



Thermo Scientific HyperSep Columns

Application Notebook – Issue 1, April 2011

Removing Uncertainty

by Applying Science to SPE

Pharmaceutical/Biotech • Environmental • Forensics • Food Safety

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Sandoz, Inc.
Restoril

Upjohn Company
Halcion, Xanax

U.S. Silica Company
Florisil

Valeant Pharmaceuticals International, Inc.
Ativan

Varian, Inc.
Varian 8400

Thermo Scientific HyperSep Columns

The Importance of SPE

Sample preparation is a critical step prior to LC or GC analysis. Over the last few years, the requirement for higher sensitivity, selectivity, accuracy, precision and sample throughput has increased significantly. This is due to reduced sample volumes, greater drug efficacy, and greater awareness of toxicity levels of pollutants.

Improved sample preparation techniques ensure accurate LC/GC and MS analysis in the following ways:

- Maximize detection selectivity
 - Reduce ion suppression
 - Reduce protein binding
 - Reduce matrix interferences
- Improve analytical system performance
 - Longer column lifetimes
 - Less maintenance on detector
 - Syringes less likely to block
 - Less contamination
- Improve sensitivity
 - Lower limits of detection
 - More accurate quantitation
 - Improved data processing

Sample Preparation Techniques

Solid phase extraction (SPE) is a sample preparation technique that is widely used by chromatographers in pharmaceutical, environmental, forensics and food safety applications. Solid phase extraction is very selective with a wide number of phases available. The technique can be automated and uses significantly smaller volumes of solvent compared to liquid/liquid extraction (LLE) and supported liquid extraction (SLE) methods.

In general, SPE can be used for three important purposes in sample preparation:

- Concentration of the analyte
- Removal of interfering compounds
- Transfer of analyte into a suitable solvent for analysis

SPE has a number of benefits over other sample preparation techniques, such as protein precipitation (PPT) and LLE.

Protein precipitation is a relatively fast technique to perform, but it has a number of limitations. The process is non-selective and only removes proteinaceous material. Because there can be significant matrix interference, subsequent cleanup stages may be required, adding costs and time to the cleanup process.

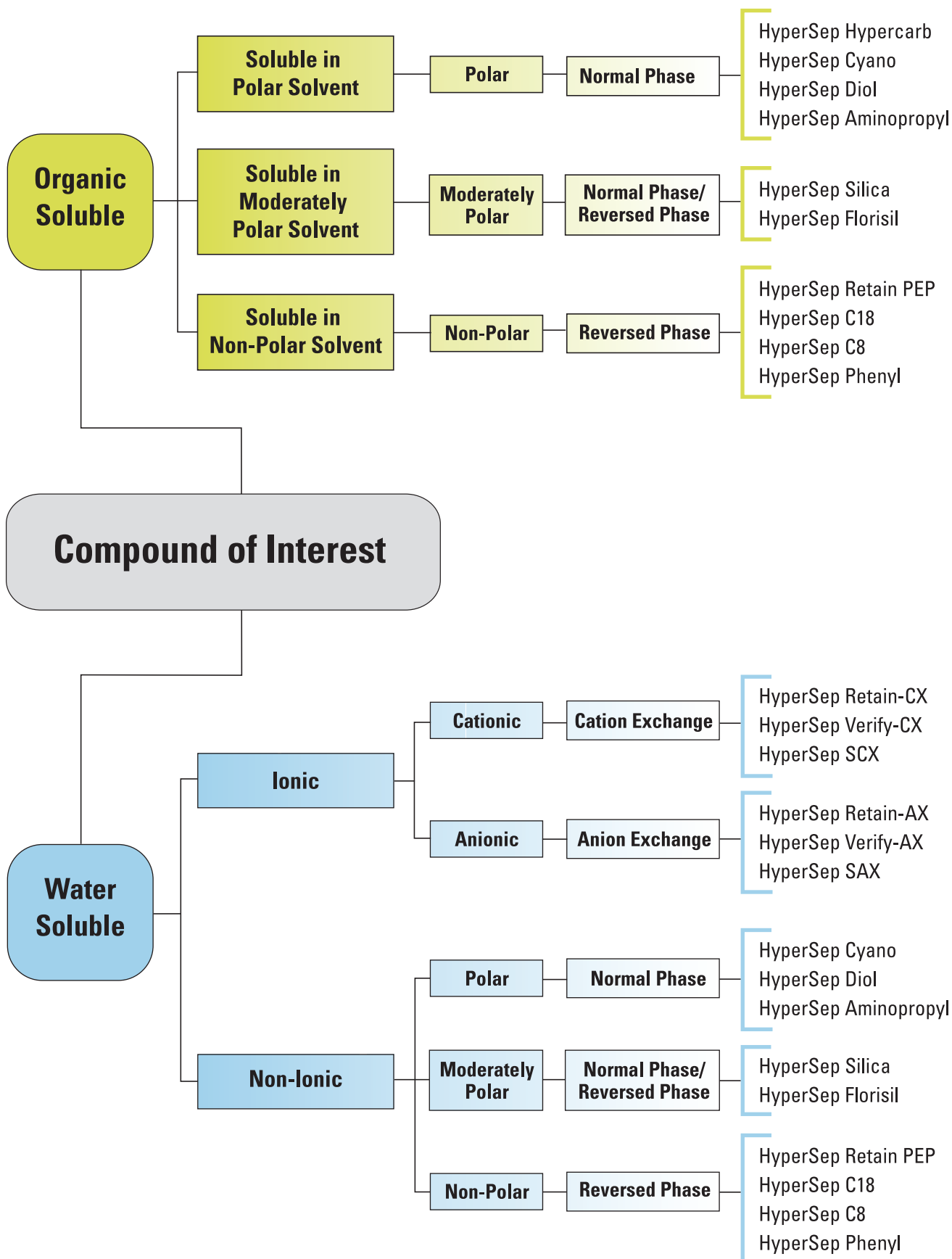
LLE presents additional drawbacks. This process uses large volumes of potentially hazardous solvent and often involves laborious method development. It is a less selective technique for polar compounds.

Selection of the Correct SPE Phase

To maximize the benefits of SPE, there are a number of factors to consider:

- Select the correct SPE column size
 - Consider the volume of the sample
- Select the correct SPE bed weight
 - Consider the concentration/amount of analyte
- Determine the correct phase for an application by considering the physicochemical properties of the analyte
 - pH , pK_a etc.
 - Solubility $\log P$

SPE Phase Selection



HyperSep SPE Phases

Polymeric



Reversed Phase Non-polar (Hydrophobic) Phases

- Non-polar-non-polar interactions
- Van der Waals or dispersive forces

HyperSep Retain PEP

Polystyrene divinylbenzene material surface modified with urea groups

- Surface area 550 to 750m²/g
- Particle size 40 to 60µm
- Pore size 55 to 90Å

A versatile polymeric material for the retention of polar and non-polar analytes ideal for applications such as drugs and metabolites in biological matrices, environmental samples and desalting of peptides in serum, plasma or biological fluids.

Mixed-Mode Phases

- Two functional groups
- Non-polar and ion exchange
- Hydrophobic and ionic retention
- Ideal for samples with complex structures

HyperSep Retain-CX

Versatile polymeric material for retention of basic compounds

- Surface area 550 to 750m²/g
- Particle size 40 to 60µm
- Pore size 55 to 90Å

Retain-CX is a versatile polymeric material for the enhanced retention of basic compounds. Typical application areas include the analysis of a wide range of drugs of abuse from biological matrices.

HyperSep Retain-AX

Versatile polymeric material for retention of acidic compounds

- Surface area 550 to 750m²/g
- Particle size 40 to 60µm
- Pore size 55 to 90Å

Retain-AX is a versatile polymeric material for the enhanced retention of acidic compounds. Typical application areas include the analysis of a range of acidic drugs of abuse from biological matrices, such as THC and its metabolites.

HyperSep Hypercarb

Unique material for retention of highly polar compounds

- 100% porous graphitic carbon material
- Retention of extremely polar compounds
- Retention properties allow low bed weights

Provides total pH stability and the retention and separation of highly polar species. It is ideal for problem analytes in SPE applications.

Reversed Phase Silica Phases



Reversed Phase Hydrophobic Phases

- Non-polar-non-polar interactions
- Van der Waals or dispersion force

HyperSep C18

Highly retentive alkyl-bonded silica phase for non-polar to moderately polar compounds

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

A silica-based material for applications such as drugs and their metabolites in biological matrices, trace organics in environmental water samples and toxins in food samples.

HyperSep C8

Less retentive alternative to C18 for non-polar to moderately polar compounds

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

A silica-based material for applications such as drugs and their metabolites in biological matrices, trace organics in environmental water samples, and toxins in food samples. C8 is used for hydrophobic compounds which tend to be retentive on C18 columns.

HyperSep Phenyl

Alternative selectivity for retention of basic compounds

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

Phenyl is a silica-based material which offers alternative selectivity for aromatic compounds due to the presence of the benzene ring within the structure. Typical applications include benzodiazepines in biological matrices and extraction of aromatic compounds.

Normal Phase Silica Phases



Normal Phase Hydrophilic Phases

- Polar-polar interactions
- Dipole-dipole interactions

HyperSep Silica

A polar sorbent primarily used to retain analytes from non-polar matrices

- Surface area 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

Silica material is primarily used to extract analytes from non-polar solvents such as hydrocarbons, less polar esters and ethers. Typical application areas include extraction of aldehydes, amines, pesticides, herbicides, carotenoids, fat soluble vitamins, aflatoxins, fatty acids and phospholipids.

HyperSep Florisil®

Ideal for the isolation of polar compounds from non-polar matrices

- Surface area 289m²/g
- Particle size 40 to 60µm
- Pore size 60Å

Florisil is a magnesia-loaded silica gel which has been specifically designed for applications such as the extraction of pesticides using AOAC and EPA methods, as well as polychlorinated biphenyls (PCBs) in transformer oil.

HyperSep Cyano

For retention of polar compounds from non-polar matrices

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

Cyano is a silica-based material of low hydrophobicity. It is less retentive than either silica or diol. Typical application areas include retention of polar compounds from hexane and oil.

HyperSep Diol

For extraction of polar compounds

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

A silica-based material which can be used for extraction of polar compounds. Typical applications include normal phase extraction and purification of polar compounds.

HyperSep Aminopropyl

A polar sorbent for both polar and anion exchange interactions

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

Aminopropyl is a silica-based material which can be used as both a polar sorbent and a weak anion exchanger. Typical applications include petroleum fractionation, saccharides, drugs and drug metabolites.

Ion Exchange Phases



Ion Exchange Phases

- Electrostatic interactions

HyperSep SAX (Strong Anion Exchanger)

Strong anion exchange sorbent for extraction of weak acids

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

SAX is ideal for the extraction of negatively charged compounds from both aqueous and non-aqueous matrices, as well as the extraction of weak acids such as carboxylic acid. Typical application areas include removal of acidic food pigments, removal of phenolic compounds, nucleic acids and surfactants.

HyperSep SCX (Strong Cation Exchanger)

Strong cation exchange sorbent for extraction of charged basic compounds

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

SCX is ideal for the extraction of positively charged compounds from both aqueous and non-aqueous matrices. Typical application areas include extraction of antibiotics, drugs, organic bases, amino acids, catecholamines and herbicides.

Mixed-Mode Phases

- Two functional groups
- Non-polar and ion exchange
- Hydrophobic and ionic retention
- Ideal for samples with complex structures

HyperSep Verify-CX

Non-polar and anionic characteristics for improved analysis of basic drugs of abuse

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

Verify-CX is a mixed-mode material based on two functional groups bonded to the silica base: a reversed phase C8 group and a strong cation exchanger. Typical application areas include the analysis of a range of basic drugs of abuse from biological matrices.

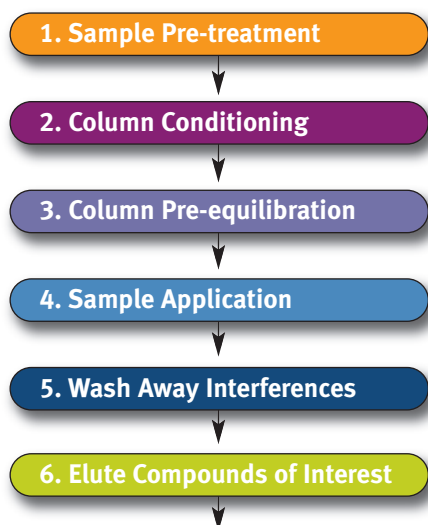
HyperSep Verify-AX

Non-polar and cationic characteristics for improved analysis of acidic drugs of abuse

- Surface area 470 to 530m²/g
- Particle size 40 to 60µm
- Pore size 60Å

Verify-AX is a mixed-mode material based on two functional groups bonded to the silica base: a reversed phase C8 group and a strong anion exchanger. Typical application areas include the analysis of a range of acidic drugs of abuse from biological matrices, including THC and its metabolites.

SPE Procedure – Six Steps for Clean Extract



1 Sample Pre-treatment

It is important to optimize the sample for effective analyte retention. Consider the following when pre-treating a sample prior to application to the SPE product:

- Adjust sample/matrix composition for proper dilution/ionic strength
- Ensure that sample is at proper pH for optimum retention
- Confirm that analytes are free in solution
- Remove any unwanted particulates via filtration or centrifugation

Sample Matrix Sample Pre-Treatment

Serum, Plasma	Dilute with an equal volume of water or suitable buffer prior to applying the sample to the SPE column. Buffer choice and pH considerations are dependent upon the compound of interest in the sample.
Whole Blood	Blood is similar to serum and plasma, apart from the presence of whole red blood cells. Dilute with an equal volume of water or buffer to ensure that compounds of interest are free in solution.
Urine	Dilute with an equal volume of water or suitable buffer prior to applying the sample to the SPE column.
Fats, Oils	Dilute samples with non-polar organic solvents such as hexane due to the non-polar nature of the matrix.
Cereals	Homogenize sample with a non-polar solvent.
Ointments and Creams	Ointments are typically either water-based or oil-based. For water-based products, dissolve in a polar solvent such as methanol. For oil-based products, dissolve in a non-polar solvent such as hexane.
Water	Pre-treatment is dependent upon the particulate content of the sample. Some samples can be applied directly to the SPE product. For samples heavily laden with particulates, filtration/centrifugation may be necessary.
Soil and Sludge	Analytes can be difficult to adsorb onto the sorbent material. Samples are typically extracted using a non-polar solvent such as hexane, then using a polar sorbent material for the SPE process.
Fruits and Vegetables	Homogenize sample with a polar solvent such as methanol and subsequently dilute with water if required.
Crude Oil Products	Dilute sample with a non-polar solvent such as hexane.
Dairy Produce	Typically diluted/homogenized with water or suitable buffer.
Meats and Soft Drinks	Dilute sample with water.

2 Column Conditioning

Prepare the sorbent for effective interaction(s) with the compounds of interest.

- Use an appropriate solvent to condition the column and activate the ligands on the chromatographic surface
- Prevent the sorbent from drying during the conditioning step (dry sorbent can affect the ability of the analytes to interact); allow about 1mm of last conditioning solvent to remain above the top tube frit

3 Column Pre-equilibration

- To re-equilibrate the column, use the same solvent that is used for the sample pre-treatment step (do not let the sorbent dry during the conditioning step)
- Allow about 1mm of last conditioning solvent to remain above the top tube frit

4 Sample Application

- Analytes are retained on the sorbent. Apply the sample at an appropriate flow rate (1mL/minute is a typical flow rate; too high a flow rate can lead to inconsistent extractions)

5 Wash Away Interferences

Remove impurities bound less strongly than the compounds of interest.

- Select a wash solvent that is strong enough to remove the interferences, but weak enough to leave the compounds of interest behind
- Selectively rinse away the less strongly bonded interferences
- Wash solvent selected according to phase mechanism and analyte properties (a typical wash solution may contain less organic or inorganic salt than the final eluent)

6 Elute Compounds of Interest

Selectively recover the analyte(s) by disrupting the analyte-sorbent interaction.

- Selectively elute the analytes of interest using different solvents
- A smaller elution volume leads to a more concentrated extract
- Select an elution solvent that leaves the strongly retained impurities behind
- Select elution solvent according to phase mechanism and analyte properties
- For best results, elute compounds of interest using two small aliquots (rather than one large aliquot)

Solvent Selection in SPE

The choice of solvent is dependent upon the sample matrix and the retention mechanism used. The table shows the differing polarities of solvents commonly used in SPE.

Polarity	Solvent	Miscibility with Water
Nonpolar 	Hexane	No
	Isooctane	No
	Petroleum Ether	No
	Cyclohexane	No
	Carbon Tetrachloride	No
	Chloroform	No
	Methylene Chloride	No
	Tetrahydrofuran	Yes
	Diethyl Ether	No
	Ethyl Acetate	Poor
	Acetone	Yes
	Acetonitrile	Yes
	Isopropanol	Yes
	Methanol	Yes
Polar	Water	Yes
	Acetic Acid	Yes

Method Development Optimization in SPE

The use of SPE as a sample preparation technique can significantly reduce the effects of ion suppression. For highest recovery levels and cleaner extracts, optimization of the SPE process is important. By optimizing the load, wash, and elution steps of the SPE process, a cleaner sample extract can be obtained, leading to benefits for the detection and robustness of the analytical instruments. A gradual elution of the compound from the cartridge results in the optimum wash and elution conditions.

A range of drugs were investigated for different HyperSep SPE phases. It was anticipated that the nature of the drug, and the pH, would determine the optimum conditions in which to perform the experiments. Of particular interest is the effect of the elution conditions, as these typically default to 100% organic, and this is not always optimal for selective extraction of an analyte.

Factors that influence levels of recovery are:

- pH levels
 - Sample loading
 - Buffers used
 - Elution
- Wash solvents
 - Must not recover analytes
- Elution solvents
 - Polarity
 - Solubility
 - Elutropic strength

Application Examples

1) Optimization of Load Step

For Reversed-Phase Interactions

- Neutral compounds are not affected by pH (no need to adjust the pH of the sample)
- For charged compounds, use at a pH at which the compound is not charged. Neutralize the molecule according to the following:
 - For basic compounds, the neutral molecule exists at least 2 pH units below the pKa of the compound
 - For acidic compounds, the neutral molecule exists at least 2 pH units above the pKa of the compound

For Normal-Phase Interactions

- pH is not normally an issue in normal phase interactions, as the solvents used are typically non-polar organic solvents, rather than water.
- There is no need to verify the sample application pH

For Ion-Exchange Interactions

- pH and pKa are important considerations
- Acidic compounds are extracted from a sample solution at least 2 pH units above the pKa of the analyte
- Basic compounds are extracted from a sample solution 2 or more pH units below the pKa of the analyte

2) Optimization of Wash and Elution Steps

Wash and elution profiles were carried out for a number of the HyperSep SPE phases to determine the optimum wash and elution solvent mixes for maximum recovery of the compound. The results show the importance of using an optimized method for best recovery levels.

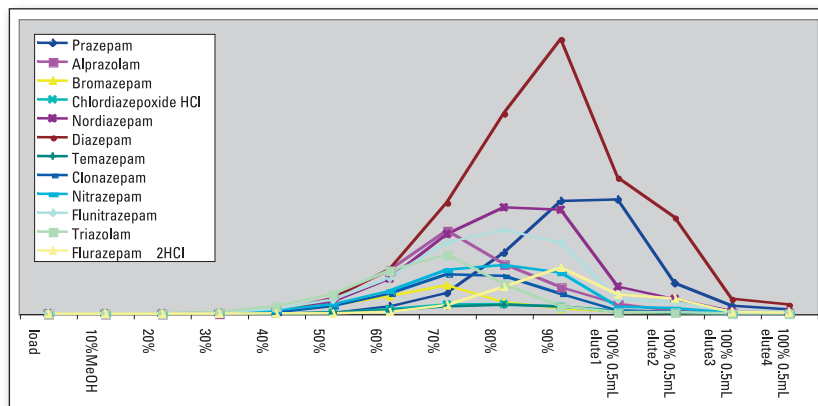
For all phases, the following compounds were investigated:

Compound	pKa Value	Structure	Compound	pKa Value	Structure
Prazepam	3.0		Temazepam	1.3	
Alprazolam	2.4		Clonazepam	10.5 (1-position), 1.5 (4-position)	
Bromazepam	11.0 2.9		Nitrazepam	2.5	
Chlordiazepoxide HCl	4.8		Flunitrazepam	1.8	
Nordiazepam	3.5		Triazolam	1.5	
Diazepam	3.3		Flurazepam	8.2 1.9	

HyperSep Retain PEP

Using a 30mg 1mL SPE Cartridge
(Part Number 60107-201)

1. Condition with 1mL methanol followed by 1mL water
2. Load 1mL of 500ng/mL sample in water
3. Wash with increasing strengths of methanol in water, starting with 0% methanol/100% water to 90% methanol/10% water, increasing methanol content by 10% each time
4. Elute 4 times with 0.5mL of 100% methanol

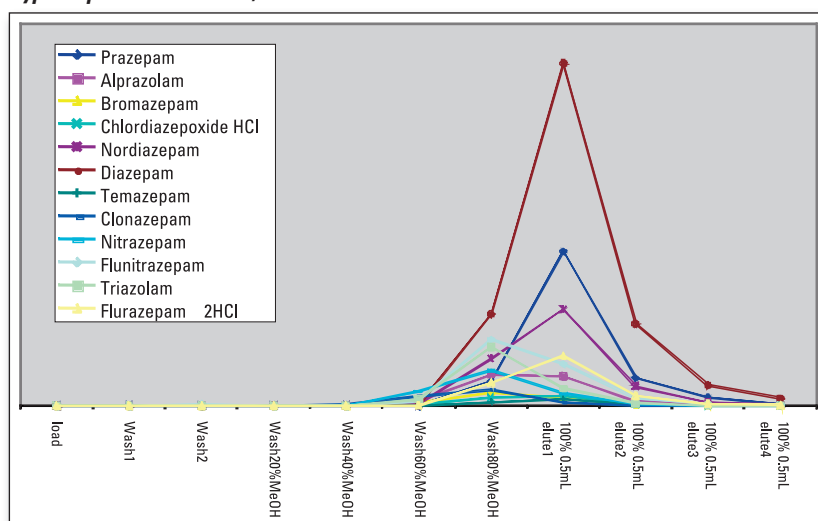
HyperSep Retain PEP Wash/Elution Profile

The results show an optimum wash profile of 30% methanol/70% water before compounds of interest start to be eluted. An elution volume of 1.5mL in three aliquots of 500µL eluted off the compounds of interest to give a high recovery level.

HyperSep Retain-CX

Using a 30mg 1mL cartridge
(Part Number 60107-301)

1. Condition with 1mL of 0.1% formic acid in methanol followed by re-equilibrium using 1mL of 0.1% formic acid in water
2. Load 1mL of 500ng/mL samples in 0.1% formic acid in water
3. Wash with 1mL of 0.1% formic acid in water
4. Wash with 1mL of 0.1% formic acid in methanol
5. Wash with increasing strengths of 5% ammonia in methanol/water, starting with 20% methanol/80% water to 80% methanol/20% water, increasing methanol content by 10% each time
6. Elute 4 times with 0.5mL of 5% ammonia in methanol

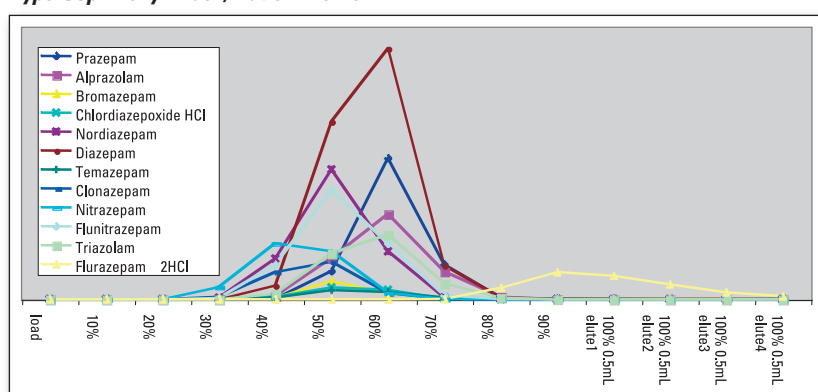
HyperSep Retain-CX Wash/Elution Profile

The results show an optimum wash profile of 5% ammonia in 50% methanol/50% water before compounds of interest start to be eluted. An elution volume of 1.5mL in three aliquots of 500µL eluted off the compounds of interest to give a high recovery level.

HyperSep Phenyl

Using 100mg 1mL cartridge
(Part Number 60108-386)

1. Condition with 1mL methanol followed by 1mL of water
2. Load 1mL of 500ng/mL sample in water
3. Wash with increasing strengths of methanol from 10% to 90%
4. Elute 4 times with 0.5mL 100% methanol

HyperSep Phenyl Wash/Elution Profile

The results show an optimum wash profile of 20% methanol/80% water before compounds of interest start to be eluted. An elution volume of 1.5mL in four aliquots of 500µL eluted off the compounds of interest to give a high recovery level.

