLEAN-UP® EXTRACTION COLUMN

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

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 CH₃OH.

5 mL of D.I. H₂O.

Dry column (5 min > 10 inches Hg).

5. ELUTE GLYCOPYRROLATE

1 x 4 mL CH₃OH / 0.5 M Ammonium Acetate buffer, pH 3.0 (95:5).

6. DRY ELUTE

Evaporate to dryness at 60 °C.

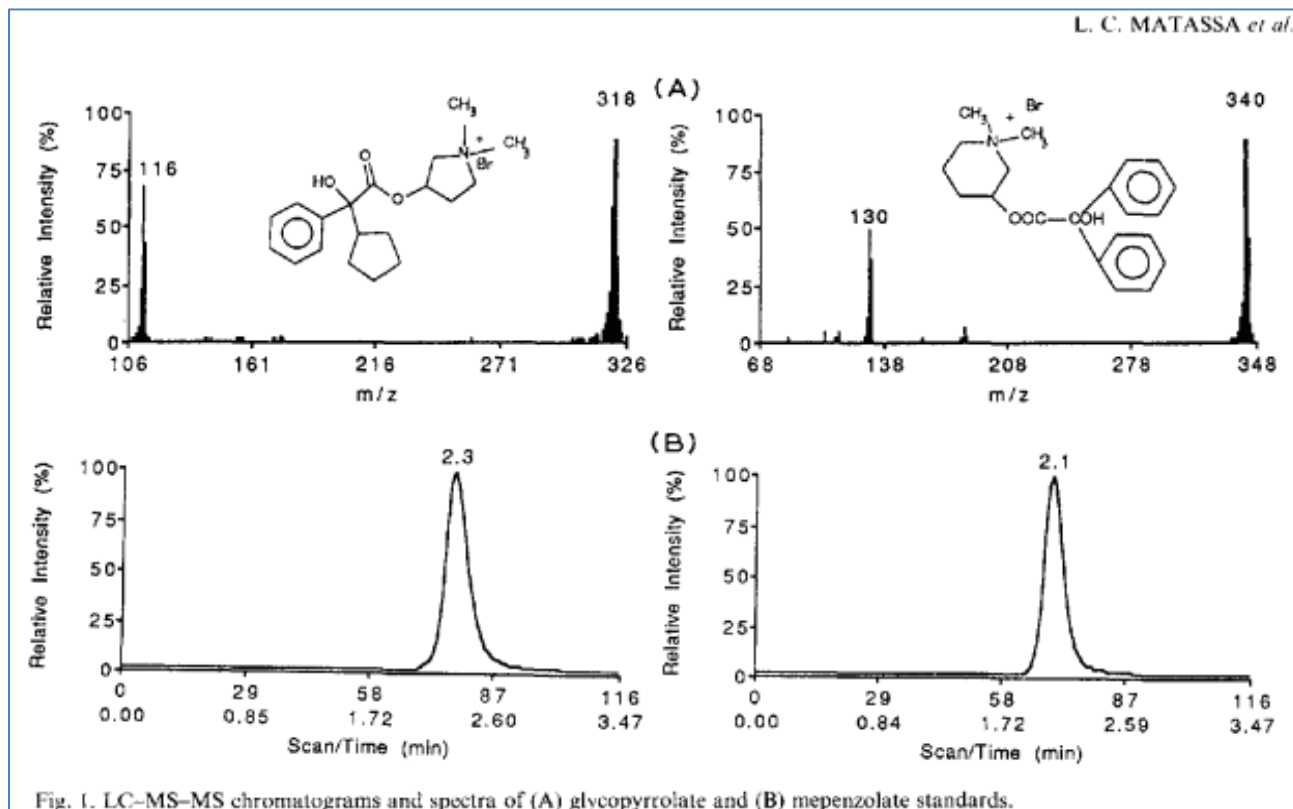
Reconstitute with 100 µL CH₃OH.

7. QUANTITATE

Inject 10 µL onto HPLC.

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM



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

Flow Rate: 0.8mL/minute

Injection Volume: 10 μ l

LC Column: Hamilton PRP-1, 150 mm x 4.1 mm I.D. 5 μ m

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.