Conclusion

- For second (organic) wash, choose the strongest solution where no compound breakthrough occurs
- For elution step, use a solution stronger than where all the compound of interest is eluted
- NB: when choosing these solutions allow some margin for error

Acetaminophen in Calf Serum

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

- Dilute 10mg of acetaminophen in 100mL of water to give a 100ppm solution
- Mix 25mL of acetaminophen (100ppm) solution in a 50mL flask and dilute to volume with water (standard solution 50ppm)
- Mix 25mL of acetaminophen (100ppm) solution in a 50mL flask and dilute to volume with serum and 1% H₃PO₄ (sample 50% serum and 1% H₃PO₄)

Condition Retain PEP Extraction Column

- 2mL of methanol
- 2mL of DI H₂O

Apply Sample

- Load 2mL sample solutions

Wash Column

- 2mL of methanol/DI H₂O (5/95, v/v)
- Dry column (5 to 10 minutes at >10" Hg/full flow for positive pressure manifold)

Elute Acetaminophen

- 2mL of methanol

Dry Eluate and Reconstitute

- Evaporate to dryness at <50°C using nitrogen
- Reconstitute sample using 1mL of mobile phase

Acetaminophen in Calf Serum

Using 60mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-303)

Sample Preparation

- Dilute 10mg of acetaminophen in 100mL of water to give a 100ppm solution
- Mix 25mL of acetaminophen (100ppm) solution in a 50mL flask and dilute to volume with water (standard solution 50ppm)
- Mix 25mL of acetaminophen (100ppm) solution in a 50mL flask and dilute to volume with serum and 1% H₃PO₄ (sample 50% serum and 1% H₃PO₄)

Condition Retain-CX Extraction Column

- 2mL of methanol
- 2mL of DI H₂O

Apply Sample

- Load 2mL sample solutions

Wash Column

- 2mL of methanol/DI H₂O (5/95, v/v)
- Dry column (5-10 minutes at >10" Hg/full flow for positive pressure manifold)

Elute Acetaminophen

- 2mL of methanol

Dry Eluate and Reconstitute

- Evaporate to dryness at <50°C using nitrogen
- Reconstitute sample using 1mL of mobile phase

Acetaminophen Extraction and Cleanup from Hyclone

Using 60mg 6mL HyperSep Retain PEP and -CX Extraction Columns (Part Number: 60107-203 and 60107-308)

Prepare Sample

- Dissolve 10mg of Ethylamino-phenol in 100mL of water (100ppm)
- Dilute 25mL of Ethylamino-phenol solution to 50mL with water
- Dilute 25mL of Ethylamino-phenol solution to 50mL with serum

Condition Column

- 1 x 2mL CH₃OH
- 1 x 2mL H₂O water 2mL

Apply Sample

- Load 2mL of sample

Wash Column

- 1 x 2mL 5% CH₃OH

Elute Sample

- 1 x 2mL CH₃OH

Results

SPE Column	Recovery (%)	
	Pretreatment 1	Pretreatment 2
HyperSep Retain PEP + 50% Serum	58.5	40.3
HyperSep Retain-CX + 50% Serum	94.6	94.6

Acetaminophen in Solution

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

Prepare a 5ppm sample in 0.2% ammonium acetate, pH 5

Condition Retain PEP Extraction Column

2mL of methanol

2mL of DI H₂O

Apply Sample

Load 4mL samples at 1-2mL/minute

Wash Column

1mL of DI H₂O

Elute Acetaminophen

5mL of methanol

Dry Eluate and Reconstitute

Evaporate to full dryness under a stream of nitrogen

Reconstitute the sample to 1mL with water

Analysis

Mobile phase: acetonitrile:1mM KH₂PO₄ (30:70, v/v)

Flow: 1mL/minute

Temperature: 30°C

Detection: UV 254nm

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 250 x 4.6mm

25005-254630

Benzodiazepines in Serum or Plasma for HPLC Analysis

Using 200mg 6mL HyperSep Verify-CX Extraction Column (Part Number: 60108-722)

Prepare Sample

To 1mL 1.0mL of 100mM phosphate buffer (pH 6.0) add internal standard(s)*

Add 1mL of serum or plasma

Mix/vortex

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Centrifuge as appropriate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH

1 x 3mL DI H₂O

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

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1mL/minute

Wash Column

1 x 2mL DI H₂O

1 x 2mL 20% acetonitrile in 100mM phosphate buffer (pH 6.0)

Dry column (10 minutes at >10" Hg)

1 x 2mL hexane

Elute Benzodiazepines

1 x 5mL ethyl acetate containing 2% ammonium hydroxide

Collect eluate at 1 to 2mL/minute

Dry Eluate

Evaporate to dryness at <40°C

Reconstitute

Reconstitute in mobile phase

Quantitate

Inject sample onto HPLC

* Suggested internal standards: Alprazolam-D5, Alphahydroxyalprazolam-D5, Diazepam, Lorazepam-D4, Oxazepam-D5, Temazepam-D5

Recommended HPLC Column

Part Number

BETASIL Phenyl/Hexyl 5µm, 150 x 4.6mm

73005-154630

Benzodiazepines in Urine for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample – β -Glucuronidase Hydrolysis

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

β -glucuronidase solution contains: 5,000 F units/mL
Patella vulgata in 100mM acetate buffer (pH=5.0)

Mix/vortex

Hydrolyze for 3 hours at 65°C

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH

1 x 3mL DI H₂O

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

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load at 1mL/minute

Wash Column

1 x 2mL DI H₂O

1 x 2mL 20% acetonitrile in 100mM phosphate
buffer (pH= 6.0)

Dry column (5 minutes at >10"Hg)

1 x 2mL hexane

Elute Benzodiazepines

1 x 5mL ethyl acetate containing 4% ammonium hydroxide
Collect eluate at 1 to 2mL/minute

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50 μ L ethyl acetate and 50 μ L BSTFA**
(with 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

Quantitate

Inject 1 to 2 μ L onto GC/MS

For mass spectrometry monitor the following ions:

Generic Name	Trade Name	Primary Ion***	Secondary	Tertiary
Nordiazepam -D5-TBDMS		332	334	333
Nordiazepam-TBDMS		327	328	329
Oxazepam-D5-TBDMS		462	519	462
Oxazepam-TBDMS	Serax	457	513	459
Temazepam-D5-TBDMS		362	390	288
Temazepam-TBDMS	Restoril®	357	359	385
Lorazepam-TBDMS	Ativan®	491	513	493
Clonazepam	Klonopin®	372	374	326
7-Aminoclonazepam –TBM		456	458	513
Diazepam	Valium®	256	283	221
Desalkylflurazepam-TBDMS		345	347	402
Prazepam*		269	241	324
α -Hydroxymidazolam-TBDMS	Versed®	398	400	440
Desmethyflunitrazepam-TBDMS		357	310	356
7-Aminoflunitrazepam-TBDMS		397	324	398
Alprazolam	Xanax®	308	279	204
α -Hydroxyalprazolam-D5-TBDMS		386	388	387
α -Hydroxyalprazolam-TBDMS		383	384	381
Triazolam	Halcion®	313	314	342
α -Hydroxytriazolam-TMS		415	417	190

* Suggested internal standard for GC/MS: Prazepam or Oxazepam-D5

** Part number TS-38831

*** Quantitation ion

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25m x 0.25 μ m

26098-1420

Benzodiazepines in Whole Blood for GC or GC/MS Confirmations

Using 100mg 1mL HyperSep Diol Extraction Column (Part Number: 60108-572)

Prepare Sample

To 1mL of pH 6 buffer add internal standards*, add 2mL of whole blood and mix/vortex

Add 5mL of pH 6 buffer

Sonicate with a probe sonifier for ~10 seconds and centrifuge at ~2700rpm for 15 minutes

Condition HyperSep Diol Extraction Column

1 x 3mL ethyl acetate

1 x 3mL MeOH

1 x 3mL DI H₂O

1 x 3mL 0.1M phosphate buffer (pH 6.0)

Apply Sample

Load sample by gravity

Wash Sample

1 x 3mL DI H₂O

1 x 3mL 5% acetonitrile in 0.1M phosphate buffer (pH 6.0)

Dry columns 5 minutes at full vacuum or >10" Hg

1 x 3mL hexane

Elute Benzodiazepines

2 x 3mL ethyl acetate

Dry Eluate

Evaporate to dryness under nitrogen at ~55°C

Add external standards*

Derivatize

Add 100µL acetonitrile and 100µL MTBSTFA w/1% t-BDMCS

Heat for 30 minutes at 70°C

Remove from heat source to cool

Inject 1µL into GC/MS-NCI

NOTE: Do not evaporate MTBSTFA solution

* Suggested internal standards: Diazepam-D5 and Lorazepam-D4.

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25mm x 0.25µm

26098-1420

Benzodiazepine Screen: Blood, Serum, Urine and Tissue for GC-MS

Using 200mg 6mL HyperSep Verify-CX Extraction Column (Part Number: 60108-722)

Prepare Sample

To 1mL of 100mM phosphate buffer (pH= 6) add internal standards*
Add 1mL blood/Urine or 1g of (1:4) tissue homogenate
Mix/vortex
Add 3mL of 100mM phosphate buffer (pH= 6)
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate
Mix/vortex and centrifuge as appropriate

Procedure for Urine

To 1mL of acetate buffer (pH 5.0) containing 5,000 F units/mL β -Glucuronidase
Add internal standards*
To this solution add 1mL of urine
Mix/vortex
Hydrolyze for 3 hours at 65°C
Allow to cool
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Add 3mL of 100mM phosphate buffer (pH 6.0) and mix

Condition HyperSep Verify-CX Extraction Column

1 x 3mL MeOH
1 x 3mL 100mM phosphate buffer (pH 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL of 5% (v/v) acetonitrile in 100mM phosphate buffer (pH6)
Dry column (5 minutes at >10" Hg)
1 x 3mL of hexane
Dry column (5 minutes at >10" Hg)

Elute Benzodiazepines

1 x 3mL ethyl acetate; ammonia (98:2 v/v)
Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°

Derivatize

Add 50 μ L acetonitrile and 50 μ L BSTFA with 1% TCMS**
Heat for 30 minutes at 70°C
Remove from heat source to cool
Inject 1 μ L into GC/MS

Compound	Primary Ion	Secondary	Tertiary
Alprazolam	308	279	204
Alprazolam-D*	513	284	
Alphahydroxyalprazolam	318	396	383
Alphahydroxyalprazolam-D5*	386	401	
Diazepam	256	283	284
Diazepam-D5*	287	289	
Lorazepam	429	430	347
Lorazepam-D4*	433	435	
Nordiazepam	34	342	343
Nordiazepam-D5*	345	347	
Oxazepam	429	313	430
Oxazepam-D5*	435	433	
Temazepam	343	257	283
Temazepam-D5*	348	262	

* Suggested internal standards: Alprazolam-D, Alphahydroxyalprazolam-D5, Diazepam-D5, Lorazepam-D4, Nordiazepam-D5, Oxazepam-D5, Temazepam-D5

** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25mm x 0.25 μ m 26098-1420

Benzodiazepine Screen: Blood, Serum, Urine and Tissue for LC/MS/MS

Using 200mg 6mL HyperSep Verify-CX Extraction Column (Part Number: 60108-722)

Prepare Sample

To 1mL of 100mM phosphate buffer (pH= 6) add internal standards*
Add 1mL blood/Urine or 1g of (1:4) tissue homogenate
Mix/vortex
Add 3mL of 100mM phosphate buffer (pH= 6)
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate
Mix/vortex
Centrifuge as appropriate

Procedure for Urine

To 1mL of acetate buffer (pH=5.0) containing 5,000 F units/mL β -Glucuronidase
Add internal standards*
To this solution add 1mL of urine
Mix/vortex
Hydrolyze for 3 hours at 65°C
Allow to cool
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Add 3mL of 100mM phosphate buffer (pH 6.0) and mix

Condition HyperSep Verify-CX Extraction Column

1 x 3mL MeOH
1 x 3mL 100mM phosphate buffer (pH= 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL of 5% (v/v) acetonitrile in 100mM phosphate buffer (pH6)
Dry column (5 minutes at >10" Hg)
1 x 3mL of hexane
Dry column (5 minutes at >10" Hg)

Elute Benzodiazepines

1 x 3mL ethyl acetate; ammonia (98:2 v/v)
Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°

Reconstitute

Reconstitute sample in 50 μ L of 0.02% formic acid (aqueous)

Instrumental Conditions: LC/MS/MS

Mobile phase 30:70 (acetonitrile:0.02% aq. formic acid)
Flow rate: 0.35mL/min
Column temperature: Ambient
Injection volume: 5 μ L on to MS triple quad

Compound	MRM Transition
Alprazolam	309.1/281.2
Alprazolam-D5*	314.1/286.2
Alphahydroxyalprazolam	325.1/242.9
Alphahydroxyalprazolam-D5*	330.1/302.2
Chlordiazepoxide	300.1/227.0
Diazepam	285.5/192.5
Diazepam*	292.2/198.2
Lorazepam	321.1/275.1
Lorazepam-D4*	325.1/279.0
Nordiazepam	271.1/140.1
Nordiazepam-D5*	275.6/140.1
Oxazepam	287.1/241.1
Oxazepam-D5*	290.2/198.2
Temazepam	301.1/255.1
Temazepam-D5*	306.1/260.1

* Suggested internal standards: Alprazolam-D5, Alphahydroxyalprazolam-D5, Diazepam, Nordiazepam-D5, Oxazepam-D5, Temazepam-D5

Recommended HPLC Column

Part Number

Hypersil GOLD PFP 3 μ m, 150 x 2.1mm 25403-152130

Beta Agonist Analysis

Using 60mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-303)

Compounds

Cimaterol, sulfamonomethoine,
clenbuterol hydrochloride, salbutamol

Sample Preparation

Extract 20g of pig liver sample in acetonitrile, dried and
spiked with standard chloric acid (10mmol) solution
containing 4 agonists

Condition Retain-CX Extraction Column

5mL of methanol
5mL of DI H₂O
5mL of 30mmol/L of chloric acid

Apply Sample

Load 2mL samples

Wash Column

5mL of DI H₂O
5mL of methanol
Dry column (5-10 minutes at >10" Hg/full flow for
positive pressure manifold)

Elute Beta Agonists

5mL of 4% ammonia/methanol

Dry Eluate and Reconstitute

Evaporate to full dryness at room temperature under a
stream of nitrogen

Beta Agonists in Urine for GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM acetate buffer (pH 4.5) add 1mL of urine
Add 2mL of 100mM
Acetate buffer (pH 4.5)
Mix/vortex
Centrifuge as appropriate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 3mL 100mM acetate buffer (pH 4.7)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

2 x 1mL acetone/methanol (1:1) aspirate
Dry column (5 minutes at >10" Hg)

Elute Beta Agonists

1 x 1mL dichloromethane/isopropanol and ammonium
hydroxide (78:20:2)
Collect the eluate at 1 to 2mL/minute (or gravity)

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Derivatization solution: Methaneboronic acid at 5mg/mL
prepared in dry ethyl acetate (use molecular sieve)
Store this solution at -20°C (freezer conditions) until use

Reaction Mixture

Add 100µL 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

Analysis

Inject 1 to 2µL sample (derivatized solution) on to GC/MS

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25mm x 0.25µm

26098-1420

Beta Blockers in Blood, Urine for GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of acetate buffer (pH= 4.5) add 1mL of blood or urine

Add 2mL of acetate buffer (pH= 4.5)

Mix/vortex

Centrifuge as appropriate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH

1 x 3mL DI H₂O

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

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

2 x 1mL acetone/methanol (1:1) aspirate

Dry column (5 minutes at >10" Hg)

Elute Beta Blockers

1 x 1mL dichloromethane/isopropanol/ammonium hydroxide (78:20:2)

Collect the eluate by gravity

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Derivatization solution: Methaneboronic acid at 5mg/mL prepared in dry ethyl acetate (use molecular sieve)

Store this solution at -20°C (freezer conditions) until use

Reaction mixture

Add 100µL 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

Analysis

Inject 1 to 2µL sample on to GC/MS

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25mm x 0.25µm

26098-1420

Caffeine, Theophylline and Theobromine in Blood, Plasma/Serum, and Urine

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM acetic acid add internal standard*

Add 1mL blood, serum/plasma, or urine

Add 2mL of 100mM acetic acid

Mix/vortex and centrifuge as appropriate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH

1 x 3mL DI H₂O

1 x 1mL 100mM acetic acid

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O

1 x 3mL 100mM acetic acid

Dry column (5 minutes at >10" Hg)

Elute Caffeine/Theobromine/Theophylline

1 x 3mL ethyl acetate:methanol (90:10)

Collect eluate at 1 to 2mL/minute

Evaporation

Combine eluates

Evaporate eluates under a gentle stream of nitrogen <40°C

Reconstitute

Reconstitute sample in 1,000µL of 0.1% formic acid (aq)

Inject 20µL on to LC

* Suggested internal standard: 8-Chlorotheophylline

Recommended HPLC Column

Part Number

Hypercarb 3µm, 50 x 2.1mm

35003-052130

Cefoperazone in Water

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

5ppm of cefoperazone in water

Condition Retain PEP Extraction Column

2mL of methanol

2mL of DI H₂O

Apply Sample

Load 10mL samples at 1 to 2mL/minute

Wash Column

1mL of methanol/DI H₂O (5:95, v/v)

Elute Cefoperazone

6mL of methanol

Analysis

Mobile phase: 0.005 M tetrabutyl ammonium phosphate
(pH 3.63)/ACN (70:30, v/v)

Flow: 1mL/minute

Temperature: 30°C

Detection: UV 254nm

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 250 x 4.6mm

25305-254630

Clenbuterol in Solution

Using 60mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-303)

Sample Preparation

Prepare a 10ppm solution of clenbuterol in 20mM
ammonium acetate

Condition Retain-CX Extraction Column

2mL of methanol

2mL of DI H₂O

2mL of 30mM HCl

Apply Sample

Load 1mL samples

Wash Column

1mL of methanol

1mL of DI water

Elute Clenbuterol

1mL of methanol with 4% ammonia

Analysis

Mobile phase: methanol:phosphoric acid (40:60, v/v)

Flow: 1mL/minute

Temperature: 30°C

Detection: UV 244nm

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 250 x 4.6mm

25305-254630

Clonazepam and 7-Aminoclonazepam in Urine for GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample: β -Glucuronidase Hydrolysis

To 2mL of urine, add internal standard(s)* and 1mL of β -Glucuronidase solution

β -Glucuronidase solution contains 5,000 F units/mL
Patella vulgata in 100mM acetate buffer (pH 5.0)

Mix/vortex

Hydrolyze for 3 hours at 65°C

Cool before proceeding

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH

1 x 3mL deionized water

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

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

1 x 2mL deionized water

1 x 2mL 20% acetonitrile in 100mM phosphate
buffer (pH 6.0)

Dry column (5 minutes at >10" Hg)

1 x 2mL hexane

Elute Clonazepam/7-Aminoclonazepam

1 x 3mL ethyl acetate with 2% NH₄OH

Collect eluate at 1 to 2mL/minute

NOTE: Prepare fresh daily

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50 μ L ethyl acetate and 50 μ L MTBSTFA (with 1%
TBDMCS)**

Mix/vortex

React 20 minutes at 90°C

Remove from heat source to cool

NOTE: Do not evaporate MTBSTFA solution

Analysis

Inject 1 to 2 μ L of sample onto GC/MS

For mass spectrometry monitor the following ions:

Compound	Primary Ion***	Secondary	Tertiary
Clonazepam-TBDMS	372	374	326
7-Aminoclonazepam-TBDMS	342	344	399
Clonazepam-D4-TBDMS	376	378	377
7-Aminoclonazepam-D4-TBDMS	346	348	403

* Suggested internal standard for GC/MS: Clonazepam-D4, 7-aminoclonazepam-D4

** Part number TS-48927

*** Quantitation ion

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25mm x 0.25 μ m	26098-1420
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Cyanuric Acid and Melamine in Food Materials for LC/MS/MS

Using 200mg 6mL HyperSep Verify-CX Extraction Column (Part Number: 60108-722)

and 200mg 6mL HyperSep Retain-AX Extraction Column (Part Number: 60107-412)

Prepare Sample

To 1 to 5g of sample add 10 to 25mL of CH₃CN/DI H₂O (50:50)
Shake for 5 minutes
Centrifuge
Transfer 5mL of supernatant to clean glass screw top tube
Add 1mL of 100mM HCl
Add 1mL of CH₂Cl₂
Shake for 5 minutes
Centrifuge
Transfer upper layer to clean glass tube
Add 2mL of DI H₂O to CH₂Cl₂
Shake for 5 minutes
Centrifuge
Add upper layer to previous aqueous portion
Apply to conditioned SPE (CSDAU206 (BCX) column

Condition HyperSep Verify – CX Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O

NOTE: Aspirate at <3 "Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute
Collect effluent for use with HyperSep Retain-AX Column SPE

Wash Column

Wash HyperSep Verify-CX Column
1 x 1mL DI H₂O
Collect wash for use with HyperSep Retain-AX Column
Remove collection tubes from manifold and go to HyperSep Retain-AX section
1 x 3mL 100mM HCl
1 x 1mL CH₃OH
Dry column (5 minutes at >10"Hg)

Elute Melamine

Insert fresh collection tubes into manifold
1 x 2mL of CH₃OH containing 5% NH₄OH
1 x 3mL of CH₃OH containing 5% NH₄OH
Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°C

Reconstitute

Reconstitute sample in 1,000µL of CH₃CN
Add external standard*
Inject 5µL to LC/MS

HyperSep Retain-AX SPE Procedure

Adjust solution from Wash and Elute Steps to pH 7 **

Condition HyperSep Retain-AX Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O

NOTE: Aspirate at <3 "Hg to prevent sorbent drying out

Apply Sample

Load sample which has been adjusted to pH7 at 1 to 2mL/minute

Wash HyperSep Retain – AX Column

1 x 3mL DI H₂O
1 x 1mL CH₃OH
Dry column (just enough to remove residual solvent)

Elute Cyanuric Acid

Insert fresh collection tubes into manifold
1 x 3mL of CH₃OH containing 1% HCl
1 x 2mL of CH₃OH containing 1% HCl
Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°C

Reconstitute

Reconstitute sample in 100µL of mobile phase
Add external standard*
Inject 5µL to LC/MS

Compound	MRM Transition
Melamine	127.1/85.1
2, 4 Diamino 6-hydroxy pyrimidine*	127.1/67.0
Cyanuric Acid	127.8/84.9

* External standard: 2, 4 Diamino 6-hydroxy pyrimidine

** Adjust pH with 100 to 200µL of 5% (v/v) (aq)/NH₄OH

Recommended HPLC Column	Part Number
Hypersil GOLD AX 3µm, 150 x 2.1mm	25503-152130

DHEA, Testosterone, and Epitestosterone in Urine for GC or GC/MS Analysis

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

Pipette 5mL of urine into borosilicate glass test tubes
Add internal standard*, adjust sample pH to 5.5 to 6.5
using concentrated sodium phosphate monobasic
or dibasic

Mix sample

Centrifuge samples at 3,000rpm for 5 minutes

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH

1 x 3mL DI H₂O

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

Apply Sample

Pour supernatant onto column

Allow to flow via gravity

Wash Column

1 x 3mL DI H₂O

Dry column (10 minutes at >10mm Hg)

Elute Steroids

1 x 3mL of CH₃OH

Collect at 1 to 2mL/minute

Enzymatic Hydrolysis

Dry eluate under a stream of nitrogen; Add 2mL of
200mM phosphate buffer (pH 7.0) and 250 units
of β -glucuronidase

Mix/vortex and allow to incubate at 50°C for 1 hour

Cool sample, cap and adjust the pH to 10 to 11 using a
1:1 mixture of NaHCO₃/Na₂CO₃

Additional Clean-up

Add 5mL of n-butyl chloride to each sample. The tubes and
shake vigorously for 10 minutes and then centrifuge at
3,000rpm for 5 minutes. Transfer the organic layer to
clean test tubes and dry under a stream of nitrogen.
Place dried sample in a desiccator and further dry
under vacuum for 30 minutes.

Derivatize

Add 50 μ L of MSTFA/NH₄I/dithioerythritol
(1,000:2:5, V/W/W) and incubate at 70°C for
20 minutes

Centrifuge sample at 3,000rpm for 1 minute and transfer
directly to GC injector vials

Quantitate

Inject 1 to 2 μ L onto GC/MS

For mass spectrometry monitor the following ions:

Compound	Primary Ion**	Secondary
Testosterone	432	417
Epitestosterone	432	417
DHEA	432	417
16 α Hydroxytestosterone*	520	259

* Suggested internal standard at 20ng/mL: 16 α Hydroxytestosterone

** Quantitation ion

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25m x 0.25 μ m	26098-1420
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Doxepin in Rat Serum

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

- Mix 10mL of doxepin aqueous solution (20mg/L) and 30mL of rat serum into a 100mL vessel
- Dilute to 100mL with 0.5% ammonia solution to give a 2ppm solution

Condition Retain PEP Extraction Column

- 2mL of methanol
- 2mL of DI H₂O

Apply Sample

- Load 2mL samples

Wash Column

- 2mL of 0.5% ammonia solution containing 5% methanol

Elute Doxepin

- 2mL of 1% acetic acid in methanol

Dry Eluate and Reconstitute

- Evaporate to full dryness at room temperature under a stream of nitrogen
- Reconstitute the sample to 1mL with ACN:20mmol sodium acetate (pH 4) (40:60, v/v)

Analysis

- Mobile phase: ACN:20mmol sodium acetate (pH 4) (40:60, v/v)
- Flow: 1.0mL/minute
- Injection: 10µL
- Temperature: 30°C
- Detection: 290nm

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 250 x 4.6mm

25005-254630

Drug Analysis in Serum

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Condition HyperSep Retain – PEP Column

- 1 x 3mL CH₃OH
- 1 x 3mL H₂O

Apply Sample

- Load spiked serum sample onto SPE cartridge

Wash Column

- 1 x 3mL H₂O
- 1 x 3mL 5% CH₃OH

Elution

- 1 x 3mL CH₃OH

Results

Analytes	Recovery (%)
Dexamethasone	97.9
Ethinylestradiol	96.3
Hydrocortisone	74.0
Triamcinolone	71.9
Levonorgestrel	93.9
Ganciclovir	54.1
Prednisone Acetate	98.6
Cefalexin	58.6
Cefradine	45.6

Gabapentin in Blood, Plasma/Serum for GC or GC/MS Analysis

Using 100mg 1mL HyperSep C18 (Part Number: 60108-302)

Prepare Sample

To 500µL of 20% acetic acid add internal standard*
Mix/vortex
Add 500µL of blood, plasma/serum
Mix/vortex
Centrifuge as appropriate

Condition HyperSep C18 Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM HCL

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 3mL ethyl acetate
1 x 3mL hexane
Dry column (5 minutes at >10" Hg) or until column is dry

Elution

1 x 1mL 2% NH₄OH in CH₃OH

Dry Eluate

Evaporate to dryness at <40°C

Derivatization

Add 50µL of ethyl acetate and 50µL of BSTFA (1% TCMS)** and 50µL ethyl acetate
Cap and heat at 70°C for 30 minutes
Remove and allow to cool

Quantitate

Inject 1 to 2µL onto GC/MS

Compound	Primary	Secondary	Tertiary
Gabapentin-TMS	210	225	182
Gabapentin-D10-TMS*	220	235	192

* Internal standard: 1-aminomethyl-1-cycloheptyl acetic acid (FID): Gabapentin-D10 (GC/MS)

** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25mm x 0.25µm 26098-1420

Gabapentin in Blood, Plasma/Serum for LC/MS Analysis

Using 200mg 6mL HyperSep Verify-CX Extraction Column (Part Number: 60108-722)

Prepare Sample

To 0.2 to 0.5mL of sample add 1mL of acetone
(dropwise) whilst vortexing
Add internal standard*
Mix/vortex and centrifuge as appropriate
Transfer organic phase to clean tube
Evaporate to dryness
Add 3mL of 100mM HCL
Mix/vortex and centrifuge as appropriate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM HCL

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 3mL ethyl acetate
1 x 3mL hexane
Dry column (10 minutes at >10" Hg)

Elute Gabapentin

1 x 3mL CH₃OH containing 2% NH₄OH
Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°
Dissolve residue in 100µL CH₃OH
Inject 5µL of sample on to LC/MS

* Suggested internal standards: Gabapentin-D10, Aminocyclohexane-propionic acid

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 2.1mm 25003-152130

Ketotifen Fumarate in Solution

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

Dilute 50mg of ketotifen fumarate in 50mL of 0.5% ammonia aqueous solution

Condition Retain PEP Extraction Column

2mL of methanol

2mL of DI H₂O

Apply Sample

Load 2mL samples

Wash Column

2mL of DI H₂O

Elute Ketotifen Fumarate

2mL of methanol

Dry Eluate and Reconstitute

Evaporate to full dryness at room temperature under a stream of nitrogen

Reconstitute sample in suitable solvent for analysis

Lovastatin in Water

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

Sample 1: 2ppm lovastatin in 10% acetonitrile aqueous solution

Sample 2: 0.2ppm lovastatin in 1% acetonitrile aqueous solution

Condition Retain PEP Extraction Column

2mL of methanol

2mL of DI H₂O

Apply Sample

Load 10mL samples at 1 to 2mL/minute

Wash Column

1mL of DI H₂O

Elute Lovastatin

4mL of acetonitrile

Dry Eluate and Reconstitute

Evaporate to full dryness under a stream of nitrogen

Reconstitute the sample to 1mL with methanol:1% formic acid solution (85:15, v/v)

Analysis

Mobile phase: methanol:1% formic acid solution (85:15, v/v)

Flow: 1mL/minute

Temperature: 30°C

Detection: UV 230nm

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 150 x 4.6mm

25305-154630

Methylmalonic Acid from Serum or Plasma for GC/MS Analysis

Using 500mg 6mL HyperSep SAX Extraction Column (Part Number: 60108-434)

Prepare Sample

Add 100µL of internal standard D3-MMA and 1mL of acetonitrile to 1mL of plasma or serum

Mix/vortex for 20 seconds

Centrifuge for 5 minutes at 2,000rpm

Condition Extraction Column

1 x 3mL CH₃OH

1 x 3mL DI H₂O

Apply Sample

Decant supernatant onto SPE column

Wash Column

1 x 10mL of DI H₂O

Dry with vacuum for 3 minutes

1 x 5mL of CH₃OH

Dry with vacuum for 3 minutes

1 x 2mL of MTBE*

Dry with vacuum for 3 minutes

Elute Methylmalonic Acid

1 x 5mL of 3% formic acid in MTBE, collect at 1 to 2mL/min

Dry Eluate

Dry under a stream of nitrogen at <35°C

Derivatize

Reconstitute with 25µL of MSTFA + 1% TMCS and 25µL ethyl acetate

Heat for 20 minutes at 60°C

Quantitate

Inject 1 to 2µL onto GC/MS

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25mm x 0.25µm

26098-1420

Nadifloxacin in Solution

Using 60mg 3mL HyperSep Retain-AX Extraction Column (Part Number: 60107-403)

Sample Preparation

Prepare a 5ppm sample in 50mM phosphate buffer
pH 7.4

Condition Retain-AX Extraction Column

1mL of methanol
1mL of 2N NaOH
1mL of DI H₂O

Apply Sample

Load 5mL samples at 1 to 2mL/minute

Wash Column

1mL of 5% ammonia aqueous solution
1mL of methanol

Elute Nadifloxacin

3mL of methanol with 4% acetic acid

Analysis

Mobile phase: acetonitrile:1mM KH₂PO₄ (pH 3)
(40:60, v/v)
Flow: 1mL/minute
Temperature: 30°C
Detection: UV 254nm

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 150 x 4.6mm

25005-154630

Nicotine and Continine in Urine or Serum for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 2mL of 100mM phosphate buffer (pH =6.0) add
internal standards*
Add 2mL of urine or serum
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or
dibasic sodium phosphate
Mix/vortex
Centrifuge as appropriate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3"Hg to prevent sorbent drying

Apply Sample

Load at 1mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 2mL 200mM HCl
Dry column (5 minutes at >10"Hg)
1 x 2mL Hexane

Wash Column

Remove rack of collection tubes to rewash columns
1 x 3mL CH₃OH
Dry column, (5 minutes at >10"Hg)

Elute Continine and Nicotine

Replace rack of collection tubes
1 x 3mL CH₂Cl₂/IPA/NH₄OH (78:20:2)
Collect eluate at 1mL/minute

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

Concentrate

Evaporate to dryness at <40°C
Take care not to over-heat or over evaporate
Reconstitute with 100µL ethyl acetate

Quantitate

Inject 1 to 2µL onto GC/MS
Monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
Nicotine	84	133	162
Nicotine-D4*	88	137	166
Cotinine	98	119	176
Cotinine-D3*	101	122	179

* Suggested internal standard: Nicotine-D4, Cotinine-D3

** Quantitation Ion

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25m x 0.25µm

26098-1420

Olanzapine in Whole Blood

Using 200mg 3mL HyperSep Cyano Extraction Column (Part Number: 60108-747)

Prepare Sample

To 1mL of DI H₂O add internal standard*
Add 1mL blood
Add 8mL of DI H₂O
Mix/vortex and centrifuge as appropriate

Condition HyperSep Cyano Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL 1% acetic acid (aq)
Dry column (5 minutes at >10" Hg)

Elute Olanzapine

2 x 3mL 1% acetic acid in CH₃OH
Collect eluate at 1 to 2mL/minute

NOTE: Prepare elution solvent daily

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°C

Reconstitute

Reconstitute sample in 100µL 0.1% trifluoroacetic acid (aq)
Inject 50µL on to LC-UV (260nm)

* Suggested internal standard: Prazepam

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 4.6mm

25003-154630

Oleic Acid and its Metabolites in Blood Plasma

Using 200mg 3mL HyperSep Retain-AX Extraction Column (Part Number: 60107-401)

Prepare Sample

Add 3% phosphoric acid to sample to 500µL of sample

Condition HyperSep Retain-AX Extraction Column

1 x 1mL CH₃OH
1 x 1mL H₂O

Apply Sample

Load 0.5mL of sample

Wash Column

1 x 1mL 1%CH₃CO₂S
1mL CH₃OH

Elute Compound

1 x 1mL ACN(1% formic acid)

Quantitation

Inject sample on to LC/MS/MS
Mobile phase: ACN:3mmol/L ammonium acetate (85:15)

Compound	MRM Transition
Oleic acid	281.2/281.2
Oleic acid metabolite	315.2/315.2
Internal label C17	269.2/269.2 (internal label C17)

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 150 x 4.6mm

25005-154630

Opiates in Human Urine – Propyl Derivatives for GC or GC/MS Confirmations

Using 200mg 6mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample Acid Hydrolysis of Glucuronides

To 2mL of urine add internal standard(s)* and 400µL concentrated HCl
Add 200µL 10% hydroxylamine solution in DI H₂O
Mix/vortex
Heat to 90°C for 40 minutes in a heating block, or an autoclave for 15 minutes on a liquid cycle
Cool before proceeding
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Add 500µL 50% ammonium hydroxide
Mix/vortex
Adjust sample pH 5 to 6 by drop wise addition with 50% ammonium hydroxide

Prepare Sample-enzymatic Hydrolysis of Glucuronides

To 2mL of urine, add internal standard(s), and 1mL of β-Glucuronidase solution
β-Glucuronidase solution contains 5,000 F units/mL Patella vulgata in 100mM acetate buffer (pH 5.0)
Hydrolyze for 3 hours at 60°C
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Adjust sample pH to 5 to 6 with 1.0 N NaOH

Condition Clean Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O then aspirate
1 x 3mL 100mM acetate buffer (pH 4.5) then aspirate
1 x 3mL CH₃OH then aspirate
Dry column (5 minutes at >10" Hg)

Elute Opiates

1 x 3mL ethyl acetate/isopropanol/ammonium hydroxide (84:12:4)

Dry Eluant

Evaporate to dryness at <40°C

Derivatize

Add 200µL 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 50µL of ethyl acetate/methanol (70:30)

Quantitate

Inject 1 to 2µL onto GC/MS
For mass spectrometry monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
Hydrocodone	299	242	214
Codeine	355	282	229
Codeine-D3*	358	285	232
Oxycodone	371	314	298
Hydromorphone	285	341	228
6-Acetylmorphine	327	268	383
Oxymorphone	357	300	413
Morphine	341	268	397
Morphine-D3*	344	271	400

* Suggested internal standard for GC/MS: Codeine-D3 and Morphine-D3

** Quantitation ion

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25m x 0.25µm	26098-1420
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Opiates in Urine-oxime TMS Procedure for GC OR GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample Acid Hydrolysis of Glucuronides

To 2mL of urine add internal standard(s)* and 400µL concentrated HCl
Add 200µL 10% hydroxylamine solution in DI H₂O
Mix/vortex
Heat to 90°C for 40 minutes in a heating block, or an autoclave for 15 minutes on a liquid cycle
Cool before proceeding
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Add 500µL 50% ammonium hydroxide
Mix/vortex
Adjust sample pH 5 to 6 by drop wise addition with 50% ammonium hydroxide

Prepare Enzyme Hydrolysis of Glucuronides

To 2mL of urine add internal standard(s)* and enzyme preparation in buffer
Mix/vortex
Heat to 60°C for sufficient time in a heating block (depends on analytes and enzyme)
Add 200µL 10% hydroxylamine solution
Heat to 60°C for 30 minutes in a heating block
Adjust pH to 5 to 6
Centrifuge for 10 minutes at 2,000rpm and discard pellet

Condition Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 3mL 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 3mL 100mM acetate buffer (pH 4.5)
1 x 3mL CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Opiates

1 x 3mL CH₂Cl₂/IPA/NH₄OH (76:20:4)
Collect eluate at 1 to 2mL/minute

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 100µL ethyl acetate and 100µL BSTFA (with 1% TMCS)**
Overlay with N₂ and cap
Mix/vortex
React 45 minutes at 70°C in a heat block
Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution

Quantitate

Inject 1 to 2µL onto GC/MS
For mass spectrometry monitor the following ions:

Compound	Quant Ion	Secondary	Tertiary
Meperidine-D4	251	222	250
Meperidine	247	218	246
Normeperidine-D4 TMS*	308	280	309
Normeperidine TMS*	305	276	304
Tramadol TMS	335	245	290
O-Desmethyiltramadol TMS	393	378	303
N-Desmethyiltramadol TMS	393	378	116
Pentazocine TMS	357	342	289
Codeine-D3 TMS*	374	359	346
Codeine-D6 TMS*	377	349	316
Codeine TMS	371	356	343
Norcodeine TMS	429	414	356
Dihydrocodeine TMS	373	315	358
Morphine-D3 TMS*	432	417	404
Morphine-D6 TMS*	435	420	404
Morphine TMS	429	414	401
Normorphine TMS	487	472	414
Diacetylmorphine	369	327	268
Hydrocodone Oxime-D3 TMS	389	300	374
Hydrocodone Oxime-D6 TMS	392	303	377
Hydrocodone Oxime TMS	386	297	371
Hydromorphone Oxime-D3 TMS	447	432	358
Hydromorphone Oxime TMS	444	429	355
Oxycodone Oxime-D3 TMS	477	462	420
Oxycodone Oxime-D6 TMS	480	465	420
Oxycodone Oxime TMS	474	459	417
Oxymorphone Oxime-D3 TMS	535	520	290
Oxymorphone Oxime TMS	532	517	287

* Suggested internal standards for GC/MS: D4-Meperidine, D4-Normeperidine, D3-Codeine, D3-Morphine D6-Hydrocodone D6-Oxycodone. Suggest trying D6-Codeine, and D6-Morphine for lowest LOD/LOQ.

** Part number TS-38831

Opiates in Urine for GC/MS Confirmations

Using 200mg 6mL HyperSep Retain-CX Extraction Column (Part Number: 60107-314)

Sample Preparation (Enzymatic Hydrolysis)

To 1mL of urine add internal standard(s) and 1.0mL β -Glucuronidase solution. (β -Glucuronidase solution contains 5,000 F units/mL *Patella Vulgata* in 100mM acetate buffer, pH 5.0). Hydrolyze for 3 hours at 60°C.

Cool, then centrifuge for 10 minutes at high speed and discard pellet

Adjust pH to 6.0 $\pm$ 0.5 with 1.0N NaOH

NOTE: For unconjugated (free) opiates; to 1mL urine, add internal standard(s) and 1mL 100mM phosphate buffer (pH 6.0). Proceed to next step.

Apply Sample

Load at a rate of 1 to 2mL/min

Was Column

1 x 1mL DI H₂O

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

1 x 1mL MeOH

Dry column (3 minutes at >10" Hg)

Elute Opiates

2 x 0.5mL CH₂Cl₂/IPA/NH₄OH (78/20/2), collect eluate at 1 to 2mL/min

Evaporate eluate to dryness at <40°C

Derivatization

Add 50 μ L ethyl acetate and 50 μ L BSTFA with 1% TMCS**, then cap, mix/vortex

React for 20 minutes at 70°C, allow to cool

NOTE: Do not evaporate BSTFA solution

Analyze

Inject 1 to 2 μ L onto GC/MS

For mass spectrometry monitor the following ions:

Analyte (TMS)	Target (Quantitation) Ion	Qualifier Ions
Codeine-TMS	371	234, 343
Codeine-D6-TMS*	377	237, 349
Morphine-TMS	429	401, 414
Morphine-D6-TMS*	435	404, 420
6-Acetylmorphine-TMS	399	400, 340
6-Acetylmorphine-D6-TMS	405	406, 343

* Suggested internal standards: Codeine-D6-TMS, Morphine-D6-TMS

** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25m x 0.25 μ m

26098-1420

Paroxetine in Blood, Plasma/Serum and Urine for LC/MS/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM phosphate buffer (pH= 6) add internal standards*

Add 1mL whole blood, serum/plasma or urine

Add 2mL of 100mM phosphate buffer (pH= 6)

Mix/vortex and centrifuge as appropriate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH

1 x 3mL DI H₂O

1 x 3mL 100mM phosphate buffer (pH=6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O

1 x 3mL 100mM acetic acid

1 x 3mL CH₃OH

Dry column (5 minutes at >10" Hg)

Elute Paroxetine

1 x 3mL Ethyl acetate:acetonitrile:ammonium hydroxide (78:20:2)

Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°C

Dissolve residue in 100 μ L CH₃OH

Injection Volume: 5 μ L onto LC/MS triple quad

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

* Suggested internal standard: Paroxetine-D6

Recommended HPLC Column

Part Number

Hypersil GOLD C8 3 μ m, 50 x 4.6mm

25203-054630

Propranolol in Rat Serum

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

- Mix 10mL of Propranolol aqueous solution (100mg/L) and 30mL of rat serum in to a 100mL vessel
- Dilute to 100mL with 0.5% ammonia solution to give a 10ppm solution

Condition Retain PEP Extraction Column

- 2mL of methanol
- 2mL of DI H₂O

Apply Sample

- Load 2mL samples

Wash Column

- 2mL of 0.5% ammonia solution containing 5% methanol

Elute Propranolol

- 2mL of 1% acetic acid in methanol

Dry Eluate and Reconstitute

- Evaporate to full dryness at room temperature under a stream of nitrogen
- Reconstitute the sample to 1mL with ACN:20mmol sodium acetate (pH 4) (30:70, v/v)

Analysis

- Mobile phase: ACN:20mmol sodium acetate (pH 4) (40:60, v/v)
- Flow: 1.0mL/minute
- Injection: 10µL
- Temperature: 30°C
- Detection: UV 290nm

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 250 x 4.6mm

25005-254630

Salbutamol in Solution

Using 60mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-303)

Sample Preparation

- Prepare a 10ppm solution of salbutamol in 20mM ammonium acetate

Condition Retain-CX Extraction Column

- 2mL of methanol
- 2mL of DI H₂O
- 2mL of 30mM HCl

Apply Sample

- Load 1mL samples

Wash Column

- 1mL of methanol
- 1mL of DI water

Elute Salbutamol

- 1mL of methanol with 4% ammonia

Analysis

- Mobile phase: methanol:phosphoric acid (40:60, v/v)
- Flow: 1mL/minute
- Temperature: 30°C
- Detection: UV 244nm

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 250 x 4.6mm

25305-254630

Sertraline and Desemethylsertraline in Blood, Plasma/Serum for HPLC Analysis

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 4mL DI H₂O add 2mL of 100mM phosphate buffer (pH= 6.0), to this add internal standard*
Add 1mL of blood, plasma/serum or urine
Mix/vortex
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH= 6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load at 1mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 1mL 100mM acetic acid
1 x 3mL CH₃OH
Dry column (5 minutes at >10"Hg)

Elute

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

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

Dry Eluate

Evaporate to dryness at <40°C

Quantitate

Reconstitute with 200µL acetonitrile:DI H₂O (1:3)
Mix/vortex vigorously for 30 seconds
Inject 100µL onto LC at wavelength 235nm
Mobile phase: 0.25 M potassium phosphate (pH 2.7) containing 30% CH₃CN
Flow rate: 2mL/minute

* Suggested internal standard: Desmethylsertraline

Tacrolimus, Cyclosporin and Rapamycin in Whole Blood

Using 200mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60107-212)

Prepare Sample

Add 50mL whole blood and 50mL of 0.1 M ZnSO₄ to a centrifuge tube
Mix/vortex
Add 500mL methanol and internal standards*
Mix/vortex
Centrifuge
Transfer supernate to a clean tube, add 500mL DI H₂O
Mix/vortex

Condition HyperSep Retain PEP Extraction Column

1 x 2mL CH₃OH
1 x 2mL DI H₂O

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Decant the sample onto the column
Load at 1 to 2mL/minute

Wash Column

1 x 2mL DI H₂O
Dry column (20 minutes at >10"Hg)

Elute Analytes

Add 750mL of ethyl acetate
Collect eluate at 1 to 2mL/minute

Analysis

Inject onto LC system

* Suggested internal standards: Cyclosporin Cyclosporin-D, Tacrolimus Ascomycin, and Rapamycin Desmethoxyrapamycin

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 4.6mm

25003-154630

Tricyclic Antidepressants in Plasma/Serum for HPLC

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 2mL of 100mM phosphate buffer (pH= 6.0) add internal standard*
Add 1mL of plasma/serum
Mix/vortex
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH= 6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load at 1mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 1mL 100mM acetic acid
1 x 3mL CH₃OH
Dry column (5 minutes at >10"Hg)

Elute

1 x 3mL CH₂Cl₂/IPA/NH₄OH (78:20:2)
Collect eluate at 1mL/minute or use gravity flow

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

Dry Eluate

Evaporate to dryness at <40°C

Quantitate

Reconstitute with 200µL ethyl acetate/DI H₂O (1:3)
Mix/vortex vigorously for 30 seconds
Inject 100µL onto HPLC

* Suggested internal standards: Trimipramine, Protriptyline

Acephate in Water

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

Prepare a 10mL, 10ppm sample of acephate in ammonium sulphate aqueous solution (w/w 20%)

Condition Retain PEP Extraction Column

2mL of methanol

2mL of DI H₂O

Apply Sample

Load 4mL samples at 1 to 2mL/minute

Wash Column

1mL of DI H₂O

Elute Acephate

5mL of methanol

Dry Eluate and Reconstitute

Evaporate to full dryness under a stream of nitrogen

Reconstitute the sample to 0.5mL with water

Analysis

Mobile phase: ACN/water (40:60, v/v)

Flow: 1mL/minute

Temperature: 30°C

Detection: UV 214nm

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 250 x 4.6mm

25005-254630

Aniline and N,N-Dimethylaniline in Solution

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

Prepare solutions of aniline and N,N-dimethylaniline in DI H₂O at concentrations of 200, 20 and 2ppm

Condition Retain PEP Extraction Column

3mL of methanol

3mL of DI H₂O

Apply Sample

Load 3mL samples at 1 to 2mL/minute

Wash Column

1mL of DI H₂O

Elute Aniline and N,N-Dimethylaniline

3mL of methanol/water (95:5, v/v)

Dry Eluate and Reconstitute

Evaporate to full dryness at room temperature under a stream of nitrogen

Reconstitute the sample to 1mL with methanol

Analysis

Mobile phase: methanol/water (50:50, v/v)

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 250 x 4.6mm

25305-254630

Results

Sample Aniline	Concentration (ppm)	Peak area (av) std	Peak area (av) Retain PEP	Recovery (%) Retain PEP
a	200	360251	213975	60
b	20	28374.536686	20672	56
c	2	3563	2870	81

Sample N,N-dimethylaniline	Concentration (ppm)	Peak area (av) std	Peak area (av) Retain PEP	Recovery (%) Retain PEP
a	200	1418380	1398427	98
b	20	174334	159897	92
c	2	19483	18386	94

Atrazine in Water

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

To 10mL of water containing 10ppm atrazine, add
20µL of acetic acid

Condition Retain PEP Extraction Column

2mL of methanol
2mL of DI H₂O

Apply Sample

Load 4mL samples at 1 to 2mL/minute

Wash Column

1mL of DI H₂O

Elute Atrazine

5mL of methanol

Dry Eluate and Reconstitute

Evaporate to full dryness under a stream of nitrogen
Reconstitute the sample to 0.5mL with water

Analysis

Mobile phase: ACN/0.2% acetic acid (10:90, v/v)
Flow: 1mL/minute
Temperature: 30°C
Detection: UV 214nm

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 250 x 4.6mm

25005-254630

Bentazone in Water

Using 500mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60107-206)

Sample Preparation

Adjust the pH of 500mL of water to ≤3 using
0.5 M H₂SO₄

Condition Retain PEP Extraction Column

3mL of tetrahydrofuran
5mL of methanol
5mL of DI H₂O

Apply Sample

Load the 500mL sample at a rate no greater than
5mL/min

Wash Column

5mL of DI H₂O
Dry column (20 minutes at >10" Hg/full flow for positive
pressure manifold)

Elute Bentazone

Use 0.8mL of methanol to replace the residual water in
the PEP packing
Discard the water which passes through the SPE column
Wait for 2 minutes to make sure the methanol infiltrates
the packing material
Elute the column with 3mL of tetrahydrofuran
Collect the eluate and reconstitute to 3mL using
mobile phase

Bentazone in Water

Alternative Derivatization

Using 500mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60107-206)

Sample Preparation

Adjust pH to 3 with H₂SO₄

Condition HyperSep Retain PEP Extraction Column

1 x 5mL THF
1 x 5mL CH₃OH
1 x 5mL H₂O

NOTE: Do not allow the cartridge to dry out

Apply Sample

Load 1 x 500mL H₂O
Elute at 5mL/minute

Wash Column

1 x 5mL H₂O
Dry column (20 minutes under N₂)

Elute

1 x 3mL THF
Concentrate sample to 3mL under N₂

Analysis

Inject onto HPLC

Chlorophenoxy Acid Herbicides in Water

Using 1g 6mL HyperSep C18 Extraction Column (Part Number: 60108-301)

Sample Preparation

Adjust pH of 1L of water sample to pH 1.0 with hydrochloric acid

Condition C18 Extraction Column

10mL of hexane/acetone (50:50)
10mL of acidified methanol (5% HCl in methanol)
10mL of DI H₂O

Apply Sample

Load 1 liter of sample at a rate of 8 to 10mL/minute

Wash Column

10mL of DI H₂O adjusted to pH 1.0 with HCl

Dry Column

Use maximum vacuum pressure for 15 to 30 minutes

Elute Chlorophenoxy Acid Herbicides

10mL of hexane/acetone (50:50)

Concentrate/Evaporate

Add 500µL of a keeper solvent (methanol, DMF, other)
Evaporate to 500µL under a nitrogen stream at room temperature

Injection/Analysis

Reconstitute with 100µL of TCTEF and inject at 1 to 2µL onto GC column

Chlorophenoxy Acid Herbicides Extracted

2,4-D acid
2,4,5-trichloro phenoxy propionic acid (Silvex)
Dicamba
Dinitro-sec-butyl phenol

Chlorophenol in Water

Using 500mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60107-206)

Sample Preparation

Collect 500mL of H₂O
Adjust pH to 2 with H₂SO₄

Condition HyperSep Retain PEP Extraction Column

1 x 5mL CH₃OH
1 x 5mL H₂O

NOTE: Do not allow the cartridge to dry out

Apply Sample

Load 1 x 500mL H₂O
Elute at 5mL/minute

Wash Column

1 x 5mL H₂O
1 x 1mL CH₃OH

Elute

1 x 3mL THF
Concentrate sample to 3mL under N₂

Analysis

Inject onto HPLC

Recommended HPLC Column

Part Number

Hypersil GOLD PFP 3µm, 150 x 4.6mm

25403-154630

2,4-Dichlorophenol in Water

Using 500mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60107-206)

Sample Preparation

Adjust pH to 2 with H₂SO₄

Condition HyperSep Retain PEP Extraction Column

1 x 5mL CH₃OH
1 x 5mL H₂O

NOTE: Do not allow the cartridge to dry out

Apply Sample

Load 1 x 500mL H₂O
Elute at 5mL/minute

Wash Column

1 x 5mL H₂O
1 x 1mL CH₃OH
Dry column (20 minutes under N₂)

Elute

1 x 3mL THF
Concentrate sample to 3mL under N₂

Analysis

Inject onto HPLC

Recommended HPLC Column

Part Number

Hypersil GOLD PFP 3µm, 150 x 4.6mm

25403-154630

2,4-Dichlorophenoxyacetic Acid in Water

Using 500mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60107-206)

Sample Preparation

Adjust the pH of 500mL of water to 1.5 to 2 using
0.5 M H₂SO₄

Condition Retain PEP Extraction Column

3mL of tetrahydrofuran
5mL of methanol
5mL of DI H₂O

Apply Sample

Load the 500mL sample at a rate no greater than
5mL/min

Wash Column

5mL of DI H₂O
Dry column (20 minutes at >10" Hg/full flow for positive
pressure manifold)

Elute 2,4-Dichlorophenoxyacetic Acid

Use 0.8mL of methanol to replace the residual water in
the PEP packing
Discard the water which passes through the SPE column
Wait for 2 minutes to make sure the methanol infiltrates
the packing material
Elute the column with 3mL of THF
Collect the eluate and reconstitute to 3mL using
mobile phase

EPA Method 508 – Analysis of Chlorinated Pesticides, Herbicides and Organohalides

Using 10g 75mL HyperSep C18 Extraction Column (Part Number: 60108-703)

Sample Preparation

1L water collected
Add MgCl₂ (final conc. 10mg/L)

Condition HyperSep C18 Extraction Column

1 x 1mL 1:1 EtAc/CH₂Cl₂
1 x 10mL CH₃OH
1 x 10mL H₂O

Apply Sample

Add 1 x 5mL CH₃OH to sample
Mix
Take 50μL of sample and mix
Load sample at 1 to 2mL/minute

Elute

Insert fresh collection tubes into manifold
1 x 10mL EtAc
1 x 10mL CH₂Cl₂
1 x 3mL EtAc/CH₂Cl₂

Evaporation

Evaporate eluates to 0.8mL under a gentle stream of
nitrogen in a heated water bath 40°C
Add internal standard
Adjust volume to 1mL

Analysis

Add 1 to 2μL onto GC

Recommended GC Column

Part Number

TraceGOLD TG-OCP | 30m x 0.25mm x 0.25μm

26078-1420

EPA Method 535 – Analysis of Chloroacetanilide and Acetamide Herbicide Degradates in Water

Using 500mg 6mL HyperSep Hypercarb Extraction Column (Part Number: 60106-402)

Sample Preparation

250mL water collected

Condition HyperSep Hypercarb Extraction Column

1 x 20mL ammonium acetate/CH₃OH

1 x 30mL H₂O

1 x 3mL H₂O to top of cartridge

Apply Sample

Load sample at 10 to 15mL/minute

Wash Column

1 x 5mL H₂O

Dry column

Elute

Insert fresh collection tubes into manifold

1 x 15mL of ammonium acetate/CH₃OH

Collect eluate at 5mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen in a heated water bath 60 to 70°C

Analysis

To sample add 1mL 5mM ammonium acetate/CH₃OH

Inject onto LC/MS/MS

Compound

MRM Transition

Propachlor OA 206/134

Flufenacet OA 224/152

Propachlor ESA 256/80

Flufenacet ESA 274/80

Dimethenamid OA 270/198

Dimethenamid ESA 320/80

Alachlor OA 264/160

Acetochlor OA 264/146

Alachlor ESA 314/80

Metolachlor OA 278/206

Acetochlor ESA 314/80

Metolachlor ESA 328/80

Dimethachlor ESA (sur) 300/80

Butachlor ESA (IS) 356/80

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 2.1mm

25003-152130

EPA Method 8330B – Explosives and Residue Analysis – Nitroaromatics, Nitroamines, Nitrate Esters

Using 500mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60107-206)

Sample Preparation

Soil

- Add 2.0g of soil into a 25mL glass vial
- Add 2g of sodium sulfate and mix
- Add 0.1mL explosives soil surrogate to all samples, (blanks/spikes)
- Add 0.5mL explosives spike to the LCS, LCSD, matrix spike, and matrix spike duplicate samples
- Add 10mL of ACN
- Mix/vortex 1 minute
- Sonicate for 18 hours (<10°C)
- Centrifuge 15 to 20 minutes
- Remove solvent layer
- Add solvent layer to 5mL (5.0 gram/L) CaCl₂
- Mix and leave to stand for 15 minutes
- Filter through 1µm Teflon filter
- Discard the first 3mL, retain the remainder
- Discard the first 3mL and retain the remainder in an appropriately labeled 12mL vial
- Store in a refrigerator

Aqueous matrices

- Do not concentrate explosives residue to dryness as they may detonate
- 1L of sample water
- Add 5.0mL of methanol and surrogate standards to all samples and blanks
- Add matrix spikes standards to sample replicates

Glass Apparatus Washing

Explosives

- 1 x 5mL ACN
- 1 x 15mL IPA
- 1 x 15mL CH₃OH

Nitramines, Nitroaromatics

- 1 x 5mL ACN
- 1 x 15mL ACN
- Draw solvents through the cartridge under low vacuum

Condition HyperSep Hypercarb Extraction Column

Use an all glass vacuum manifold

Explosives

- 1 x 20mL ACN
- 1 x 20mL ACN
- 1 x 50mL DI H₂O
- 1 x 50mL DI H₂O

Nitramines, Nitroaromatics

- 1 x 15mL ACN
- 1 x 30mL DI H₂O

Apply Sample

- Load sample at 10mL/min
- Dry column under vacuum for 15 minutes

Elute

Explosives

- 1 x 4mL ACN
- Collect eluate at low flow
- Store in freezer

Nitramines, Nitroaromatics

- 1 x 5mL ACN
- Collect eluate at low flow
- Store in freezer

Evaporation

- Evaporate eluates to 0.7mL under a gentle stream of nitrogen <40°C

Analysis

- Add internal standard to 0.7mL sample
- Inject 100µL onto LC/MS
- Flow rate: 0.50mL/minute
- Mobile phase: 50:50 methanol:water

Recommended HPLC Columns

Part Number

Hypersil GOLD 5µm, 250 x 4.6mm	25005-254630
Hypersil GOLD C8 3µm, 150 x 4mm	25203-154030
Hypersil GOLD CN 5µm, 250 x 4.5mm	25805-254630
Betasil Phenyl Hexyl 5µm, 250 x 3mm	73005-253030

Ethametsulfuron in Solution

Using 60mg 3mL HyperSep Retain-AX Extraction Column (Part Number: 60107-403)

Sample Preparation

Prepare a 10ppm solution of nicosulfuron diluted in 2% ammonia aqueous solution

Condition Retain-AX Extraction Column

1mL of methanol

1mL of DI H₂O

Apply Sample

Load 2mL samples

Wash Column

1mL of 2% ammonia hydroxide

1mL of methanol

Elute Ethametsulfuron

2mL of methanol with 2% acetic acid

Analysis

Mobile phase: methanol:2% acetic acid (60:40, v/v)

Flow: 1mL/minute

Temperature: 30°C

Detection: UV 355nm

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 250 x 4.6mm

25305-254630

Glyphosate and Glufosinate by Solid-Phase Anion Exchange Extraction

Using 1g 6mL HyperSep Verify-AX Extraction Column (Part Number: 60108-732)

Sample Preparation

1L H₂O adjusted to pH 6

Condition HyperSep Verify-AX Extraction Column

1 x 5mL CH₃OH

1 x 10mL DI H₂O

Apply Sample

Load sample at 1 to 3mL/minute

Wash Column

1 x 10mL DI H₂O

Dry column (10 minutes at >10" Hg)

Elute

1 x 4mL of 1 mol/L HCl/CH₃OH (4/1)

Add eluant draw through at 1mL/minute

Evaporate

Evaporate under a gentle stream of nitrogen in a water bath heated to 50°C

Analysis

Add 50µL of MTBSTFA* and 50µL of dimethylformamide for derivatization

Sonicate for 2 minutes

Heat to 80°C for 30 minutes

Cool to room temperature

Inject onto GC/MS

* Part number TS-48920

Recommended GC Column

Part Number

TraceGOLD TG-17MS 30m x 0.25mm x 0.25µm

26089-1420

Metal Extraction by SPE

Using HyperSep Aminopropyl, HyperSep Retain-AX, HyperSep SAX
and HyperSep Verify-AX Extraction Columns

Sample Preparation

Prepare sample as appropriate

Condition appropriate HyperSep Extraction Column

NOTE: Column selection depends on volume of sample,
concentration of metal to be extracted

For a 1mL column

1 x 3mL CH₃OH

1 x 3mL H₂O

NOTE: Do not allow the cartridge to dry out

Apply Sample

Load 10 to 50mL of sample water at 1 to 3mL/minute

Wash Column

1 x 10mL H₂O

Dry column (1 minute at >10" Hg)

Elute

Acid

Prepare 100mM nitric acid solution

1 x 3mL of nitric acid (100mM) solution to the column

Flow through at 1 to 3mL/minute

Dilute eluant with H₂O for analysis

Base

1 x 3mL of triethylamine to the column

Flow through at 1 to 3mL/minute

Dilute eluant with H₂O for analysis

Analysis

Prepare calibration curves for use with atomic absorption
(AA) or Inductively

Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)
using appropriate metals standards

4'4'-Methylenedianiline in Serum

Using 100mg 1mL HyperSep C18 Extraction Column (Part Number: 60108-302)

Prepare Sample

None

Condition Verify-CX Extraction Column

3mL of methanol

3mL of DI H₂O

Apply Sample

Load sample at 1mL/minute

Wash Column

1mL of DI H₂O

Elute 4'4'-Methylenedianiline

0.25mL of methanol containing 1 M ammonium hydroxide

Analysis

Inject 10µL onto HPLC system

Extracted 100 µg/mL sample

Retention time: 3.162 seconds

Mobile phase: methanol/water (50:50)

Flow rate: 1.2mL/min

Injection volume: 10µL

Wavelength: UV 254nm

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 250 x 4.6mm

25005-254630

Miticides and Agrochemicals in Honey Bees, Wax and Pollen

Using QuEChERS Methodology (Part Number: 60105-210, 60105-213, 60105-230 and 60105-308)

Sample Preparation

Wrap sample in aluminum foil
Store on dry ice at -80°C
Beebread and brood – remove from combs store with beeswax at -20°C until processed
Place 3 grams of sample into a 50mL centrifuge tube
Add 100µL of control solution
Add 1 x 27mL 44% DI water, 55% acetonitrile and 1% glacial acetic acid
Add 1 x 100µL of internal standard
For beebread, reduce particle size by use of a high speed disperser for 1 minute
For comb wax melt the sample at 80°C in a water bath followed by cooling to room temperature
Add the contents of 60105-210
Shake
Centrifuge for 1 minute

Clean-Up

Transfer 1mL of supernatant to 60105-230 microcentrifuge tube
Mix/vortex 1 minute
Transfer supernatant to an autosampler vial for LC analysis

Analysis

LC

LC analysis required for neonicotinoids, polar pesticides and their metabolites

GC

Prepare a dual layer solid-phase extraction cartridge 60105-308 by adding about 80mg of anhydrous magnesium sulfate to the top frit
Add 1 x 4.0mL of acetone/toluene (7:3 v:v)
Elute solvent to waste under vacuum (60104-230)
Add 1 x 2mL of supernatant to the top of the cartridge
Elute cartridge using 3 to 4mL of acetone/toluene (7:3, v:v)
Evaporator sample at 50°C
Dry eluate to 0.4mL
Inject into GC

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 2.1mm

25003-152130

Nicosulfuron in Solution

Using 60mg 3mL HyperSep Retain-AX Extraction Column (Part Number: 60107-403)

Sample Preparation

Prepare a 10ppm solution of nicosulfuron in DI H₂O

Condition Retain-AX Extraction Column

1mL of methanol
1mL of DI H₂O

Apply Sample

Load 2mL samples

Wash Column

1mL of 2% ammonia hydroxide
1mL of methanol

Elute Nicosulfuron

2mL of methanol with 2% acetic acid

Analysis

Mobile phase: methanol:2% acetic acid (60:40, v/v)
Flow: 1mL/minute
Temperature: 30°C
Detection: UV 355nm

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 250 x 4.6mm

25305-254630

Nitroanilines in Solution

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

Prepare solutions of 2-nitroaniline and 4-nitroaniline in 0.1% ammonia aqueous at concentrations of 20, 2 and 0.2ppm

Condition Retain PEP Extraction Column

3mL of methanol
3mL of DI H₂O

Apply Sample

Load 3mL samples at 1 to 2mL/minute

Wash Column

1mL of 0.1% ammonia aqueous solution

Elute Nitroanilines

3mL of methanol/1% formic acid (95:5, v/v)

Dry Eluate and Reconstitute

Evaporate to full dryness at room temperature under a stream of nitrogen

Reconstitute the sample to 1mL with methanol

Analysis

Mobile phase: methanol/1% formic acid (10:90, v/v)

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 250 x 4.6mm

25305-254630

Results

Sample 4-nitroaniline	Concentration (ppm)	Peak Area (av) std	Peak Area (av) Retain PEP	Recovery (%) Retain PEP
a	20	296740.5	270652	91
b	2	28374.5	31517	111
c	0.2	2830.5	2947	104

Sample 2-nitroaniline	Concentration (ppm)	Peak Area (av) std	Peak Area (av) Retain PEP	Recovery (%) Retain PEP
a	20	464657.5	456445	98
b	2	45113.5	46537	103
c	0.2	3489.5	4234	121

Nitrobenzene in Water

Using 500mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60107-206)

Sample Preparation

Ensure that a 497.5mL sample of water is pH neutral
To this sample, add 2.5mL of methanol

Condition Retain PEP Extraction Column

3mL of hexane
5mL of methanol
5mL of DI H₂O

Apply Sample

Load the 500mL sample at a rate no greater than 5mL/min

Wash Column

5mL of DI H₂O
Dry column (20 minutes at >10"Hg/full flow for positive pressure manifold)

Elute Nitrobenzene

Use 0.8mL of acetone to replace the residual water in the PEP packing

Discard the water which passes through the SPE column
Wait for 2 minutes to make sure the acetone infiltrates the packing material

Connect the Retain PEP column to a column packed with 5g of anhydrous sodium sulphate which has been washed with 3mL of acetone, 3mL of hexane and 3mL acetone

Elute the column series with 10mL of hexane/acetone (90:10, v/v)

Collect the eluate and concentrate to 1mL with nitrogen at 40°C

Nitrobenzene in Water

Alternative Derivatization

Using 500mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60107-206)

Sample Preparation

Add CH₃OH to water sample to provide a 0.5% solution
Adjust pH to 7

Condition HyperSep Retain PEP Extraction Column

1 x 3mL n-hexane
1 x 5mL CH₃OH

NOTE: Do not allow the cartridge to dry out

Apply Sample

Load 1 x 10mL H₂O
Elute at 5mL/minute

Wash Column

1 x 10mL H₂O
Dry column (20 minutes at >10"Hg)

Elute

1 x 10mL n-hexane/acetone (90:10, V/V)
Concentrate sample to 1mL

Analysis

Inject onto HPLC

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 4.6mm

25003-154630

Organochlorine Pesticides and PCB Extraction

Using 10g 75mL HyperSep C18 Extraction Column (Part Number: 60108-703)

Sample Preparation

Adjust pH of sample to 2 using sulfuric acid
Add 1 x 5mL CH₃OH

Condition HyperSep C18 Extraction Column

1 x 10mL methylene Chloride
1 x 10mL acetone
1 x 10 CH₃OH
1 x 20mL DI H₂O

NOTE: Do not allow the cartridge to dry out

Apply Sample

Load sample at 1 to 3mL/minute

Wash Column

1 x 5mL acetone
Dry column (1 minute at >10"Hg)

Elute

3 x 10mL CH₃Cl
Dry the extract by passing it through anhydrous sodium sulfate
Rinse the collection device with CH₃Cl
Add the solvent to the sodium sulfate

Analysis

Concentrate the extract
Analyze as appropriate via GC/MS

Recommended GC Column

Part Number

TraceGOLD TG-OCP I 30m x 0.25mm x 0.25µm

26078-1420

Clean-Up of Organochlorine Pesticides and PCB Extracts

Using 1g 6mL HyperSep Florisil Column (Part Number: 60108-431)

Sample Preparation

Add hexane to sample

Condition HyperSep Florisil Extraction Column

1 x 9mL 90:10 hexane/acetone

Apply Sample

Load 2mL of sample at 1 to 2mL/minute

Elute

1 x 9mL of 90:10 hexane/acetone
Collect eluate at 1 to 2mL/minute

Evaporate

Evaporate eluates to 1mL under a gentle stream of nitrogen <40°C

Reconstitute

Reconstitute sample to a final volume of 2mL with hexane

Pesticides in Water

Using 1g 6mL HyperSep C18 Extraction Column (Part Number: 60108-301)

Sample Preparation

None

Condition C18 Extraction Column

10mL of hexane/acetone (50:50)

10mL of methanol

10mL of DI H₂O

Apply Sample

Load 1 liter of sample at a rate of 8 to 10mL/minute

Wash Column

20mL of DI H₂O

Dry Column

Use maximum vacuum pressure for 15 to 30 minutes

Elute Chlorophenoxy Acid Herbicides

10mL of hexane/acetone (50:50)

Concentration/Evaporation

Add 500μL of a keeper solvent (methanol, DMF, other)

Evaporate to 500μL under a nitrogen stream at room temperature

Pesticides Extracted

α-hexachlorocyclohexane	4,4'-DDE
Lindane	Dieldrin
β-hexachlorocyclohexane	Endrin
Heptachlor	4,4'-DDD
δ-hexachlorocyclohexane	Endosulfan II
Aldrin	4,4'-DDT
Heptachlor	Endrin aldehyde
Endosulfan 1	Enrin sulfate

GC/MS Determination of Phenols in Drinking Water

Using 500mg 6mL HyperSep Retain PEP Extraction Column (Part Number: 60108-732)

Sample Preparation

Collect 1L of H₂O

Adjust pH to 2 with 6N HCl

Condition HyperSep Retain PEP Extraction Column

1 x 3mL CH₃Cl

1 x 3mL CH₃OH

1 x 3mL 0.05N HCl

NOTE: Do not allow the cartridge to dry out

Apply Sample

Load 1L of water sample at 20mL/minute

Dry column for 10 to 15 minutes

Wash Column

1 x 10mL H₂O

Dry column (1 minute at >10"Hg)

Elute

1 x 10mL CH₃Cl

1 x 3mL CH₃Cl

Concentrate the extract to 0.9mL in water bath (40°C) under a gentle stream of nitrogen

Analysis

Adjust final volume to 1.0mL with CH₃Cl

Analyze the extract with GC/MS

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25mm x 0.25μm

26098-1420

Phenols in Tap Water

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

To a sample of tap water add formic acid 1% and 0.5 to 1ppm phenols internal standard

Condition Retain PEP Extraction Column

2mL of methanol
2mL of 1% formic acid

Apply Sample

Load 10mL samples at 1 to 2mL/minute

Wash Column

1mL of 1% formic acid

Elute Phenols

2mL of methanol

Dry Eluate and Reconstitute

Evaporate to full dryness under a stream of nitrogen
Reconstitute the sample to 1mL with methanol/1% formic acid (1:1)

Analysis

Mobile phase: methanol/formic acid (1:1 v/v)

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 250 x 4.6mm

25305-254630

Results

	Standard	Blank	Standard (n=3)			Average	Std	Relative Std
			1	2	3			
Phenol	1.472	0.000	1.367	1.541	1.524	1.477	0.096	100.3
4-nitro phenol	1.469	0.000	1.229	1.308	1.430	1.322	0.101	90.0
m-methylphenol	1.374	0.000	1.294	1.540	1.548	1.461	0.144	106.3
2-chlorophenol	0.613	0.000	0.527	0.684	0.641	0.617	0.081	100.6
2,4-dichlorophenol	1.630	0.000	1.305	1.613	1.621	1.513	0.180	90.392.8
2,4,6-trichlorophenol	1.655	0.000	1.365	1.609	1.511	1.495	0.123	95.690.3
Pentachlorophenol	1.470	0.000	1.259	1.487	1.472	1.406	0.128	95.6

Determination of Phenols in Water

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

Collect H₂O

Condition HyperSep Retain PEP Extraction Column

1 x 3mL methyl tertiary butyl ether (10:90, V/V)
1 x 3mL CH₃OH
1 x 3mL H₂O

NOTE: Do not allow the cartridge to dry out

Apply Sample

Load 1L of water sample at 20mL/minute
Dry column for 10 to 15 minutes

Wash Column

1 x 10mL DI H₂O
Dry column (20 minutes at >10"Hg)

Elute

1 x 2mL CH₃OH
1 x 2mL methyl tertiary butyl ether (10:90 V/V)
Concentrate the collected elute to 1mL with a stream of nitrogen

Analysis

Inject onto HPLC

Recommended HPLC Column

Part Number

Hypersil GOLD PFP 3µm, 150 x 4.6mm

25403-154630

LC/MS/MS Analysis of Phenoxyacetic Acid Herbicides

Using 10g 75mL HyperSep C18 Extraction Column (Part Number: 60108-703)

Sample Preparation

- Collect 10 to 100 grams of soil sample
- Add DI H₂O to form a slurry
- Mix for 15 minutes
- Adjust pH to 2 using 50% aq H₂SO₄
- Filter sample through previously acidified filter media

Condition HyperSep C18 Extraction Column

- 1 x 5mL CH₃OH
- 1 x 5mL DI H₂O

NOTE: Do not allow the cartridge to dry out

Apply Sample

- Load sample at 10mL/minute

Elute Phenoxyacetic acid Herbicides

- 2 x 5mL CH₃OH
- Collect at 1 to 2mL/minute
- Evaporate to dryness at <40°C using N₂

Analysis

- Reconstitute in 100µL of mobile phase
- Inject 10 to 100µL onto LC/MS
- Mobile phase: 0.1 M ammonium acetate (A):methanol (B)

% B	Time
25	0
60	15

Compound	+ve Ion m/z	-ve Ion m/z
Dalapon		141
Dicamba	238	184
2,4-D	238	184
MCPA	218	199
Dichloroprop	252	235
MCP	232	213
2,4,5-T	272	218
2,4,5-TP Silvex	286	269
Dinoseb	228	240
2,4-DB	266	247
2,4-D, butoxy ethanol ester	321	185
2,4-T, butoxy ethanol ester	372	195
2,4,5-T, butoxy ethanol ester	328	195
2,4-D, ethyl hexyl ester	350	161

Recommended HPLC Columns	Part Number
ODS-Hypersil C18 5µm, 100 x 2mm	30105-102130
MOS2-Hypersil C18 3µm, 100 x 2mm	30303-102130

Polychlorinated Biphenyls in Pond Water

Using 2g 15mL HyperSep C18 Extraction Column (Part Number: 60108-701)

Sample Preparation

- Filter water sample through a 0.45µm filter
- Add 2mL of methanol to 200mL of filtered water sample
- Mix and degas sample for 2 minutes

Condition C18 Extraction Column

- 15 to 20mL of hexane
- 15 to 20mL of methanol
- 10mL of DI H₂O

Apply Sample

- Load 200mL of sample at a rate of 8 to 10mL/minute

Wash Column

- 20mL of DI H₂O

Dry Column

- Use maximum vacuum pressure for 20 to 30 minutes

Elute Polychlorinated Biphenyls

- 20mL of hexane

Concentration/Evaporation

- Evaporate to dryness under a nitrogen stream at room temperature

Injection/Analysis

- Reconstitute with 100µL of methanol and inject 1 to 2µL aliquot onto GC column

Pesticides Extracted

- Aroclor 1026
- Aroclor 1221
- Aroclor 1232
- Aroclor 1242
- Aroclor 1248
- Aroclor 1254
- Aroclor 1260

Extraction of Polycyclic Aromatic Hydrocarbons from Fish

Using QuEChERS Methodology (Part Number: 60105-211 and 60105-206)

Sample Preparation

- Add 5.0g of homogenized fish to a 50mL centrifuge tube 60105-211
- Add 10mL of acetonitrile
- Mix by shaking
- Mix/vortex for 3 minutes
- Centrifuge for 3 minutes at 3,400rpm

Clean-up

- Add 3mL of supernatant to centrifuge tube 60105-206
- Shake for 1 minute
- Centrifuge for 1 minute at 3,400rpm
- Filter supernatant through 0.20µm PTFE membrane filter (F2500-4)

Analysis

- Inject: 15µL on to HPLC
- Flow rate: 0.8mL/minute
- Mobile phase: 50:50 ACN:water
- Gradient: linear for 15 minutes, total run 40 minutes

Compound

Wavelength nm

naphthalene, acenaphthene and fluorene	315/260
Phenanthrene	366/260
anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and dibenzo(a,l)pyrene	430/260
(indeno(1,2,3-cd)pyrene)	505/290

Recommended HPLC Column

Part Number

Hypersil GREEN PAH 5µm, 150 x 4.0mm	31105-154030
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Determination of Polycyclic Aromatic Hydrocarbon in Water

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

- Add 1 x 20mL of 10% nitric acid into 1L of H₂O

Condition HyperSep Retain PEP Extraction Column

- 1 x 5mL IPA
- 1 x 5mL H₂O

NOTE: Do not allow the cartridge to dry out

Apply Sample

- Load 1L of water sample at 20mL/minute

Wash Column

- 1 x 5mL (H₂O 300mL+CH₃OH 700mL+Na₂HPO₄ 2.1g+KH₂PO₄ 2g)
- Dry column (20 minutes at >10" Hg)

Elute

- 1 x 4mL (IPA 90mL+acetic acid 10mL+toluene 200mL+petroleum ether 1L)
- Concentrate sample

Analysis

- Inject onto HPLC

Recommended HPLC Column

Part Number

Hypersil Green PAH 3µm, 150 x 4mm	31103-154030
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Polynuclear Aromatic Hydrocarbons in Pond Water

Using 500mg 3mL HyperSep NH₂ Extraction Column (Part Number: 60108-518)

Sample Preparation

Filter water sample through a 0.45µm filter
Add 2mL of methanol to 200mL of filtered water sample
Mix and degas sample for 2 minutes

Condition NH₂ Extraction Column

15 to 20mL of methylene
chloride/trichlorotrifluoroethylene (TCTFE)
15 to 20mL of TCTFE
Dry for 5 minutes
15 to 20mL of methanol
20mL of DI H₂O

Apply Sample

Load 200mL of sample at a rate of 8 to 10mL/minute

Wash Column

20mL of DI H₂O

Dry Column

Use maximum vacuum pressure for 15 to 30 minutes

Elute Polynuclear Aromatic Hydrocarbons

20mL of TCTFE

Concentration/Evaporation

Evaporate to dryness under a nitrogen stream at room temperature

Injection/Analysis

Reconstitute with 100µL of TCTFE and inject 1 to 2µL aliquot onto GC

Polynuclear Aromatic Hydrocarbons Extracted

Naphthalene	Chrysene
Fluorene	B(e)pyrene
Acenaphthene	B(b)fluoranthene
Phenanthrene	B(k)fluoranthene
Anthracene	B(a)pyrene
Fluoranthene	D(a,h)anthracene
Pyrene	B(g,hi)perylene
B(a)anthracene	Indeno(1,2,3-cd)pyrene

Praziquantel in Water

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

To 10mL of water/methanol (95:5, v/v) containing
10ppm atrazine, add 20µL of acetic acid

Condition Retain PEP Extraction Column

2mL of methanol
2mL of DI H₂O

Apply Sample

Load 4mL samples at 1 to 2mL/minute

Wash Column

1mL of methanol/DI H₂O (5:95, v/v)

Elute Praziquantel

4mL of methanol

Analysis

Mobile phase: ACN/water (40:60, v/v)
Flow: 1mL/minute
Temperature: 30°C
Detection: UV 214nm

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 250 x 4.6mm

25005-254630

Thiourea Herbicides in Soil

Using 60mg 3mL HyperSep Retain PEP Extraction Column (Part Number: 60107-203)

Sample Preparation

Homogenize a soil sample containing thiourea herbicides in phosphate buffer solution acid (pH 2.5)

Condition Retain PEP Extraction Column

5mL of methanol

5mL of phosphate buffer (pH 2.5)

Apply Sample

Load 10mL samples at 1 to 2mL/minute

Wash Column

3mL of phosphate buffer (pH 2.5)

Elute Thiourea Herbicides

5mL of ACN/phosphate buffer (pH 7.8) (9:1, v/v)

Dry Eluate and Reconstitute

Evaporate to full dryness under a stream of nitrogen

Reconstitute the sample to 1mL with methanol

Analysis

Mobile phase: ACN-methanol-water (0.2% acetic acid)

Flow: 1mL/minute

Temperature: 30°C

Detection: mass spectrometry

Results

Compound	Recovery (%) Retain PEP
Nicosulfuron	91
Thifensulfuron-methyl	89
Metsulfuron-methyl	89
Sulfometuron-methyl	86
Chlorsulfuron	99
Ethametsulfuron-methyl	81
Tribenuron	15
Bensulfuron-methyl	82
Pyrazosulfuron-ethyl	93
Chlorimuron-ethyl	107

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 250 x 4.6mm	25005-254630
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6-Acetylmorphine in Urine

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Sample Preparation

- 1 x 2mL of 100mM phosphate buffer (pH= 6.0)
- Add internal standard*
- Mix/vortex
- Add 4mL of sample
- Centrifuge for 10 minutes at 2,000rpm
- Discard pellet
- Sample pH should be 6.0±0.5
- Adjust pH to 6 with 100mM monobasic or dibasic sodium phosphate

Condition HyperSep Verify-CX Extraction Column

- 1 x 3mL CH₃OH
- 1 x 3mL DI H₂O
- 1 x 1mL 100mM phosphate buffer (pH=6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

- Load at 1mL/minute

Wash Column

- 1 x 3mL DI H₂O
- 1 x 2mL 100mM acetate buffer (pH 4.5)
- 1 x 3mL CH₃OH
- Dry column (10 minutes at >10 "Hg)

Elute

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

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

- Evaporate to dryness at <40°C

Derivatize

- Add 50µL ethyl acetate
- Mix/vortex
- Add 50µL BSTFA (with 1% TMCS)**
- Overlay with N₂ and cap
- Mix/vortex
- React 45 minutes at 70°C
- Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution

Analysis

- Inject 1 to 2µL onto GC/MS
- For mass spectrometry analysis monitor the following ions:

Compound	Primary Ion***	Secondary	Tertiary
D6-6-AM-TMS*	405	406	343
6-AM-TMS	399	400	340

* Suggested internal standard for GC/MS:

** Part number TS-38831

*** Quantitation ion

Recommended GC Column

Part Number

TraceGOLD TG-5SiIMS 30m x 0.25mm x 0.25µm	26096-1420
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Amphetamines in Urine, Oxidation with Periodate for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 2mL of urine add internal standard(s)*, 1mL of 100mM phosphate buffer (pH 6.0) and 1mL of 0.35 M sodium periodate

Mix/vortex

Incubate at room temperature for 20 minutes

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

1mL of 100mM acetic acid

3mL of CH₃OH

Dry column (5 minutes at >10" Hg)

Elute Amphetamines

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Concentrate Eluate

Add 30µL silylation grade DMF*** to eluate

Evaporate to 30µL at <40°C

Fluoroacylate with PFPA (PFAA)

Add 50µL PFPA (PFAA)****

Overlay with N₂ and cap

Improve derivatization by addition of 50µL PFPOH

React for 20 minutes at 70°C

Evaporate to dryness at <40°C

Quantitate

Inject 1 to 2µL onto gas chromatograph

For mass spectrometry monitor the following ions:

Analyte (TMS)	Primary Ion**	Secondary	Tertiary
D ₅ -amphetamine*	194	92	123
Amphetamine	190	91	118
D ₅ -methamphetamine*	208	92	163
Methamphetamine	204	91	160

* Suggested internal standards

** Quantitation ion

*** Part number TS-20672 (50mL vial)

**** Part number TS-65193 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Amphetamines, Opiates and Phencyclidine in Oral Fluids

Using 60mg 6mL HyperSep Retain-CX Extraction Column (Part Number: 60107-308)

Sample Preparation

Add 100 to 500µL of oral fluid sample to a clean tube
Add internal standard(s) and let sit for 10 minutes at room temperature
Add 800µL of 100mM phosphate buffer (pH= 6.0)
Mix/vortex for 10 seconds
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition HyperSep Retain-CX Extraction Column

1 x 200µL CH₃OH
1 x 200µL DI H₂O
1 x 200µL 100mM phosphate buffer (pH=6.0)

Apply Sample

Do not exceed 1mL/minute

Wash Column

1 x 500µL DI H₂O
1 x 500µL 100mM acetic acid
1 x 500µL CH₃OH
Dry column (5 minutes at >10" Hg)

Elute

1 x 800µL CH₂Cl₂/IPA/NH₄OH (70:26:4)
Do not exceed 1mL/minute

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

For amphetamines and PCP:

Add 100µL of 5% trifluoroacetic acid in methanol after 5 minutes
Dry for 5 minutes drying
Evaporate to full dryness at <40°C under a stream of N₂

Derivatize

For Amphetamines*:

Add 50µL PFPA (PFAA)
Mix/vortex
Overlay with N₂ and cap
React 20 minutes at 70°C
Evaporate to dryness at <40°C
Reconstitute with 50µL ethyl acetate

For Opiates*:

Add 200µL of a 1:1 solution of propionic anhydride/pyridine
Make fresh daily
Mix/vortex
React 60 minutes at 40°C
Evaporate to dryness at <40°C
Reconstitute with 50µL ethyl acetate

Analysis

Inject 2µL onto gas chromatograph

* Alternate derivatizations may be used
Phencyclidine does not derivatize

Amphetamines, Opiates and Phencyclidine in Oral Fluid for GC/MS Confirmations

Using 50mg 1mL HyperSep Verify-CX Extraction Column (Part Number: 60108-741)

Prepare Sample

- Add 100 to 500µL of neat sample to a clean test tube
- Add internal standard(s) and let sit for 10 minutes at room temperature
- Add 800µL of 100mM phosphate buffer (pH 6.0)
- Mix/vortex for 10 seconds
- Sample pH should be 6.0±0.5
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

- 200µL of CH₃OH
- 200µL of DI H₂O
- 200µL of 100mM phosphate buffer (pH 6.0)

Apply Sample

- Load sample at 1mL/minute (do not exceed this flow rate)

Wash Column

- 500µL of DI H₂O
- 500µL of 100mM acetic acid
- 500µL of CH₃OH
- Dry column (5 minutes at >10" Hg)

Elute Compounds

- 800µL of CH₂Cl₂/IPA/NH₄OH (70:26:4)
- Collect eluate at 1mL/minute (do not exceed this rate)

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

Dry Eluate

- For amphetamines and PCP, add 100µL of 5% trifluoroacetic acid in methanol after 5 minutes of drying (5 minutes drying removes ammonia, addition of acid ionizes volatile analytes preventing loss)
- Evaporate to full dryness at <40°C under a stream of nitrogen

Derivatize

For Amphetamines*:

- Add 50µL PFPA (PFAA)** then vortex. Overlay with nitrogen and cap. React for 20 minutes at 70°C.
- Evaporate to dryness at <40°C. Reconstitute with 50µL ethyl acetate.

For Opiates*:

- Add 200µL of a 1:1 solution of PFPA (PFAA)** (10 x 1mL ampules, Cat. No. TS-65193)
- Vortex then react for 60 minutes at 40°C
- Reconstitute with 50µL ethyl acetate

Quantitate

- Inject 2µL onto gas chromatograph

* Alternative derivatizations may be used

** Part number TS-65193 (10 x 1mL ampules)

NOTE: Phencyclidine does not derivatize

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Anabolic Steroids in Urine for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample – β -Glucuronidase Hydrolysis

To 5mL of urine add suitable internal standards and 2mL of β -Glucuronidase

β -Glucuronidase: 5,000 F units/mL *Patella vulgata* in 100mM acetate buffer (pH 5.0)

Mix/vortex

Hydrolyze for 3 hours at 65°C

Cool before proceeding

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Adjust sample pH to 6.0 $\pm$ 0.5 with approximately 700 μ L of 1.0N NaOH

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of 10% (v/v) CH₃OH in DI H₂O

Dry column (5 minutes at >10" Hg)

1mL hexane or hexane/ethyl acetate (50:50)

Elute Anabolic Steroids

Option a: 3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2); collect eluate at 1 to 2mL/minute

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

Option b: 3mL CH₂Cl₂/IPA (80:20)

Option c: 3mL ethyl acetate

Option d: 3mL CH₃OH

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50 μ L ethyl acetate and 50 μ L MSTFA (with 3% trimethylsilyliodide)

Overlay with N₂ and cap

Mix/vortex

React for 20 minutes at 70°C

Remove from heat source to cool

NOTE: Do not evaporate MSTFA solution

Quantitate

Inject 1 to 2 μ L onto gas chromatograph

Monitor the ions (GC/MS) in the table below:

Compound	Primary Ion*	Secondary	Tertiary	Other
Testosterone-TMS	432	301	209	
19-nortestosterone-TMS	405	315	225	
Oxymethalone	640	52	462	370,143
Dehydroepiandrosterone-2TMS	432	327	297	
10-nortestosterone-2TMS	418	287	194	
Oxymethalone metabolite #1	640	52	462	143
Oxymethalone metabolite #2	625	462	370	143
11- β -hydroxyandosterone	522	417	158	
Methandienone	409	313	281	
19-norandosterone-2TMS	405	315	225	
Alpha-hydroxytestosterone	504	417		
17- α -epitestosterone-TMS	432	341	327	209
Stanozolol	472	381	342	149

* Quantitation ion

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 μ m	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 μ m	26AC497P

Antidepressant/Painkillers in Blood and Urine

Using 60mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-303)

Sample Preparation

To 1mL of 100mM phosphate buffer (pH= 6) add internal standard*

Add 1mL of blood or urine

Add 2mL of 100 phosphate buffer (pH= 6)

Mix/vortex

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Mix/vortex

Centrifuge as appropriate

Condition HyperSep Retain-CX Extraction Column

1 x 3mL CH₃OH

1 x 3mL H₂O

1 x 1mL 100mM phosphate buffer (pH= 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O

1 x 3mL 1% acetic acid

1 x 3mL methanol

Dry column (5 minutes at >10" Hg)

Elute

1 x 3mL ethyl acetate; ACN:ammonia (78:20:2 v/v)

Collect eluate at 1 to 2mL/minute

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

Analysis

Reconstitute sample in 100µL of methanol

Inject 5µL onto GC/MS

Compound	MRM Transition
Amitriptyline	278.8/91.1
Amitriptyline-D3*	281.2/91.2
Diphenhydramine	256.2/167.1
Diphenhydramine-D3*	259.2/167.1
Doxepin	280.2/107.1
EDDP	278.2/234.2
EDDP-D3*	281.4/234.3
Methadone	310.2/105.1
Methadone-D9*	319.2/268.3
Nortriptyline	264.2/91.1
Norpropoxyphene	326.2/44.1
Norpropoxyphene-D5*	331.1/267.1
Propoxyphene	340.2/58.1
Propoxyphene-D11*	351.3/64.0
Sertraline	308.1/161.0
Tramadol	264.2/58.1
Tramadol-D3*	268.2/58.0
Venlafaxine	278.2/58.2
Zolpidem	308.2/235

* Suggested internal standards

Recommended HPLC Column	Part Number
Hypersil GOLD 3µm, 150 x 2.1mm	25003-152130

Antidepressants/Painkillers in Urine and Blood for LC/MS/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

- To 1mL of 100mM phosphate buffer (pH 6.0) add internal standard*
- Add 1mL of urine or blood
- Add 2mL of 100mM phosphate buffer (pH 6.0)
- Mix/vortex
- Sample pH should be 6.0±0.5
- Adjust pH accordingly with 100mM of monobasic or dibasic sodium phosphate
- Mix/vortex
- Centrifuge as appropriate

Condition Verify-CX Extraction Column

- 3mL of CH₃OH then aspirate
- 3mL of DI H₂O then aspirate
- 1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 3mL of DI H₂O
- 3mL of 1% acetic acid
- 3mL of CH₃OH
- Dry column (5 minutes at >10" Hg)

Elute Antidepressants/Painkillers

- 3mL of ethyl acetate: acetonitrile: ammonia (78:20:2, v/v/v)
- Collect eluate at 1 to 2mL/minute

Evaporate

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

Reconstitute

- Reconstitute sample in 100µL of methanol
- Inject 5µL onto LC system

Quantitate

- Mobile phase: acetonitrile: 0.1% formic acid (33:67, v/v)
- Flow rate: 0.35mL/min

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 2.1mm 25003-152130

Compound	Detection Ions
Amitriptyline	278.8/91.1
Amitriptyline-D3*	281.2/91.2
Diphenhydramine	256.2/167.1
Diphenhydramine-D3*	259.2/167.1
Doxepin	280.2/107.1
EDDP	278.2/234.2
EDDP-D3*	281.4/234.3
Methadone	310.2/105.1
Methadone-D9*	319.2/268.3
Nortriptyline	264.2/91.1
Norpropoxyphene	326.2/44.1
Norpropoxyphene-D5*	331.1/267.1
Propoxyphene	340.2/58.1
Propoxyphene-D11*	351.3/64.0
Sertraline	308.1/161.0
Tramadol	264.2/58.1
Tramadol-D3*	268.2/58.0
Venlafaxine	278.2/58.2
Zolpidem	308.2/235

* Suggested internal standards

Barbiturates in Urine for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 2mL of urine add internal standard(s)* and 1mL of 100mM phosphate buffer (pH 5.0)

Mix/vortex

Sample pH should be 5.0±0.5

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

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 5.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

1mL of 100mM acetic acid

Dry column (5 minutes at >10" Hg)

2mL of hexane

Elute Barbiturates

3mL of hexane/ethyl acetate (50:50); Collect eluate at 1 to 2mL/minute

Dry Eluate

Evaporate to dryness at <40°C

Reconstitute with 100µL ethyl acetate

Optional Derivatization

Add 25 to 50µL of 0.2M TPAH***

Reaction occurs in the injection port

Quantitate

Inject 1 to 2µL onto gas chromatograph

For mass spectrometry, monitor the following ions (GC/MS):

Underivatized

Drug	Primary Ion**	Secondary	Tertiary
Amobarbital	156	141	157
Butalbital	156	141	157
Butalbital	168	167	181
Hexobarbital*	221	157	236
Pentobarbital	156	141	197
Phenobarbital	204	232	117
Secobarbital	168	167	195
Thiopental	172	157	173

Derivatized

Drug	Primary Ion**	Secondary	Tertiary
D ₅ -butalbital	201	214	
Butalbital	196	195	209
Amobarbital	169	184	185
Pentobarbital	169	184	112
¹³ C ₄ -secobarbital	200		185
Secobarbital	196	195	181
D ₅ -phenobarbital	237	151	
Phenobarbital	232	146	175

* Suggested internal standard

** Quantitation ions (target ions in bold)

*** Part number TS-49301 (MethElut reagent 12x1mL vials)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Basic Drugs in Urine, Blood, Plasma/Serum and Tissue for HPLC Analysis

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Mix/vortex

Add 1 to 5mL of urine, 1mL of blood, plasma or serum, or 1g (1:4) tissue homogenate

Mix/vortex

Add 2mL of 100mM phosphate buffer (pH 6.0)

Sample pH should be 6.0 ± 0.5 ; adjust sample pH accordingly with 100mM monobasic or dibasic sodium phosphate

Centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH_3OH then aspirate

3mL of $\text{DI H}_2\text{O}$ then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at $<3''\text{Hg}$ to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of $\text{DI H}_2\text{O}$

1mL of 100mM acetic acid

3mL of methanol

Dry column (5 minutes at $>10''\text{Hg}$)

Elute Bases

2mL of $\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$ (98:2)

Collect eluate at 1 to 2mL/minute

NOTE: Prepare elution solvent daily

Extract

To the eluate add 2.0mL of $\text{DI H}_2\text{O}$ and 500 μL of methylene chloride

Mix/vortex

Centrifuge at 2,000rpm for 10 minutes

Transfer organic lower layer to a clean test tube

Evaporate

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

Quantitate

Reconstitute in mobile phase and inject onto the HPLC system

Benzodiazepines in Urine

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Sample Preparation

To 2mL of urine add internal standard(s)* and 1mL of β -glucuronidase solution
 β -glucuronidase solution contains: 5,000 F units/mL
 Patella vulgata in 100mM acetate buffer (pH=5.0)
 Mix/vortex
 Hydrolyze for 3 hours at 65°C
 Centrifuge for 10 minutes at 2,000rpm and discard pellet

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
 1 x 3mL DI H₂O
 1 x 1mL 100mM phosphate buffer (pH= 6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load at 1mL/minute

Wash Column

1 x 2mL DI H₂O
 1 x 2mL 20% ACN in 100mM phosphate buffer (pH= 6.0)
 Dry column (5 minutes at >10" Hg)
 1 x 2mL hexane

Elute

1 x 5mL ethyl acetate containing 4% ammonium hydroxide
 Collect eluate at 1 to 2mL/minute
 Evaporate to dryness at <40°C

Derivatize

Add 50 μ L ethyl acetate and 50 μ L BSTFA**
 (with 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

Analysis

Inject 1 to 2 μ L onto GC/MS
 For mass spectrometry monitor the following ions:

Generic Name	Trade Name	Primary Ion***	Secondary	Tertiary
Alprazolam	Xanax®	308	279	204
α -Hydroxyalprazolam-TMS		381	396	383
Chlordiazepoxide	Librium®	282	283	284
Clonazepam	Klonopin®	387	352	306
Diazepam	Valium®	256	283	221
Desalkylflurazepam-TMS		359	341	245
Hydroxyethylflurazepam-TMS		288	360	389
Lorazepam-TMS	Ativan®	429	430	347
Nordiazepam-TMS		341	342	343
Oxazepam-TMS	Serax	429	430	313
Prazepam*		269	241	324
Temazepam-TMS	Restoril®	343	283	257
Triazolam	Halcion®	313	314	342
α -Hydroxytriazolam-TMS		415	417	190

* Suggested internal standard for GC/MS: Prazepam or Oxazepam-D5

** Part number TS-38831

*** Quantitation ion

NOTE: Flurazepam does not extract under these conditions; However metabolites such as desalkylflurazepam and hydroxyethylflurazepam will extract with high recovery.

Recommended GC Column

Part Number

TraceGOLD TG-5SiIMS 30m x 0.25m x 0.25 μ m 26096-1420

Benzodiazepines in Urine

Alternative Derivatization

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Derivatize

To 2mL of urine add internal standard(s)* and 1mL of β -Glucuronidase
 β -Glucuronidase: 5,000 F units/mL Patella vulgate in 100mM acetate buffer (pH 5.0)
 Mix/vortex
 Hydrolyze for 3 hours at 65°C
 Centrifuge for 10 minutes at 2,000rpm and discard pellet

Analysis

Inject 1 to 2 μ L onto GC/MS
 For mass spectrometry monitor the following ions:

Generic Name	Trade Name	Primary Ion**	Secondary	Tertiary
Nordiazepam -D5-TBDMS		332	334	333
Nordiazepam-TBDMS		327	328	329
Oxazepam-D5-TBDMS		462	519	462
Oxazepam-TBDMS	Serax	457	513	459
Temazepam-D5-TBDMS		362	390	288
Temazepam-TBDMS	Restoril®	357	359	385
Lorazepam-TBDMS	Ativan®	491	513	493
Clonazepam	Klonopin®	372	374	326
7-Aminoclonazepam -TBMS		456	458	513
Diazepam	Valium®	256	283	221
Desalkylflurazepam-TBDMS		345	347	402
Prazepam*		269	241	324
α -Hydroxymidazolam-TBDMS	Versed®	398	400	440
Desmethyflunitrazepam-TBDMS		357	310	356
7-Aminoflunitrazepam-TBDMS		397	324	398
Alprazolam	Xanax®	308	279	204
α -Hydroxyalprazolam-D5-TBDMS		386	388	387
α -Hydroxyalprazolam-TBDMS		383	384	381
Triazolam	Halcion®	313	314	342
α -Hydroxytriazolam-TBDMS		415	417	190

* Suggested internal standard

** Quantitation ion

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 μ m	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 μ m	26AC497P

Benzodiazepines in Serum or Plasma

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Sample Preparation

To 1mL H₂O add 1.0mL of 100mM phosphate buffer (pH 6.0)
Add internal standard(s)*
Add 1mL of serum or plasma
Mix/vortex
Sample pH should be 6.0
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate
Centrifuge

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH= 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1mL/minute

Wash Column

1 x 2mL DI H₂O
1 x 2mL 20% ACN in 100mM phosphate buffer (pH 6.0)
Dry column (10 minutes at >10" Hg)
1 x 2mL hexane

Elute

1 x 5mL ethyl acetate containing 2% ammonium hydroxide
Collect eluate at 1 to 2mL/minute.
Evaporate to dryness at <40°C

Reconstitute

Reconstitute in mobile phase

Analysis

Inject sample onto HPLC

* Suggested internal standards: Alprazolam-D5, Alphahydroxyalprazolam-D5, Diazepam, Lorazepam-D4, Oxazepam-D5, Temazepam-D5

Recommended HPLC Column

Part Number

BETASIL Phenyl/Hexyl 5µm, 150 x 4.6mm

73005-154630

Benzodiazepines in Whole Blood

Using 500mg 6mL HyperSep Diol Extraction Column (Part Number: 60108-575)

Sample Preparation

To 1mL of pH 6 buffer add internal standards*
Add 2mL of whole blood
Mix/vortex
Add 5mL of pH 6 buffer
Sonicate for 10 seconds
Centrifuge at ~2700rpm for 15 minutes

Condition HyperSep Diol Extraction Column

1 x 3mL ethyl acetate
1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 3mL 0.1M phosphate buffer (pH 6.0)

Apply Sample

Load sample by gravity

Wash Column

1 x 3mL DI H₂O
1 x 3mL 5% ACN in 0.1M phosphate buffer (pH 6.0)
Dry columns 5 minutes at full vacuum or >10" Hg
1 x 3mL Hexane

Elute

2 x 3mL ethyl acetate
Evaporate to dryness under nitrogen at ~55°C
Add external standards*

Derivatize

Add 100µL ACN and 100µL MTBSTFA w/1% t-BDMCS
Heat for 30 minutes at 70°C
Remove from heat source to cool
Inject 1µL into GC/MS-NCI

NOTE: Do not evaporate MTBSTFA solution

Analysis

Inject onto GC/MS

* Suggested standards: Diazepam-D5 and Lorazepam-D4.

Recommended GC Column

Part Number

TraceGOLD TG-5SiIMS 30m x 0.25mm x 0.25µm

26096-1420

Benzodiazepine Screening in Blood, Serum, Urine and Tissue

Using 200mg 3mL HyperSep Diol Extraction Column (Part Number: 60108-573)

Sample Preparation

To 1mL of 100mM phosphate buffer (pH= 6) add internal standards*
Add 1mL blood/urine or 1g of (1:4) tissue homogenate
Mix/vortex
Add 3mL of 100mM phosphate buffer (pH= 6)
Sample pH should be 6.0±0.5
Adjust pH with 100mM monobasic or dibasic sodium phosphate
Mix/vortex
Centrifuge

Procedure for Urine

To 1mL of acetate buffer (pH 5.0) containing 5,000 F units/mL β -Glucuronidase
Add internal standards*
To this solution add 1mL of urine
Mix/vortex
Hydrolyze for 3 hours at 65°C
Allow to cool
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Add 3mL of 100mM phosphate buffer (pH 6.0) and mix

Condition HyperSep Diol Extraction Column

1 x 3mL CH₃OH
1 x 3mL 100mM phosphate buffer (pH 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL of 5% (v/v) ACN in 100mM phosphate buffer (pH6)
Dry column (5 minutes at >10"Hg)
1 x 3mL of hexane
Dry column (5 minutes at >10"Hg)

Elute

1 x 3mL ethyl acetate; ammonia (98:2 v/v)
Collect eluate at 1 to 2mL/minute
Evaporate eluates under a gentle stream of nitrogen <40°C

Derivatize

Add 50 μ L ACN and 50 μ L BSTFA with 1% TCMS
Heat for 30 minutes at 70°C
Remove from heat source to cool

Analysis

Inject 1 μ L into GC/MS

Compound	Primary Ion	Secondary	Tertiary
Alprazolam	308	279	204
Alprazolam-D*	513	284	
Alphahydroxyalprazolam	318	396	383
Alphahydroxyalprazolam-D5*	386	401	
Diazepam	256	283	284
Diazepam-D5*	287	289	
Lorazepam	429	430	347
Lorazepam-D4*	433	435	
Nordiazepam	34	342	343
Nordiazepam-D5*	345	347	
Oxazepam	429	313	430
Oxazepam-D5*	435	433	
Temazepam	343	257	283
Temazepam-D5*	348	262	

* Suggested internal standards: Alprazolam-D, Alphahydroxyalprazolam-D5, Diazepam-D5, Lorazepam-D4, Nordiazepam-D5, Oxazepam-D5, Temazepam-D5

Recommended GC Column

Part Number

TraceGOLD TG-5SiIMS 30m x 0.25mm x 0.25 μ m	26096-1420
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Beta Agonists in Urine

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Sample Preparation

To 1mL of 100mM acetate buffer (pH 4.5)
Add 1mL of urine
Add 2mL of 100mM acetate buffer (pH 4.5)
Mix/vortex
Centrifuge as appropriate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 3mL 100mM acetate buffer (pH 4.7)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

2 x 1mL acetone/methanol (1:1) aspirate
Dry column (5 minutes at >10" Hg)

Elute

1 x 1mL dichloromethane/isopropanol and ammonium hydroxide (78:20:2)
Collect the eluate at 1 to 2mL/minute (or gravity)

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

Evaporate to dryness at <40°C

Derivatize

Derivatization solution: Methaneboronic acid at 5mg/mL prepared in dry ethyl acetate (use molecular sieve)
Store this solution at -20°C (freezer conditions) until use

Reaction Mixture

Add 100µL 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

Analysis

Inject 1 to 2µL sample (derivatized solution)

Beta Blockers in Urine and Blood for GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of urine or blood add 1mL of 100mM acetate buffer (pH 4.5)
Add 2mL of acetate buffer (pH 4.5)
Mix/vortex
Centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
3mL of 100mM acetate buffer (pH 4.5) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

2 x 1mL of acetone/methanol (1:1) then aspirate
Dry column (5 minutes at >10" Hg)

Elute Beta Blockers

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

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Derivatization solution: Methaneboronic acid at 5mg/mL prepared in dry ethyl acetate (use molecular sieve)

Store this solution at -20°C (freezer conditions) until use

Reaction mixture: Add 100µL of the methaneboronic acid solution (see above)

Mix/vortex
React for 15 minutes at 70°C
Remove from heat source to cool

NOTE: Do not evaporate this solution

Analysis

Inject 1 to 2µL of sample

Blood GHB Extraction

Using 200mg 3mL HyperSep Retain-AX Extraction Column (Part Number: 60107-404)

Sample Preparation

To 1mL blood sample add internal standard* and 0.5mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

Rock for 10 minutes

Centrifuge for 10 minutes at 2,700rpm

Condition HyperSep Retain-AX Extraction Column

1 x 3mL CH₃OH

1 x 3mL DI H₂O

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

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Place centrifuge tubes into vacuum manifold for collection

The sample loading is collected

Decant sample onto column

Aspirate at about 1"Hg

After the sample is off the columns apply full vacuum for about 15 seconds to remove any residual blood

Elute

Remove centrifuge tubes, set aside

Place clean centrifuge tubes into vacuum manifold for collection

1 x 2mL of CH₃OH/NH₄OH (99:1)

Aspirate at about 1"Hg

Concentrate

Remove test tubes from vacuum manifold

Mix/vortex the sample prior to concentrating

Evaporate to dryness at 60°C using a stream of nitrogen

Sample Clean-up

Add 200µL of dimethylformamide

Add 1mL of hexane saturated with dimethylformamide

Rock for 5 minutes

Centrifuge at 5 minutes at 2,700rpm

Transfer lower dimethylformamide layer to a clean test tube

Evaporate to dryness at 50°C using a stream of air or nitrogen

Derivatize

Add 25µL ethyl acetate and 25µL BSTFA (with 1% TMCS**)

Mix/vortex

Heat at 70°C for 30 minutes

Analysis

Inject a 1 to 2µL of the sample onto GC/MS

Compound	Primary Ion *	Secondary	Tertiary
GHB-D6-di-TMS*	239	240	241
GHB-di-TMS	233	234	235

* Suggested internal standard for GC/MS: D6-GHB

** Part number TS-38831

Recommended GC Column	Part Number
TraceGOLD TG-5SiIMS 30m x 0.25m x 0.25µm	26096-1420

Buprenorphine and Norbuprenorphine in Blood and Urine

Using 500mg 6mL HyperSep Aminopropyl Extraction Column (Part Number: 60108-519)

Sample Preparation

1mL of 100mM acetate buffer (pH= 5)
Add internal standard*
Mix/vortex
Add 1mL of blood, plasma/serum
Add 2mL of 100mM acetate buffer (pH= 5)
Mix/vortex
Sample pH should be 6.0±0.5
Adjust pH with 100mM monobasic or dibasic sodium phosphate
Centrifuge

Enzyme Hydrolysis of Glucuronides

1mL of 100mM acetate buffer
Add internal standard*
Add 1 to 5mL of blood or urine
Mix/vortex
Add 2mL of 100mM acetate buffer (pH= 5)
Hydrolyze with helix pomatia (5,000 units/mL)
Heat for 3 hours at 60°C
Cool before proceeding

Condition HyperSep Aminopropyl Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM acetate buffer (pH=5.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

1 x 2mL DI H₂O
1 x 3mL 100mM acetate buffer (pH=5.0)
1 x 3mL methanol
Dry column (5 to 10 minutes at greater than 10"Hg/full flow for positive pressure manifold)

Elute

1 x 3mL methylene chloride/iso-propano/ammonium hydroxide (78/20/12) (make elution solvent fresh)
Collect eluate at 1 to 2mL/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.

Evaporate to dryness at <40°C

Derivatize

Add 50µL ethyl acetate and 50µL BSTFA (with 1% TMCS)**
React 20 minutes at 70°C
Remove from heat source to cool

NOTE: Do not evaporate BSTFA

Analysis

Inject 1 to 2µL onto GC/MS

For mass spectrometry monitor the following ions:

Compound	Primary Ion	Secondary	Tertiary
Buprenorphine-D4-TMS*	454	486	510
Buprenorphine-TMS	450	482	506
Norbuprenorphine-TMS	468	500	524
Norbuprenorphine-D3-TMS*	471	503	527

* Internal standards: Buprenorphine-D4-TMS and Norbuprenorphine-D3-TMS

** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-5SiIMS 30m x 0.25mm x 0.25µm	26096-1420
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Buprenorphine and Norbuprenorphine in Urine and Blood for GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM acetate buffer (pH 5.0) add internal standard*

Mix/vortex and add 1mL of urine or blood

Add 2mL of 100mM acetate buffer (pH 5.0)

Mix/vortex

Sample pH should be 6.0 ± 0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Centrifuge as appropriate

Enzyme Hydrolysis of Glucuronides

To 1mL of 100mM acetate buffer add internal standard*

Add 1 to 5mL of urine or blood

Mix/vortex then add 2mL of 100mM acetate buffer (pH 5.0)

Hydrolyze with Helix Pomatia (5,000 units/mL), heat for 3 hours at 60°C

Cool before proceeding

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

3mL of 100mM acetate buffer (pH 5.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

2mL of DI H₂O

3mL of 100mM acetate buffer (pH 5.0)

3mL of methanol

Dry column (5 to 10 minutes at >10" Hg/full flow for positive pressure Manifold)

Elute Buprenorphine/Norbuprenorphine

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/minute

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

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)

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50μL of ethyl acetate and 50μL of BSTFA (with 1% TMCS)***

React for 20 minutes at 70°C

Remove from heat source to cool

NOTE: Do not evaporate BSTFA

Quantitate

Inject 1 to 2μL of sample onto the gas chromatograph/mass spectrometer

For mass spectrometry, monitor the following ions:

Analyte	Primary Ion	Secondary	Tertiary
Buprenorphine-D4-TMS*	454	486	510
Buprenorphine-TMS	450	482	506
Norbuprenorphine-TMS	468	500	524
Norbuprenorphine-D3-TMS*	471	503	527

* Suggested internal standard

** Suggested quantitation ion

*** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25μm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33μm	26AC497P

Buprenorphine and Norbuprenorphine in Urine, Blood and Plasma/Serum for HPLC Analysis

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM phosphate buffer (pH 5.0) add internal standards*

Add 1mL of urine, whole blood, plasma/serum

Add 2mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex and centrifuge as appropriate

Total (sum of free and conjugated Buprenorphine/Norbuprenorphine)

To 1mL of acetate buffer (pH 5.0) containing 5,000 F units/mL of β -Glucuronidase, add internal standards*

To this solution, add 1mL of urine or blood

Mix/vortex

Hydrolyze for 3 hours at 65°C

Allow to cool, then add 3mL of 100mM phosphate buffer (pH 6.0) and mix

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

2mL of DI H₂O

3mL of 100mM acetic acid

3mL of methanol

Dry column (5 minutes at >10" Hg/full flow for positive pressure Manifold)

Elute Buprenorphine/Norbuprenorphine

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2, v/v/v)

or

3mL of ethyl acetate/acetonitrile/ammonia (78:20:2, v/v/v)

Collect eluate at 1 to 2mL/minute

Evaporate

Evaporate to dryness at <40°C

Reconstitute

Add 50 μ L of methanol

Quantitate

Inject 5 μ L of sample onto the LC/MS system

For mass spectrometry, monitor the following ions:

Compound	Detection Ions
Buprenorphine	468.4/55.1
Buprenorphine-D4*	472.4/59.1
Norbuprenorphine	414.3/83.1
Norbuprenorphine-D3*	417.4/55.1

* Suggested internal standards

Mobile phase: acetonitrile: 0.1% formic acid (50:50, v/v)

Flow rate: 0.35mL/min

Recommended HPLC Column

Part Number

Hypersil GOLD Phenyl 3 μ m, 50 x 2.1mm	25903-054630
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Caffeine, Theophylline and Theobromine in Urine, Blood and Plasma/Serum for LC/PDA Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM acetic acid add internal standard
Add 1mL of urine, blood or plasma/serum
Add 2mL of 100mM acetic acid
Vortex and centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
3mL of 100mM acetic acid

NOTE: Aspirate at <3"Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
3mL of 100mM acetic acid
Dry column (5 minutes at >10"Hg)

Elute Beta Caffeine/Theophylline/Theobromine

3mL of ethyl acetate/methanol (90:10, v/v)
Collect eluate at 1 to 2mL/minute

Evaporate

Evaporate to dryness at <40°C

Reconstitute

1,000µL of 0.1% formic acid (aq)

Analysis

Inject 20µL of sample onto LC/PDA system
Mobile phase: acetonitrile:0.1% formic acid (10:90, v/v)
Flow rate: 0.1mL/min
Detector: diode array (200 to 350nm)
Column temperature: ambient

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 2.1mm

25003-152130

Carboxy-delta 9-THC in Urine

Using 500mg 6mL HyperSep Aminopropyl Extraction Column (Part Number: 60108-519)

Sample Preparation

To 2mL of urine add internal standard*
Add 100µL of 10 M NaOH
Mix/vortex
Hydrolyze for 20 minutes at 60°C
Cool before proceeding
Adjust sample pH to 3.0 with approx. 1.0mL of glacial acetic acid

Condition HyperSep Aminopropyl Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL acetate buffer (pH=3.0)

NOTE: Aspirate at <3"Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

1 x 2mL DI H₂O
1 x 2mL 100mM HCl/ACN (95:5)
Dry column (5-10 minutes at greater than 10"Hg)
1 x 200µL hexane then aspirate

Elute

1 x 3mL hexane/ethyl acetate (50:50)
Collect eluate at 1 to 2mL/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.

Evaporate to dryness at <40°C

Derivatize

Add 50µL ethyl acetate
Add 50µL BSTFA (with 1% TMCS)**
Mix/vortex
React 20 minutes at 70°C
Remove from heat source to cool

NOTE: Do not evaporate BSTFA

Analysis

Inject 1 to 2µL onto GC/MS
For mass spectrometry monitor the following ions:

Compound (TMS)	Primary Ion***	Secondary	Tertiary
Carboxy-delta 9-THC-D3*	374	476	491
Carboxy-delta 9-THC-D9*	380	479	497
Carboxy-delta 9-THC	371	473	488

* Suggested internal standard for GC/MS: -Carboxy-delta 9-THC-D9 and 9-THC-D3

** Part number TS-38831

*** Quantitation ion

Recommended GC Column

Part Number

TraceGOLD TG-35MS 30m x 0.25mm x 0.25µm

26094-1420

Carboxy-delta-9-THC (pKa 4.5) in Urine for GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample – Base Hydrolysis of Glucuronides

To 2mL of urine add internal standard* and 100µL of 10 M NaOH

Mix/vortex

Hydrolyze for 20 minutes at 60°C

Cool before proceeding

Adjust sample pH to 3.0 with approximately 1.0mL of glacial acetic acid (check pH to ensure that the pH value is $\approx$ 3.0)

Condition Verify-CX Extraction Column

3mL of CH₃OH

3mL of DI H₂O

1mL of acetate buffer (pH 3.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

2mL of DI H₂O

2mL of 100mM HCl/acetonitrile (95:5)

Dry column (5 to 10 minutes at >10 "Hg/full flow for positive pressure manifold)

200µL of hexane then aspirate (additional step to remove any residual moisture)

Elute Carboxy THC

3mL of hexane/ethyl acetate (50:50)

Collect eluate at 1 to 2mL/min

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)

Dry Eluate

Evaporate to full dryness at $<40^\circ\text{C}$ under a stream of nitrogen

Derivatize**

Add 50µL of ethyl acetate and 50µL of BSTFA (with 1% TMCS)***

Mix/vortex then react for 20 minutes at 70°C

Remove from heat source to cool

NOTE: Do not evaporate BSTFA

Quantitate

Inject 1 to 2µL onto gas chromatograph

For GC/MS monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
D ₃ -Carboxy-delta ₉ -THC	374	476	491
D ₉ -Carboxy-delta ₉ -THC*	380	479	497
Carboxy-delta ₉ -THC	371	473	488

* Suggested internal standard

** Quantitation ion

*** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Carboxy-delta-9-THC, Delta-9-THC (parent), Delta-9-Hydroxy THC in Whole Blood for GC or GC/MS Confirmations

Using 200mg 6mL HyperSep Verify-AX Extraction Column (Part Number: 60108-730)

Prepare Sample

To 1 to 2mL of whole blood add internal standard(s)*
Mix/vortex
Vortex and add dropwise 1mL of ice cold acetonitrile
Centrifuge and transfer acetonitrile to a clean tube
Adjust sample pH to 3.0±0.5 with approximately
2.0mL of 100mM sodium acetate buffer (check pH of
buffer to ensure that the pH value is ≈ 3.0)

Condition Verify-AX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of acetate buffer (pH 3.0) then aspirate

NOTE: Aspirate at <3"Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
2mL of 100mM HCl/acetonitrile (95:5)
Dry column (5 to 10 minutes at >10"Hg/full flow for
positive pressure manifold)
200μL of hexane then aspirate (Additional step to remove
any residual moisture. Could substitute
200μL MeOH for hexane.)

Optional: Dry column (5 minutes at >10"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 200μL of hexane in
the wash/dry step (the 200μL hexane wash step can be eliminated
if the column is allowed to dry longer under vacuum or by positive
pressure gas flow)

Elute THC (metabolites)

2mL of hexane (optional, contains delta-9-THC)
3mL of hexane/ethyl acetate (50:50)
Collect eluate at 1 to 2mL/minute

NOTE: Before proceeding, ensure that there are no water droplets
at the bottom of the collection tube (this may increase drying
time and decrease BSTFA derivatizing agent efficiency)

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50μL of ethyl acetate and 50μL of BSTFA
(with 1% TMCS)**
Mix/vortex
React for 20 minutes at 70°C
Remove from heat source to cool

NOTE: Do not evaporate BSTFA

Quantitate

Inject 1 to 2μL onto gas chromatograph
For mass spectrometry, monitor the following ions:

Compound (TMS)	Primary Ion	Secondary	Tertiary
D ₃ -Carboxy-delta-9-THC*	374	476	491
D ₉ -Carboxy-delta-9-THC*	380	479	497
Carboxy-delta-9-THC	371	473	488
D ₃ -Hydroxy-delta-9-THC*	374	462	477
Hydroxy-delta-9-THC	371	459	474
D ₃ -delta-9-THC*	374	389	
Delta-9-THC (303, 315, 330, 343)**	371	386	

* Suggested internal standard

** Ions common to deuterated delta-9-THC and non-deuterated compounds

*** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25μm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33μm	26AC497P

Carboxy THC in Urine

Using 30mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-302)

Sample Preparation

2mL of urine
Add internal standard*
Add 100µL 10N NaOH
Mix/vortex
Hydrolyze for 20 minutes at 60°C
Cool before proceeding
Adjust sample pH to 3.5±0.5 with 1.0mL glacial acetic acid

Apply Sample

Load at a rate of 1 to 2mL/min

Condition HyperSep Retain-CX Extraction Column

1 x 1mL DI H₂O
1 x 1mL 0.1M HCl/ACN (70/30)
Dry column (3 minutes at >10" Hg)
1 x 200µL hexane
2 x 0.5mL hexane/ethyl acetate (50:50)
Collect eluate at 1 to 2mL/min
Evaporate eluate to dryness at <40°C

Derivatize

Add 50µL ethyl acetate
Mix/vortex
Add 50µL BSTFA (1% TMCS)**
Cap
Mix/vortex
Heat for 20 minutes at 70°C
Allow to cool

NOTE: Do not evaporate BSTFA solution

Analysis

Inject 1 to 2µL onto GC/MS
For mass spectrometry monitor the following ions:

Compound (TMS)	Target (Quantitation) Ion	Qualifier Ions
Carboxy-THC-TMS	371	473, 488
Carboxy-THC-D3-TMS*	374	476, 491

* Suggested internal standards: Carboxy-THC-D3-TMS

** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-35MS 30m x 0.25mm x 0.25µm	26094-1420
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Carisoprodol and Meprobamate in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM phosphate buffer (pH 3.0) add internal standard*
Add 1mL of urine, blood, plasma/serum or 1g (1:4) tissue homogenate
Add 2mL of 100mM phosphate buffer (pH 3.0)
Mix/vortex
Centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 3.0) then aspirate

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

4mL of DI H₂O
2mL of 100mM HCl
Dry column (5 minutes at >10" Hg)
3mL of hexane

Elute Carisoprodol/Meprobamate

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

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

Dry Eluate

Evaporate to dryness at <40°C

Quantitate

Reconstitute with 100µL ethyl acetate
Inject 1 to 2µL onto gas chromatograph
For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
Carisoprodol	158	104	245
Meprobamate	83	114	144
Hexobarbital*	221	157	81
Meprobamate-D7*	90	121	151

* Suggested internal standard

** Quantitation ion

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Clonazepam and 7-Aminoclonazepam in Urine for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample – β -Glucuronidase Hydrolysis

To 2mL of urine add internal standard(s)* and 1mL of β -Glucuronidase

β -Glucuronidase: 5,000 F units/mL Patella vulgate in 100mM acetate buffer (pH 5.0)

Mix/vortex

Hydrolyze for 3 hours at 65°C

Cool before proceeding

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

2mL of DI H₂O

2mL of 20% acetonitrile in 100mM phosphate buffer (pH 6.0)

Dry column (5 minutes at >10" Hg)

2mL of hexane

Elute Clonazepam and 7-Aminoclonazepam

3mL of ethyl acetate With 2% NH₄OH

Collect eluate at 1 to 2mL/minute

NOTE: Prepare elution solution fresh daily

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50 μ L ethyl acetate and 50 μ L MTBSTFA (with 1% TBDMCS)***

Mix/vortex

React for 20 minutes at 90°C

Remove from heat source to cool

NOTE: Do not evaporate MTBSTFA solution

Quantitate

Inject 1 to 2 μ L onto gas chromatograph

For mass spectrometry, monitor the following ions (GC/MS):

Compound	Primary Ion**	Secondary	Tertiary
Clonazepam-TBDMS	372	374	326
7-Aminoclonazepam-TBDMS	342	344	399
Clonazepam-D4-TBDMS	376	378	377
7-Aminoclonazepam-D4-TBDMS	346	348	403

* Suggested internal standard

** Quantitation ion

*** Part number TS-48927 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 μ m	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 μ m	26AC497P

Cocaine and Benzoylecgonine and Cocaethylene in Serum, Plasma Whole Blood, Urine and Tissue

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Sample Preparation

- 1mL of 100mM phosphate buffer (pH 6)
- Add internal standard*
- Add 1mL of whole blood, serum/plasma, urine, or 1g tissue homogenate (1:4)
- Add 2mL of 100mM phosphate buffer (pH 6)
- Mix/vortex
- Centrifuge

Condition HyperSep Verify-CX Extraction Column

- 1 x 3mL CH₃OH
- 1 x 3mL DI H₂O
- 1 x 1mL 100mM phosphate buffer (pH 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 1 x 3mL DI H₂O
- 1 x 3mL 100mM HCl
- 1 x 3mL CH₃OH Dry column (5 minutes at >10" Hg)

Elute

- 1 x 3mL ethyl acetate; ACN:ammonia (78:20:2 v/v)
- Collect eluate at 1 to 2mL/minute
- Evaporate eluates to dryness under a gentle stream of nitrogen

Analysis

- Reconstitute sample in 50µL of CH₃OH
- Inject 5µL on to LC/MS

Compound	MRM Transition
Cocaine	304.2/182.3
Cocaine-D3*	307.2/185.2
Benzoylecgonine	290.1/168.0
Benzoylecgonine-D8*	298.2/171.3
Cocaethylene	318.2/196.2
Cocaethylene-D8*	326.2/204.2

* Internal standards: Cocaine-D3, Benzoylecgonine-D8, Cocaethylene-D8

Recommended HPLC Column	Part Number
Hypersil GOLD 3µm, 150 x 2.1mm	25003-152130

Cocaine and Benzoylecgonine in Oral Fluid

Using 60mg 6mL HyperSep Retain-CX Extraction Column (Part Number: 60107-308)

Sample Preparation

- Add 100 to 500µL of neat oral fluid sample to a clean tube
- Add internal standard(s)* and let sit for 10 minutes at room temperature
- Add 800µL of 100mM phosphate buffer (pH= 6.0)
- Mix/vortex for 10 seconds
- Sample pH should be 6.0
- Adjust pH with 100mM monobasic or dibasic sodium phosphate

Condition HyperSep Retain-CX Extraction Column

- 1 x 200µL CH₃OH
- 1 x 200µL DI H₂O
- 1 x 200µL 100mM HCl

Apply Sample

- Do not exceed 1mL/minute

Wash Column

- 1 x 500µL DI H₂O
- 1 x 500µL 100mM HCl acid
- 1 x 500µL CH₃OH/DI H₂O (50:50)
- Dry column (5 minutes at >10" Hg)

Elute

- 1 x 800µL CH₂Cl₂/IPA/NH₄OH (70:26:4)
- Do not exceed 1mL/minute

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

- Evaporate at <40°C under a stream of N₂

Derivatize

Fluoroalkylate:

- Add 100µL PFPA (PFAA) or HFIP
- Overlay with N₂ and cap
- React 20 minutes at 70°C
- Evaporate to dryness at <40°C
- Reconstitute with 50µL ethyl acetate

TMS:

- Add 25µL BSTFA (w. 1% TMCS)**
- Add 25µL ethyl acetate
- Overlay with N₂ and cap
- Mix/vortex
- React 30 minutes at 70°C
- Remove from heat and allow to cool

NOTE: Do not evaporate BSTFA solution

Analysis

- Inject 2µL onto GC/MS
- For mass spectrometry monitor the following ions:

Compound	Target (Quantitation) Ion	Qualifier Ions
Cocaine	182	198, 303
Cocaine-D3*	185	201, 306
Benzoylecgonine-TMS	240	256, 361
Benzoylecgonine-D8-TMS*	243	259, 369

* Internal standards: Cocaine-D3, Benzoylecgonine-D8-TMS

** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25m x 0.25µm	26098-1420
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Cocaine and Benzoylecgonine in Oral Fluid for GC/MS Confirmation

Using 50mg 1mL HyperSep Verify-CX Extraction Column (Part Number: 60108-741)

Prepare Sample

- Add 100 to 500µL of neat oral fluid sample to a clean test tube
- Add internal standard(s) and let sit for 10 minutes at room temperature
- Add 800µL of 100mM phosphate buffer (pH 6.0)
- Mix/vortex for 10 seconds (Sample pH should be 6.0±0.5)
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

- 200µL of CH₃OH
- 200µL of DI H₂O
- 200µL of 0.1N HCl

Apply Sample

- Load sample at 1mL/minute (do not exceed this flow rate)

Wash Column

- 500µL of DI H₂O
- 500µL of 100mM HCl
- 500µL of CH₃OH/DI H₂O (50:50)
- Dry column (5 minutes at >10" Hg)

Elute Compounds

- 800µL of CH₂Cl₂/IPA/NH₄OH (70:26:4)
- Collect eluate at 1mL/minute (do not exceed this rate)

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

Concentrate Eluate

- Evaporate to full dryness at <40°C under a stream of nitrogen

Derivatize

Fluoroalkylate*:

- Add 100µL PFPA (PFAA)**
- Overlay with nitrogen and cap
- React for 20 minutes at 70°C
- Evaporate to dryness at <40°C
- Reconstitute with 50µL ethyl acetate

TMS*:

- Add 25µL of BSTFA (with 1% TMCS)*** and 25µL of ethyl acetate
- Overlay with nitrogen and cap
- Mix/vortex then react for 30 minutes at 70°C
- Remove from heat and allow to cool

NOTE: Do not evaporate BSTFA solution

Quantitate

- Inject 2µL onto gas chromatograph
- For mass spectrometry, monitor the following ions:

Analyte	Target (Quantitation) Ion	Qualifier Ions
Cocaine	182	198, 303
Cocaine-D3*	185	201, 306
Benzoylecgonine	240	256, 361
Benzoylecgonine-D8-TMS*	243	259, 369

* Alternative derivatizations may be used

** Part number TS-65193 (10 x 1mL ampules)

*** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Cocaine and Benzoylecgonine in Serum, Plasma and Whole Blood

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Sample Preparation

4mL of DI H₂O
Add internal standards
1mL of sample (Serum, Plasma or Whole Blood)
Add internal standard(s)
Mix/vortex and let stand 5 minutes
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Add 2mL of 100mM phosphate buffer (pH =6.0)
Mix/vortex
Sample pH should be 6.0
Adjust pH with 100mM monobasic or dibasic sodium phosphate

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 2mL 100mM HCl
1 x 3mL CH₃OH
Dry column (5 minutes at >10" Hg)

Elute

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

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

Evaporate to dryness at <40°C

Analysis

Reconstitute in mobile phase for injection into HPLC

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 4.6mm

25003-154630

Cocaine and Benzoylecgonine in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
2mL of 100mM HCl
3mL of CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Cocaine and Benzoylecgonine

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

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50µL ethyl acetate and 50µL BSTFA (with 1% TMCS)**
Overlay with N₂ and cap
Mix/vortex
React for 20 minutes at 70°C
Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution

Quantitate

Inject 1 to 2µL onto gas chromatograph
For mass spectrometry, monitor the following ions (GC/MS):

Compound	Primary Ion**	Secondary	Tertiary
D ₃ -cocaine*	185	201	306
Cocaine	182	198	303
D ₃ -benzoylecgonine-TMS*	243	259	364
Benzoylecgonine-TMS	240	256	361

* Suggested internal standards

** Quantitation ion

*** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Cocaine and its Metabolites from Meconium for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

Vortex 0.5 to 1g of meconium with 2mL of CH₃OH
Centrifuge and transfer the supernatant to a clean tube
To each tube add 3mL of 100mM phosphate buffer
(pH 6.0), internal standard and vortex
Matrix must be more aqueous than organic for good
extraction to occur

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute
Allow to dry

Wash Column

3mL of DI H₂O
2mL of 100mM HCl
3mL of CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Cocaine and Benzoyllecgonine

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

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

Evaporate

Evaporate the elution solvent to dryness without heating

Derivatize

Add 50μL ethyl acetate and 50μL BSTFA
(with 1% TMCS***)
Overlay with N₂ and cap
Mix/vortex
React for 20 minutes at 70°C
Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution

Quantitate

Inject 1 to 2μL onto gas chromatograph
For mass spectrometry, monitor the following ions (GC/MS):

Compound	Primary Ion**	Secondary	Tertiary
D ₃ -Cocaine*	185	201	306
Cocaine	182	198	303
D ₃ -Benzoyllecgonine-TMS*	243	259	364
Benzoyllecgonine-TMS	240	256	361

* Suggested internal standards for GC/MS: D₃-Cocaine and D₃-Benzoyllecgonine

** Quantitation ion

*** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25μm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33μm	26AC497P

Delta 9-THC, Delta 9-Hydroxy THC, Carboxy-Delta-9-THC in Whole Blood

Using 200mg 6mL HyperSep Verify-AX Extraction Column (Part Number: 60108-730)

Sample Preparation

- 1 to 2mL whole blood
- Add internal standards* prepared in alcohol
- Add drop-wise 2mL ice cold ACN
- Mix thoroughly and centrifuge
- Decant ACN into a clean tube
- Evaporate ACN under a stream of air or nitrogen to 200µL
- Add 2mL distilled H₂O (pH~6.0-7.0)

NOTE: The ACN should be cold and added slowly to prevent precipitation

Apply Sample

- Load sample directly to column without any preconditioning

Wash Column

- Wash with 1mL (84/15/1) water/ACN/NH₄OH
- Dry column thoroughly under vacuum

NOTE: It is important to dry the column properly to achieve the highest recovery of compounds

Elute

- 1 x 3mL hexane/ethyl acetate/glacial acetic acid (49:49:2)
- Collect at 1 to 2mL/minute
- Evaporate fraction(s) to dryness under stream of dry air or nitrogen at <40°C

Derivatize

- Add 50µL ethyl acetate
- Mix/vortex
- Add 50µL BSTFA (with 1% TMCS)***
- React 20 minutes at 70°C
- Remove from heat source to cool

NOTE: Do not evaporate BSTFA

Analysis

- Inject 2µL onto GC/MS
- For mass spectrometry monitor the following ions:

Derivatization procedure:

Derivatizing Agent	THC {T-005}** (D3 THC) {T-003}**	THC-OH {H-041}** (D3 THC-OH) {H-027}**	THC-COOH {T-006}** (D9 THC-COOH) {T007}**
BSTFA	371, 343, 386 (374, 346, 389)	371, 459, 474 (374, 462, 477)	371, 473, 488 (380, 479, 497)

* Suggested internal standard for GC/MS: D9-Carboxy-delta 9-THC, D3-Hydroxy- delta 9-THC, D3-delta 9-THC

** Ions common to deuterated delta-9 THC and non-deuterated compounds

*** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-35MS 30m x 0.25mm x 0.25µm 26094-1420

Dextromethorphan and Phencyclidine in Whole Blood and Urine

Using 200mg 6mL HyperSep Retain-CX Extraction Column (Part Number: 60107-314)

Sample Preparation

1mL of 100mM phosphate buffer (pH 6)
Add internal standard*
Add 1mL blood, urine
Add 2mL of 100mM phosphate buffer (pH 6)
Mix/vortex
Centrifuge

Condition HyperSep Retain-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 3mL 100mM acetic acid
1 x 3mL CH₃OH
Dry column (10 minutes at >10" Hg)

Elute

1 x 3mL ethyl acetate:ACN:ammonium hydroxide (78:20:2)
Collect eluate at 1 to 2mL/minute
Evaporate eluates under a gentle stream of nitrogen <40°

Analysis

Dissolve residue in 50µL CH₃OH, and inject 5µL of sample into LC/MS

Compound	MRM Transition
PCP	244.3/159.2
PCP-D5	249.3/264.1
Dextromethorphan	272.1/128.1
Dextromethorphan-D3*	275.1/131.0

* Suggested internal standard: Dextromethorphan-D3

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 4.6mm	25003-154630
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DHEA, Testosterone and Epitestosterone in Urine for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

Pipette 5mL of urine into borosilicate glass test tubes
Add internal standard* and adjust the sample pH to 5.5 to 6.5 using concentrated monobasic or dibasic sodium phosphate
Mix sample
Centrifuge samples at 3,000rpm for 5 minutes

Condition Verify-CX Extraction Column

3mL of CH₃OH
3mL of DI H₂O
1mL of 100mM phosphate buffer (pH 6.0)

Apply Sample

Pour supernatant onto column
Allow to flow via gravity

Wash Column

3mL of DI H₂O
Dry column (10 minutes at >10" Hg)

Elute Steroids

3mL of CH₃OH
Collect eluate at 1 to 2mL/min

Enzymatic Hydrolysis

Dry eluate under a stream of nitrogen; add 2mL of 0.2M phosphate buffer (pH 7.0) and 250 units of β -glucuronidase
Mix/vortex and allow to incubate at 50°C for 1 hour
Cool sample, cap and adjust the pH to 10-11 using a 1:1 mixture of NaHCO₃/Na₂CO₃

Additional Clean-up

Add 5mL of n-butyl chloride to each sample. Cap the tubes and shake vigorously for 10 minutes and then centrifuge at 3,000rpm for 5 minutes. Transfer the organic layer to a clean test tube and dry under a stream of nitrogen. Place the dried sample in a desiccator and further dry under vacuum for 30 minutes.

Derivatize

Add 50 μ L of MSTFA**/NH₄/dithioerythritol (1000:2:5, v/w/w) and incubate at 70°C for 20 minutes
Centrifuge sample at 3,000rpm for 1 minute and transfer directly to GC injector vials

Quantitate

Inject 1 to 2 μ L onto the gas chromatograph
For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary
Testosterone	432	417
Epitestosterone	432	417
DHEA	432	417
16 α hydroxyl-testosterone*	520	259

* Suggested internal standard at 20ng/mL

** Quantitation ion

*** Part number TS-48910 (10 x 1mL ampules)

Recommended GC Columns	Part Number
TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 μ m	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 μ m	26AC497P

Duloxetine in Blood and Urine

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Sample Preparation

1mL of 100mM phosphate buffer (pH= 6)
Add internal standard*
Add 1mL of blood or urine
Add 2mL of 100 phosphate buffer (pH= 6)
Mix/vortex
Sample pH should be 6.0±0.5
Adjust pH with 100mM monobasic or dibasic sodium phosphate
Mix/vortex
Centrifuge

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH= 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 3mL 100mM acetic acid
1 x 3mL CH₃OH
Dry column (5 minutes at >10"Hg)

Elute

1 x 3mL dichloromethane/isopropanol/ammonia (78:20:2 v/v)
Collect eluate at 1 to 2mL/minute
Evaporate eluate under a gentle stream of nitrogen <40°C

Analysis

Reconstitute sample in 200µL of 0.1% formic acid
Inject 5µL onto LC/MS

Compound	MRM Transition
Ethyl Morphine*	314.2/152.2
Duloxetine	298.1/44.1

* Internal standard: Ethyl Morphine

Fentanyl and Analogues in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM phosphate buffer (pH 6.0) add internal standards*
Add 1 to 5mL of urine, blood, plasma/serum, or 1g (1:4) of tissue homogenate
Mix/vortex
Add 2mL of 100mM phosphate buffer (pH 6.0)
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate
Centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
1mL of 100mM acetic acid
3mL of CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Fentanyls

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

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

Concentrate

Evaporate to dryness at <40°C
Reconstitute with 50µL ethyl acetate

Quantitate

Inject 1 to 2µL onto gas chromatograph
For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
Fentanyl	245	146	189
D ₅ -Fentanyl*	250	151	194
α-Methylfentanyl	259	203	146
Para-Fluorofentanyl	263	164	207
3-Methylfentanyl	259	160	203
Thienfentanyl	245	146	189
Sufentanil	289	140	
Carfentanil	303	187	
Lofentanil	317	201	289
Alfentanil	289	268	194

* Suggested internal standard

** Quantitation ion

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Fentanyl/Norfentanyl in Oral Swabs

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Preparation of Standards

Separate tube add 0, 1, 5, 10ng of Fentanyl/Norfentanyl in CH₃OH
Evaporate solvent
Add 100µL of drug free oral fluid
Mix/vortex
Stand for 30 minutes
Take clean, dry swab and swab up the oral fluid
Allow to stand for 15 minutes
Remove oral swab

Sample Preparation

200µL of CH₃OH (pH 6)
Add internal standard*
Insert oral swab into CH₃OH and mix for 1 minute,
Add 100µL of CH₃OH
Allow to stand for 10 minutes
Remove swab and 3mL of 100mM phosphate buffer (pH 6)
Mix/vortex
Centrifuge

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL H₂O
1 x 1mL 100mM phosphate buffer (pH 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 3mL 1% acetic acid
1 x 3mL methanol
Dry column (5 minutes at >10" Hg)

Wash Column

1 x 3mL ethyl acetate; ACN:ammonia (78:20:2 v/v)
Collect eluate at 1 to 2mL/minute
Evaporate eluates under a gentle stream of nitrogen <40°C

Analysis

Reconstitute sample in 20µL of methanol
Inject 5µL onto LC/MS

Compound	MRM Transition
Fentanyl	333.2/188.3
Fentanyl-D5*	342.2/188.2
Norfentanyl	233.2/84.1
Norfentanyl-D5*	238.3/84.1

* Internal standard: Fentanyl-D5 and Norfentanyl-D5

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 5µm, 50 x 3mm	25305-053030
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Fentanyl/Norfentanyl in Urine, Blood and Plasma/Serum for LC/MS/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Add 1mL of urine, blood, plasma/serum

Add 2mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

Centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

1mL of 100mM acetic acid

3mL of CH₃OH

Dry column (5 minutes at >10" Hg)

Elute Fentanyl/Norfentanyl

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Evaporate

Evaporate to dryness at <40°C

Reconstitute sample in 100µL of methanol

Quantitate

Inject 5µL onto LC/MS/MS system

Monitor the following ions:

Compound	MRM Transition
Fentanyl	333.2/188.3
Fentanyl-D5*	342.3/188.2
Norfentanyl	233.2/84.1
Norfentanyl-D5*	238.3/84.1

* Suggested internal standards

Mobile phase: acetonitrile: 0.1% formic acid (50:50, v/v)

Flow rate: 0.35mL/min

Column temperature: ambient

Recommended HPLC Column	Part Number
Hypersil GOLD PFP 3µm, 150 x 2.1mm	25403-152130

Flunitrazepam and Metabolites in Urine for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample – β -Glucuronidase Hydrolysis

To 2mL of urine add internal standard(s)* and 1mL of β -Glucuronidase

β -Glucuronidase: 5,000 F units/mL *Patella vulgata* in 100mM acetate buffer (pH 5.0)

Mix/vortex

Hydrolyze for 3 hours at 65°C

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

2mL of 20% acetonitrile in 100mM phosphate buffer (pH 6.0)

Dry column (5 minutes at >10 "Hg)

2mL hexane

Elute Flunitrazepam, 7-Aminoflunitrazepam and Desmethylflunitrazepam

3mL of ethyl acetate with 2% NH₄OH

Collect eluate at 1 to 2mL/min

NOTE: Prepare elution solvent daily

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50 μ L of ethyl acetate and 50 μ L MTBSTFA (with 1% TBDMCS)***

Overlay with N₂ and cap

Mix/vortex

React for 20 minutes at 70°C

Remove from heat source to cool

NOTE: Do not evaporate MTBSTFA solution

Quantitate

Inject 1 to 2 μ L onto gas chromatograph

For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
Flunitrazepam	312	286	266
7-aminoflunitrazepam	283	255	254
Desmethylflunitrazepam	356	357	310
D ₅ -oxazepam*	462	464	463

* Suggested internal standard

** Quantitation ion

*** Part number TS-48927 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 μ m	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 μ m	26AC497P

Gabapentin in Serum, Plasma or Whole Blood for GC or GC/MS Confirmations

Using 100mg 1mL HyperSep C18 Extraction Column (Part Number: 60108-302)

Prepare Sample

To 500µL of blood, plasma or serum, add internal standard*

Vortex tube and add 500µL of 20% acetic acid and vortex tube again

Centrifuge as appropriate

Condition C18 Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM HCl

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

3mL of ethyl acetate

3mL hexane

Dry column (5 minutes at >10" Hg or until column is dry)

Elute Gabapentin

1mL of 2% NH₄OH in CH₃OH

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50µL of ethyl acetate and 50µL of BSTFA (with 1% TMCS)** or

Add 50µL of MTBSTFA (with 1% TBDMCS)*** and 50µL ethyl acetate

Cap and heat at 70°C for 30 minutes

Remove and allow to cool

Quantitate

Inject 1 to 2µL onto gas chromatograph

For mass spectrometry, monitor the following ions:

Compound	Primary	Secondary	Tertiary
Gabapentin	210	225	182
Gabapentin-D10*	220	235	192

* Suggested internal standard

** Part number TS-38831 (10 x 1mL ampules)

*** Part number TS-48927 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Reference

Wolf, C.E. II, Sady J., and Pokalis, A. (1996). Determination of gabapentin in serum using solid phase extraction and gas chromatography. *Journal of Analytical Toxicology*, 20, 498-501.

Gamma-Hydroxybutyrate (GHB) in Blood, Urine and Tissue

Using 200mg 6mL HyperSep Retain-AX Extraction Column (Part Number: 60107-412)

Sample Preparation

GHB standard; 200µg/mL in H₂O

GHB -D6 internal standard; 100µg/mL

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

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

Add 25µL of internal standard*

Add 1mL of acetone

Mix/vortex 15 seconds

Centrifuge

Transfer acetone layer to culture tubes

Evaporate extracts with N₂ at 70°C

Reconstitute extracts with 200µL of 100mM Phosphate Buffer (pH 6.0)

Mix/vortex 15 seconds

Condition HyperSep Retain AX Extraction Column

1 x 3mL of CH₃OH

1 x 3mL of DI H₂O

1 x 1mL of 100mM Phosphate Buffer (pH 6.0)

Apply Sample

Add sample with Eppendorf pipette

Elute

Place clean test tubes into vacuum manifold

Add 1mL of CH₃OH/NH₄OH (99:1) to original sample test tube

Mix/vortex

Decant onto column and collect extract

Aspirate ~1" Hg

Concentrate

Remove test tube from Vacuum Manifold

Evaporate to dryness at 70°C using N₂

Derivatize

Add 100µL of ethyl acetate and 100µL of BSTFA with 1% TCMS**

Mix/vortex

Heat at 70°C for 30 minutes

Analysis

Inject 1 to 2µL onto GC/MS

For mass spectrometry monitor the following ions:

Compound	Primary Ion *	Secondary	Tertiary
GHB-D6-di-TMS*	239	240	241
GHB-di-TMS	233	234	235

* Suggested internal standard for GC/MS: D6-GHB

** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-35MS 30m x 0.25mm x 0.25µm	26094-1420
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Gamma-Hydroxybutyrate (GHB) in Urine without Conversion to Gamma-Butyrolactone (GBL)

Using 200mg 6mL HyperSep Retain-AX Extraction Column (Part Number: 60107-412)

Sample Preparation

200µL of urine
Add internal standard* and 100µL of 100mM phosphate buffer (pH 6.0)
Mix/vortex

Condition HyperSep Retain AX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH= 6.0)

Apply Sample

Place test tubes into vacuum manifold for collection
The sample loading and wash are both collected
Decant sample onto column. Aspirate at ~1"Hg

Wash Column

Add 1mL of CH₃OH/NH₄OH (99:1) to original sample test tube
Mix/vortex
Decant wash onto column
Evaporate to dryness at 60°C using a stream of air or N₂

Sample Clean-Up

Add 200µL of dimethylformamide
Add 1mL of hexane saturated with dimethylformamide
Mix by inversion for 5 minutes
Centrifuge at 3,000rpm for 5 minutes
Transfer lower dimethylformamide layer to a clean test tube
Evaporate to dryness at <50°C using a stream of air or N₂

Derivatize

Add 100µL ethyl acetate and 100µL BSTFA (with 1% TMCS)**
Mix/vortex

Analysis

Inject 1 to 2µL onto GC/MS
For mass spectrometry monitor the following ions:

Compound	Primary Ion *	Secondary	Tertiary
GHB-D6-di-TMS*	239	240	241
GHB-di-TMS	233	234	235

* Suggested internal standard for GC/MS: GHB-D6

** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-35MS 30m x 0.25mm x 0.25µm	26094-1420
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Ketamine in Urine, Blood, Plasma/Serum for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Add 1 to 2mL of urine, blood or plasma/serum

Mix/vortex

Add 2mL of 100mM phosphate buffer (pH 6.0)

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

3mL of 100mM acetic acid

3mL CH₃OH

Dry column (5 minutes at >10" Hg or until column is dry)

Elute Ketamine

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/minute using minimal vacuum

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

Dry Eluate

Evaporate to dryness at <40°C

Reconstitute with 100µL ethyl acetate

Quantitate

Inject 1 to 2µL onto gas chromatograph

For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
D ₄ -ketamine*	184	213	156
Ketamine	180	209	152

* Suggested internal standard

** Quantitation ion

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Lysergic Acid Diethylamide (LSD) in Serum, Plasma or Whole Blood for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of blood, plasma or serum, add 4mL of DI H₂O and internal standard*

Mix/vortex and let stand for 5 minutes

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Add 2mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

3mL of 100mM acetic acid

3mL CH₃OH

Dry column (5 minutes at >10" Hg)

Elute LSD

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/minute using minimal vacuum

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 20µL ethyl acetate and 20µL BSTFA (with 1% TMCS)**

Overlay with N₂ and cap

Mix/vortex

React for 30 minutes at 70°C

Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution

Quantitate

Inject 1 to 2µL onto gas chromatograph

For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
D ₃ -LSD-TMS*	298	296	271
LSD-TMS	395	293	268

* Suggested internal standard

** Quantitation ion

*** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Lysergic Acid Diethylamide (LSD) in Urine for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

- To 5mL of urine add internal standard and 2mL of 100mM phosphate buffer (pH 6.0)
- Mix/vortex
- Sample pH should be 6.0 ± 0.5
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate
- Centrifuge as appropriate

Condition Verify-CX Extraction Column

- 3mL of CH_3OH then aspirate
- 3mL of $\text{DI H}_2\text{O}$ then aspirate
- 1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at $<3''\text{Hg}$ to prevent sorbent drying

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 3mL of $\text{DI H}_2\text{O}$
- 3mL of 100mM acetic acid
- 3mL CH_3OH
- Dry column (5 minutes at $>10''\text{Hg}$)

Elute LSD

- 3mL of $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78:20:2)
- Collect eluate at 1 to 2mL/minute using minimal vacuum

NOTE: Prepare elution solvent fresh daily; add $\text{IPA}/\text{NH}_4\text{OH}$ mix, then add CH_2Cl_2 (pH 11-12)

Dry Eluate

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

Derivatize

- Add 20 μL ethyl acetate and 20 μL BSTFA (with 1% TMCS)***
- Overlay with N_2 and cap
- Mix/vortex
- React for 20 minutes at 70°C
- Remove from heat source to cool

NOTE: Do not evaporate BSTFA solution

Quantitate

- Inject 1 to 2 μL onto gas chromatograph
- For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
$\text{D}_3\text{-LSD-TMS}^*$	298	296	271
LSD-TMS	395	293	268

* Suggested internal standard

** Quantitation ion

*** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 μm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 μm	26AC497P

LSD and Metabolites in Blood, Plasma, Serum and Urine

Using 200mg 3mL HyperSep Aminopropyl Extraction Column (Part Number: 60108-425)

Sample Preparation

1mL of 100mM phosphate buffer (pH 6)
Add internal standards*
Add 1mL of whole blood, serum/plasma, urine
Add 2mL of 100mM phosphate buffer (pH 6)
Mix/vortex
Centrifuge

Condition HyperSep Aminopropyl Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH 6)

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 3mL 100mM acetic acid
1 x 3mL CH₃OH
Dry column (5 minutes at >10" Hg)

Elute

1x 3mL ethyl acetate; ACN:ammonia (78:20:2 v/v)
Collect eluate at 1 to 2mL/minute
Evaporate eluates to dryness under a gentle stream of nitrogen

Analysis

Reconstitute sample in 50µL of CH₃OH

Inject 5µL on to LC/MS

Mobile phase:

Time%	ACN%	0.1% Formic Acid
0	30	70
3.0	90	10
3.1	30	70
5.0	30	70

Flow rate: 0.5mL/minute

Column temperature: ambient

Detector: Thermo Scientific TSQ Triple Quadrupole

Compound	MRM Transition
LSD	324.2/223.1
Iso-LSD	324.2/281 (223.1)
Nor-LSD	310.2/209.1
OH-LSD	356.2/338.1
LSD-D3*	327.2/226.1

* Suggested internal standard: LSD-D3

Recovery: >90% (N=10)

LOD: 0.1ng/mL

Recommended HPLC Column	Part Number
Hypersil GOLD PFP 3µm, 100 x 2.1mm	25403-102130

Meperidine and Normeperidine in Blood, Plasma, Serum and Urine

Using 200mg 3mL HyperSep Aminopropyl Extraction Column (Part Number: 60108-425)

Sample Preparation

1mL of 100mM phosphate buffer (pH 6)
Add internal standard*
Add 1mL of whole blood, serum/plasma, urine
Add 2mL of 100mM phosphate buffer (pH 6)
Mix/vortex
Centrifuge

Condition HyperSep Aminopropyl Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O
1 x 1mL 100mM phosphate buffer (pH 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 3mL 100mM acetic acid
1 x 3mL CH₃OH
Dry column (5 minutes at >10" Hg)

Elute

1 x 3mL ethyl acetate; ACN:ammonia (78:20:2 v/v)
Collect eluate at 1 to 2mL/minute
Evaporate eluates to dryness under a gentle stream of nitrogen

Analysis

Reconstitute sample in 50μL of CH₃OH

Inject 5μL onto LC/MS

Mobile phase:

Time %	ACN %	0.1% Formic Acid
0	90	10
5	30	70
6	90	10
10	90	10

Flow rate: 0.35mL/minute

Column temperature: ambient

Detector: Thermo Scientific TSQ Triple Quadrupole

Compound	MRM Transition
Meperidine	248.2/220.0
Meperidine-D4*	252.2/224.1
Normeperidine	234.1/160.0
Normeperidine-D4*	238.1/164.0

* Suggested internal standard: Meperidine-D4 and Normeperidine-D4

Recovery: >90% (n=10)

LOD: 10ng/mL

Recommended HPLC Column

Part Number

Hypersil GOLD 3μm, 150 x 2.1mm

25003-152130

Methadone/EDDP in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Condition Verify-CX Extraction Column

- 3mL of CH₃OH then aspirate
- 3mL of DI H₂O then aspirate
- 1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 3mL of DI H₂O
- 1mL of 100mM acetic acid
- 3mL CH₃OH
- Dry column (5 minutes at >10" Hg)

Elute Methadone/EDDP

- 3mL of ethyl acetate/acetonitrile/ammonia (78:20:2, v/v/v)
- Collect eluate at 1 to 2mL/min

NOTE: Prepare elution solvent fresh daily

Evaporate

- Evaporate to dryness at <40°C

Reconstitute

- Reconstitute sample in 100µL of methanol

Analysis

- Inject 5µL of sample onto LC/MS system
- Monitor the following ions:

Compound	MRM Transition
Methadone	310.2/105.1
Methadone-D9*	319.2/268.3
EDDP	278.2/234.2
EDDP-D3*	281.4/234.3

* Suggested internal standards

Mobile phase:

Time (min)	% Acetonitrile	% 0.1% Formic Acid
0	25	75
5	25	75
14	90	10
15	25	75
20	25	75

Flow rate: 0.35mL/min

Column temperature: ambient

Recommended HPLC Column	Part Number
Hypersil GOLD aQ 3µm, 150 x 2.1mm	25303-152130

Methadone in Urine for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

- To 2mL of urine add internal standard* and 1mL of 100mM phosphate buffer (pH 6.0)
- Mix/vortex
- Sample pH should be 6.0±0.5
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

- 3mL of CH₃OH then aspirate
- 3mL of DI H₂O then aspirate
- 1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 3mL of DI H₂O
- 1mL of 100mM acetic acid
- 3mL CH₃OH
- Dry column (5 minutes at >10" Hg)

Elute Methadone

- 3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)
- Collect eluate at 1 to 2mL/min

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

Concentrate

- Evaporate to dryness at <40°C
- Reconstitute with 100µL acetonitrile

Quantitate

- Inject 1 to 2µL onto gas chromatograph
- For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
D ₉ -Methadone*	78	226	303
Methadone	72	223	294

* Suggested internal standard

** Quantitation ion

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Methaqualone in Urine, Blood, Plasma/Serum for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

- To 2mL of urine, blood, plasma/serum, add internal standard* and 1mL of 100mM phosphate buffer (pH 6.0)
- Mix/vortex
- Add 2mL of 100mM phosphate buffer (pH 6.0)
- Mix/vortex
- Sample pH should be 6.0±0.5
- Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate
- Centrifuge as appropriate

Condition Verify-CX Extraction Column

- 3mL of CH₃OH then aspirate
- 3mL of DI H₂O then aspirate
- 1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 3mL of DI H₂O
- Dry column (5 minutes at >10" Hg)
- 2mL of hexane

Elute Methaqualone

- 3mL of hexane/ethyl acetate (50:50); collect eluate

Dry Eluate

- Evaporate to dryness at <40°C
- Reconstitute with 100µL ethyl acetate

Quantitate

- Inject 1 to 2µL onto gas chromatograph
- For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
Methaqualone	235	250	233
Hexobarbital*	221	157	156
Methaqualone-D7*	240	257	240

* Suggested internal standard

** Quantitation ion

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Methaqualone in Urine, Blood, Plasma/Serum for LC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Add 1mL of urine, whole blood, plasma/serum

Add 2mL of 100mM phosphate buffer (pH 6.0)

Vortex and centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

Dry column (5 minutes at >10"Hg)

2mL of hexane

Elute Methaqualone

3mL of hexane/ethyl acetate (50:50)

Collect eluate at 1 to 2mL/min

Dry Eluate

Evaporate to dryness at <40°C

Reconstitute

Reconstitute with 100µL of methanol

Analysis

Inject 5µL of sample onto LC/MS system

Monitor the following ions:

Compound	MRM Transition
Methaqualone	251.2/132.1
Methaqualone-D7*	258.2/138.2

* Suggested internal standard

Mobile phase: acetonitrile: 0.1% formic acid (30:70, v/v)

Flow rate: 0.35mL/min

Column temperature: ambient

Recommended HPLC Column

Part Number

Hypersil GOLD aQ 3µm, 150 x 2.1mm 25303-152130

Nicotine and Continine in Urine or Serum for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 2mL of urine or serum add internal standard(s)* and 2mL of 100mM of phosphate buffer (pH 6.0)

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Mix/vortex and centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

2mL of 200mM HCl

Dry column (5 minutes at >10"Hg)

2mL of hexane

Wash Column

Remove rack of collection tubes to re-wash columns

3mL of CH₃OH

Dry column (5 minutes at >10"Hg)

Elute Nicotine and Continine

Replace rack of collection tubes

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Concentrate

Evaporate to dryness at <40°C

Take care not to overheat or over evaporate

Reconstitute with 100µL ethyl acetate

Quantitate

Inject 1 to 2µL onto gas chromatograph

Monitor the following ions (GC/MS):

Compound	Primary Ion**	Secondary	Tertiary
Nicotine	84	133	162
Nicotine-D4*	88	137	166
Continine	98	119	176
Continine-D3*	101	122	179

* D₃-Continine and D₄-Nicotine are available as deuterated internal standards

** Quantitation ions

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Opiates in Urine – Oxime TMS Procedure for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample – Acid Hydrolysis of Glucuronides

To 2mL of urine add internal standard(s)* and 400µL of concentrated HCl

Add 200µL of 10% Hydroxylamine solution

Mix/vortex

Heat to 90°C for 40 minutes in a heating block, or an autoclave for 15 minutes on a liquid cycle

Cool before proceeding

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Add 500µL of 50% ammonium hydroxide

Mix/vortex

Adjust sample pH to 5-6 by drop-wise addition of 50% ammonium hydroxide

Prepare Sample – Enzyme Hydrolysis of Glucuronides

To 2mL of urine add internal standard(s)* and enzyme preparation in buffer

Mix/vortex

Heat to 60°C for a sufficient time in a heating block (The time depends upon analytes and enzyme)

Add 200µL of 10% hydroxylamine solution

Heat to 60°C for 30 minutes in a heating block

Adjust pH to 5-6

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3"Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

3mL of 100mM acetate buffer (pH 4.5)

3mL of CH₃OH

Dry column (5 minutes at >10"Hg)

Elute Opiates

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 100µL of ethyl acetate and 100µL of BSTFA (with 1% TMCS)***

Overlay with N₂ and cap

Mix/vortex

React for 45 minutes at 70°C in a heat block

Remove from the heat source to cool

NOTE: Do not evaporate BSTFA solution

Quantitate

Inject 1 to 2µL onto gas chromatograph

For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
D ₄ -meperidine*	251	222	250
Meperidine	247	218	246
D ₄ -normeperidine TMS*	308	280	309
Normeperidine TMS*	305	276	304
Tramadol TMS	335	245	290
O-desmethyiltramadol TMS	393	378	303
N-desmethyiltramadol TMS	393	378	116
Pentazocine TMS	357	342	289
D ₃ -codeine TMS*	374	359	346
D ₆ -codeine TMS*	377	349	316
Codeine TMS	371	356	343
Norcodeine TMS	429	414	356
Dihydrocodeine TMS	373	315	358
D ₃ -morphine TMS*	432	417	404
D ₆ -morphine TMS*	435	420	404
Morphine TMS	429	414	401
Nomorphine TMS	487	472	414
Diacetylmorphine	369	327	268
D ₃ -hydrocodone Oxime TMS	389	300	374
D ₆ -hydrocodone Oxime TMS*	392	303	377
Hydrocodone Oxime TMS	386	297	371
D ₃ -hydromorphone Oxime TMS	447	432	358
Hydromorphone Oxime TMS	444	429	355
D ₃ -oxycodone Oxime TMS	477	462	420
D ₆ -oxycodone Oxime TMS*	480	465	420
Oxycodone Oxime TMS	474	459	417
D ₃ -oxymorphone Oxime TMS	535	520	290
Oxymorphone Oxime TMS	532	517	287

* Suggested internal standards for GC/MS; suggest trying D₆-codeine and D₆-morphine for lowest LOD/LOQ

** Quantitation ion

*** Part number TS38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Opiates in Urine – Propyl Derivatives for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample – Acid Hydrolysis of Glucuronides

To 2mL of urine add internal standard(s)* and 400µL of concentrated HCl

Add 200µL of 10% hydroxylamine solution

Mix/vortex

Heat to 90°C for 40 minutes in a heating block, or an autoclave for 15 minutes on a liquid cycle

Cool before proceeding

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Add 500µL of 50% ammonium hydroxide

Mix/vortex

Adjust sample pH to 5-6 by drop-wise addition of 50% ammonium hydroxide

Prepare Sample – Enzyme Hydrolysis of Glucuronides

To 2mL of urine add internal standard(s)* and 1mL of β-Glucuronidase solution (β-Glucuronidase solution contains 5,000 F units/mL)

Hydrolyze for 3 hours at 60°C

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Adjust sample pH to 5-6 with 1.0 M NaOH

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

3mL of 100mM acetate buffer (pH 4.5)

3mL of CH₃OH

Dry column (5 minutes at >10" Hg)

Elute Opiates

3mL of CH₂Cl₂/IPA/NH₄OH (84:12:4)

Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 200µL of a 1:1 solution of propionic anhydride:pyridine

NOTE: Make this solution fresh daily

Mix/vortex

React for 60 minutes at 60°C in a heat block

Remove from the heat source to cool

Evaporate to dryness at <40°C

Reconstitute the residue with 50µL of ethyl acetate/methanol (70:30, v/v)

Quantitate

Inject 1 to 2µL onto gas chromatograph

For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
Hydrocodone	299	242	214
Codeine	355	282	229
Codeine-D3*	358	285	232
Oxycodone	371	314	298
Hydromorphone	285	341	228
6-Acetylmorphine	327	268	383
Oxymorphone	357	300	413
Morphine	341	268	397
Morphine-D3*	344	271	400

* Suggested internal standards

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Free (Unbound) Opiates in Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM phosphate buffer (pH 6.0) add internal standards*
Add 1mL of blood, plasma/serum or 1g (1:4) of tissue homogenate
Mix/vortex and allow to stand for 5 minutes
Add 2mL of 100mM phosphate buffer (pH 6.0)
Mix/vortex
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate
Centrifuge for 10 minutes at 2,000rpm and discard pellet

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
3mL of 100mM acetate buffer (pH 4.5)
3mL of CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Opiates

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

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50µL of ethyl acetate and 50µL BSTFA (with 1% TMCS)**
Overlay with N₂ and cap
Mix/vortex
React for 30 minutes at 70°C
Remove from the heat source to cool

NOTE: Do not evaporate BSTFA solution

Quantitate

Inject 1 to 2µL onto gas chromatograph
For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
Codeine-D3-TMS*	374	237	346
Codeine-TMS	371	234	343
Morphine-D3-TMS*	432	290	327
Morphine-TMS	429	287	324

* Suggested internal standard

** Quantitation ion

***Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Paroxetine in Urine, Blood and Plasma/Serum for LC/MS/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Add 1mL of urine, blood or plasma/serum

Add 2mL of 100mM phosphate buffer (pH 6.0)

Vortex and centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

3mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

3mL of 100mM acetic acid

3mL of CH₃OH

Dry column (5 minutes at >10"Hg)

Elute Paroxetine

3mL of ethyl acetate/acetonitrile/ammonium hydroxide (78:20:2, v/v/v)

Collect eluate at 1 to 2mL/minute

Evaporate

Evaporate to dryness at <40°C

Reconstitute

100µL of CH₃OH

Analysis

Inject 5µL of sample onto LC/MS/MS system

Monitor the following ions:

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

* Suggested internal standard

Mobile phase:

Time (min)	% Acetonitrile	% 0.1% Formic Acid
0	10	90
15	50	50
16	10	90
20	10	90

Flow rate: 0.35mL/min

Column temperature: ambient

Recommended HPLC Column

Part Number

Hypersil GOLD Phenyl 3µm, 150 x 2.1mm

25903-152130

Phencyclidine in Urine

Using 30mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-302)

Sample Preparation

1mL of urine

Add internal standard* and 1mL 100mM phosphate buffer (pH 6.0)

Mix/vortex

Condition HyperSep Retain-CX Extraction Column

Load at a rate of 1 to 2mL/min

Wash Column

1 x 1mL DI H₂O

1 x 1mL 100mM acetic acid

1 x 1mL CH₃OH

Dry column (3 minutes at >10"Hg)

Elute

2 x 0.5mL CH₂Cl₂/IPA/NH₄OH (78/20/2)

Collect eluate at 1 to 2mL/min

Add 1 drop 1% HCl in CH₃OH to eluate before evaporating

Evaporate to dryness at <40°C

Reconstitute with 100µL ethyl acetate

Analysis

Inject 1 to 2µL onto GC/MS

For mass spectrometry monitor the following ions:

Compound	Target (Quantitation) Ion	Qualifier Ions
Phencyclidine	200	91, 242
Phencyclidine-D5*	205	96, 247

* Suggested internal standard: Phencyclidine-D5

Recommended GC Column

Part Number

TraceGOLD TG-5SiIMS 30m x 0.25mm x 0.25µm

26096-1420

Phencyclidine in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
3mL of 100mM acetic acid
3mL of CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Phencyclidine

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

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

Dry Eluate

Evaporate to dryness at <40°C
Remove immediately upon completion
Reconstitute with 100µL ethyl acetate

Quantitate

Inject 1 to 2µL onto gas chromatograph
For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
D ₅ -phencyclidine*	205	96	247
Phencyclidine	200	91	242

* Suggested internal standard

** Quantitation ion

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Phencyclidine in Urine, Blood and Plasma/Serum for LC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM phosphate buffer (pH 6.0) add internal standard(s)*
Add 1mL of urine, blood, plasma/serum
Add 2mL of 100mM phosphate buffer (pH 6.0)
Mix/vortex
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate
Mix/vortex and centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
3mL of 100mM acetic acid
3mL of CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Phencyclidine

3mL of ethyl acetate/acetonitrile/ammonia (78:20:2, v/v/v)
Collect eluate at 1 to 2mL/min

NOTE: Prepare elution solvent fresh daily

Evaporate

Evaporate to dryness under a stream of nitrogen at <40°C

Reconstitute

Reconstitute sample in 100µL of CH₃OH

Analysis

Inject 5µL of sample onto LC/MS/MS system
Monitor the following ions:

Compound	MRM Transition
Phencyclidine	244.3/86.1
Phencyclidine-D ₅ *	249.3/86.1

* Suggested internal standard

Mobile phase: acetonitrile/0.1% formic acid (33:67, v/v)
Flow rate: 0.35mL/min
Column temperature: ambient

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 2.1mm	25003-152130
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Propoxyphene in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
3mL of 100mM acetic acid
3mL of CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Propoxyphene

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

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

Concentrate

Evaporate to dryness at <40°C
Reconstitute with 100µL ethyl acetate

Quantitate

Inject 1 to 2µL onto gas chromatograph
For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary	Other
D ₅ -propoxyphene*	63	120	213	255,270
Propoxyphene	58	115	208	250,265

* Suggested internal standard

** Quantitation ion

NOTE: To improve the analysis for Norpropoxyphene, the primary metabolite of Dextropropoxyphene, add 1 drop of 35% sodium hydroxide solution to the urine sample and, then after mixing, bring the pH to 6 for SPE extraction (this step converts the Norpropoxyphene to Norpropoxyphene amide, a more stable compound)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Propoxyphene and Norpropoxyphene in Urine, Blood, Plasma/Serum and Tissue for LC/MS/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of 100mM phosphate buffer (pH 6.0) add internal standard*
Add 1mL of urine, blood, plasma/serum, or 1g (1:4) of tissue homogenate
Add 2mL of 100mM phosphate buffer (pH 6.0)
Vortex and centrifuge as appropriate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
3mL of 100mM acetic acid
3mL of CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Propoxyphene/Norpropoxyphene

3mL of ethyl acetate/acetonitrile/ammonia (78:20:2, v/v/v) OR
3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2, v/v/v)
Collect eluate at 1 to 2mL/min

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

Evaporate

Evaporate to half volume under a stream of nitrogen at <40°C
Add 100μL of 0.1% HCl in CH₃OH
Mix/vortex
Continue evaporation to dryness at <40°C

Reconstitute

Reconstitute sample in 100μL of CH₃OH

Analysis

Inject 5μL of sample onto LC/MS/MS system
Monitor the following ions:

Compound	MRM Transition
Propoxyphene	340.0/58.0
Propoxyphene-D11*	351.2/64.0
Norpropoxyphene	326.0/252.0
Norpropoxyphene-D5*	331.0/257.0

Flow rate: 0.35mL/min

Column temperature: ambient

Mobile phase:

Time (min)	% Acetonitrile	% of 0.1% Formic Acid
0	30	70
10	30	70

Recommended HPLC Column	Part Number
Hypersil GOLD Phenyl 3μm, 50 x 2.1mm	25903-052130

Psilocin in Urine for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 5mL of urine add internal standard* (Psilocin-D10-TMS) and 2mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

Add 12,500 to 25,000 units of β -glucuronidase, then mix/vortex

Place the sample into a water bath at 45°C for 90 minutes

Remove from the bath and allow to cool

Centrifuge at 3,000rpm for 10 minutes

Use the clear filtrate (discard the plug) for SPE

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

2mL of 20% acetonitrile in water

1mL of 100mM acetic acid

Dry column (3 minutes at >10" Hg)

2mL of hexane

3mL of hexane/ethyl acetate (50:50)

3mL of CH₃OH

Dry column (3 minutes at >10" Hg)

Elute Psilocin

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at <35°C

Derivatize

Add 50 μ L of ethyl acetate

Mix/vortex

Add 50 μ L of MSTFA***

React at 70°C for 30 minutes

Remove from heat

NOTE: Do not evaporate MSTFA solution

Quantitate

Inject 1 to 2 μ L onto gas chromatograph

For mass spectrometry, monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
PSILOCIN-TMS	290	348	73 (291)
PSILOCIN-D10-TMS*	300	358	83 (301)

* Suggested internal standard

** Quantitation ion

*** Part number TS-48910 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 μ m	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 μ m	26AC497P

Source

Greishaber A., Moore, K. Levine, B. and Smith M. (1999, November). The detection of psilocin in human urine. Presented at the TRI Services Meeting.

Quetiapine in Urine, Blood, Plasma/Serum and Tissue for LC/PDA Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Condition Verify-CX Extraction Column

- 3mL of CH₃OH then aspirate
- 3mL of DI H₂O then aspirate
- 1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 3mL of phosphate buffer
- 3mL of 100mM acetic acid
- 3mL of CH₃OH
- Dry column (5 minutes at >10" Hg)
- 3mL of hexane
- Dry column (5 minutes at >10" Hg)

Elute Quetiapine

- 3mL of ethyl acetate/acetonitrile/ammonia (78:20:2, v/v/v)
- Collect eluate at 1 to 2mL/min

NOTE: Prepare elution solvent fresh daily

Evaporate

- Evaporate eluates gently under a stream of nitrogen at <40°C

Reconstitute

- Reconstitute sample in 100µL of 0.1% trifluoroacetic acid (aq)

Analysis

- Inject 50µL of sample onto LC/MS/MS system
- Monitor the following ions:

Compound

Quetiapine

Quinidine*

* Suggested internal standard

Flow rate: 1mL/min

Column temperature: ambient

Mobile phase: acetonitrile:0.1% trifluoroacetic acid (25:75)

Detector: diode array (250nm)

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 4.6mm

25003-154630

Screening in Whole Blood (Manual Method for Immunoassay)

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of blood add 4mL of H₂O (pH 5 to 7)
Mix/vortex
Let sample stand for 5 minutes to lyse red blood cells
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Add 2mL of 100mM phosphate buffer (pH 6.0)
Mix/vortex
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
3mL of acetic acid
Dry column (5 minutes at >10"Hg)
2mL of hexane

Elute Acidic and Neutral Drugs

3mL of hexane/ethyl acetate (50:50)
Collect eluate at <5mL/minute
Remove collection tubes

Wash Column

3mL of CH₃OH
Dry column (5 minutes at >10"Hg)

Elute Basic Drugs

Replace collection tubes from elution of acidic and neutral drugs
3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)
Collect eluate at 1 to 2mL/minute using minimal vacuum

NOTE: Elute into the tubes containing the acidic and neutral drugs. Prepare elution solvent fresh daily; add IPA/NH₄OH mix, then add CH₂Cl₂ (pH 11-12)

Dry Elute – Combine Eluates Acidic and Neutral drugs and Basic Drugs

Evaporate to a volume 100µL at <40°C

Reconstitute

Add 900µL of normal saline (sample volume is now its original 1.0mL)

Analyze as Appropriate

Process according to urine drug screening protocols provided by immunoassay manufacturer

Sertraline and Desmethylertraline in Serum, Plasma or Whole Blood for HPLC Analysis

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 4mL of DI H₂O add 2mL of 100mM phosphate buffer (pH 6.0)
Add internal standard
Add 1mL of urine, blood or plasma/serum
Mix/vortex
Centrifuge for 10 minutes at 2,000rpm and discard pellet
Sample pH should be 6.0±0.5
Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
1mL of 100mM acetic acid
3mL of CH₃OH
Dry column (5 minutes at >10"Hg)

Elute Sertraline and Desmethylertraline

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

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

Dry Eluate

Evaporate to dryness at <40°C

Reconstitute

Reconstitute with 200µL of ethyl acetate/DI H₂O (1:3)
Mix/vortex vigorously for 30 seconds

Analysis

Inject 100µL onto Isocratic LC system at wavelength 235nm
Mobile phase: 0.25 M potassium phosphate (pH 2.7) containing 30% CH₃CN
Flow rate: 2mL/minute

Recommended HPLC Column

Part Number

Hypersil GOLD C8 3µm, 150 x 4.6mm

25203-154630

Free and Conjugated Silocin in Urine

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Sample Preparation

- 1mL of 100mM phosphate buffer (pH 6)
- Add internal standard*
- Add 1mL of urine sample
- Add 2mL of 100mM phosphate buffer (pH 6)
- Mix/vortex
- Centrifuge

Urine Hydrolysis

- 1mL of urine
- Add internal standard* and 1mL of β -glucuronidase solution (β -glucuronidase solution contains: 5,000 F units/mL Patella vulgata in 100mM acetate buffer (pH=5.0))
- Mix/vortex
- Hydrolyze for 3 hours at 65°C
- Centrifuge for 10 minutes at 2,000rpm and discard pellet

Condition HyperSep Verify-CX Extraction Column

- 1 x 3mL CH₃OH
- 1 x 3mL DI H₂O
- 1 x 1mL 100mM phosphate buffer (pH 6)

NOTE: Aspirate at <3" Hg to prevent sorbent drying out

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 1 x 3mL DI H₂O
- 1 x 3mL 100mM acetic acid
- 1 x 3mL CH₃OH
- Dry column (5 minutes at >10"Hg)

Elute

- 1 x 3mL ethyl acetate; ACN:ammonia (78:20:2 v/v)
- Collect eluate at 1 to 2mL/minute
- Evaporate eluates to dryness under a gentle stream of nitrogen

Analysis

- Reconstitute sample in 50 μ L of CH₃OH
- Inject 5 μ L onto LC/MS
- Mobile phase:

Time	% ACN	%0.1% Formic Acid
0	20	80
5	20	80

- Flow rate: 0.20mL/minute
- Column temperature: ambient
- Detector: Thermo Scientific TSQ Triple Quadrupole

Compound	MRM Transition
Psilocin	205.2/58.2
Psilocin-D10*	215.2/68.2

* Suggested internal standard: Psilocin-D10

- Recovery >90% (n=10)
- LOD: 10ng/mL

Recommended HPLC Column

Part Number

Hypersil GOLD PFP 3 μ m, 100 x 2.1mm	25403-102130
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Sympathomimetic Amines in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Alternative Drying Procedure – Fluoroacylate PFPA (PFAA) Derivative

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

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

Condition Verify-CX Extraction Column

- 3mL of CH₃OH then aspirate
- 3mL of DI H₂O then aspirate
- 1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 3mL of DI H₂O
- 1mL of 100mM acetic acid
- 3mL of CH₃OH
- Dry column (5 minutes at >10 "Hg)

Elute SMA

- 3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)
- Collect eluate at 1 to 2mL/min

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

Concentrate Eluate

- Add 30μL silylation grade DMF*** to eluate
- Evaporate to 30μL at $<40^{\circ}\text{C}$

Alternate Drying Procedure – Fluoroacylate PFPA (PFAA) Derivative

- Add 50μL PFPA (PFAA)****
- Overlay with N₂ and cap
- Improved derivatization by addition of 50μL PFPOH†
- React for 20 minutes at 70°C
- Evaporate to dryness at $<40^{\circ}\text{C}$
- Reconstitute with 100μL ethyl acetate

Quantitate

- Inject 1 to 2μL onto gas chromatograph
- For mass spectrometry, monitor the following ions:

Analyte (PFPA)	Primary Ion**	Secondary	Tertiary
D ₅ -amphetamine*	194	92	123
Amphetamine	190	91	118
D ₅ -methamphetamine*	208	92	163
Methamphetamine	204	91	160
Pseudoephedrine	204	160	119
Ephedrine	204	160	119
Phenylephrine	190	119	267
Methylenedioxymphetamine	135	162	325
Methylenedioxymethamphetamine	204	162	339

* Suggested internal standards for GC/MS: D₅-amphetamine and D₅-methamphetamine

** Quantitation ion

*** Part number TS-20672 (50mL vial)

**** Part number TS-65193 (10 x 1mL ampules)

† Part number TS-65195 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25μm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33μm	26AC497P

Sympathomimetic Amines in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Alternative Drying Procedure – Form TMS Derivative

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 2mL of urine add internal standard(s)* and 1mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

1mL of 100mM acetic acid

3mL of CH₃OH

Dry column (5 minutes at >10"Hg)

Elute SMA

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Concentrate Eluate

Add 30μL silylation grade DMF to eluate

Evaporate to 30μL at <40°C

Alternate Drying Procedure – Form TMS Derivative

Add 50μL BSTFA (with 1% TMCS)**** and 50μL of ethyl acetate

React for 45 minutes at 70°C

Quantitate

Inject 1 to 2μL onto gas chromatograph

For mass spectrometry, monitor the following ions:

Analyte (TMS)	Primary Ion**	Secondary	Tertiary
D ₅ -amphetamine*	120	197	92
D ₆ -amphetamine*	120	198	93
D ₁₀ -amphetamine*	120	202	97
D ₁₁ -amphetamine*	120	203	98
Amphetamine	116	192	91
D ₅ -methamphetamine*	134	211	92
D ₈ -methamphetamine*	137	214	92
D ₉ -methamphetamine*	137	215	93
Methamphetamine	130	206	91
Pseudoephedrine	130	147	294
Ephedrine	130	147	294
Methylenedioxyamphetamine	116	236	135
Methylenedioxymethamphetamine	130	250	131
Para-Methoxamphetamine	116	222	121

* Suggested internal standards for GC/MS

** Quantitation ion

*** Part number TS-20672 (50mL vial)

**** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25μm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33μm	26AC497P

Sympathomimetic Amines in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

Alternative Drying Procedure – Form 4-CB (4-Carbethoxyhexafluorobutyl chloride) Derivative

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 2mL of urine add internal standard(s)* and 1mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

1mL of 100mM acetic acid

3mL of CH₃OH

Dry column (5 minutes at >10" Hg)

Elute SMA

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Concentrate Eluate

Add 30µL silylation grade DMF*** to eluate

Evaporate to 30µL at <40°C

Alternate Drying Procedure – Form 4-CB (4-Carbethoxyhexafluorobutyl chloride) Derivative

Add 20µL 4-CB and 100µL of ethyl acetate

React for 45 minutes at 70°C

Quantitate

Inject 1 to 2µL onto gas chromatograph

For mass spectrometry, monitor the following ions:

Analyte (4-CB)	Primary Ion**	Secondary	Tertiary
D ₅ -amphetamine*	298	270	399
Amphetamine	294	266	248
D ₅ -methamphetamine*	312	284	266
Methamphetamine	308	280	262
D ₅ -methylenedioxyamphetamine*	136	434	270
Methylenedioxyamphetamine	162	429	266
D ₅ -methylenedioxymethamphetamine*	312	284	266
Methylenedioxymethamphetamine	308	280	262
D ₆ -methylenedioxyethylamphetamine*	328	165	300
Methylenedioxyethylamphetamine	322	162	294

* Suggested internal standards

** Quantitation ion

*** Part number TS-20672 (50mL vial)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Tear Gas, Extraction of Chloroacetophenone (CS), O-chlorobenzylidenemalononitrile (CN) and Trans-8-methyl-N-vanillyl-6-nonenamide (OC) from Cloth for GC/MS

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

If suspected tear gas is on clothing, cut out a portion of the sprayed area and a negative control sample. Extract each of these samples into hexane. For canisters of suspected tear gas, spray onto a Kimwipe® or equivalent product and extract the sprayed area and a negative control into hexane.

Condition Verify-CX Extraction Column

3mL of CH₃OH
3mL of DI H₂O
1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3" Hg to prevent sorbent drying

Apply Sample

Load sample at 1mL/minute

Wash Column

3mL of DI H₂O
3mL of hexane
Dry column (5 minutes at >10" Hg)

Elute Analytes

1mL of CH₃OH

Dry Eluate

Evaporate to full dryness at <40°C under a stream of nitrogen

Reconstitute

Add 200µL of CH₃OH
Mix/vortex then transfer to a GC/MS vial and cap

Quantitate

Inject 1 to 2µL onto gas chromatograph

Tetrahydrocannabinol (THC) in Oral Fluid for GC/MS Confirmation

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of oral fluid add 50ng/mL internal standard (THCA D9-TMS) and let sit for 10 minutes at room temperature

Vortex for 10 seconds

Add 0.5mL of glacial acetic acid and vortex for 10 seconds

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate
3mL of DI H₂O then aspirate
1mL of 0.1N HCl then aspirate

Apply Sample

Load sample at 1mL/minute (do not exceed this flow rate)

Wash Column

2mL of DI H₂O
2mL of 0.1N HCl/acetonitrile (70:30)
Dry column (5 minutes at >10" Hg)
200µL of hexane

Elute THC

2mL of hexane/ethyl acetate (50:50)
Collect eluate at 1mL/minute (do not exceed this rate)

Dry Eluate

Evaporate to full dryness at <40°C under a stream of nitrogen

Derivatize

Add 50µL of MSTFA***
Vortex for 10 seconds then heat for 20 minutes at 60°C
Vortex for 10 seconds while hot
Reconstitute in 50µL of ethyl acetate

Quantitate

Inject 2µL onto gas chromatograph
Monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
THCA-TMS	371	386	387
THCA D9-TMS*	380	479	

* Suggested internal standard

** Quantitation ion

*** Part number TS-48910 (10 x 1mL ampule)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Source

Janet Putnam, Assistant Laboratory Director/RP Advanced Toxicology Network, Memphis, TN

Tetrahydrocannabinol (THC) in Oral Fluid for GC/MS Confirmation

Using 50mg 1mL HyperSep Verify-CX Extraction Column (Part Number: 60108-741)

Prepare Sample

- Add 100 to 500µL of neat sample to a clean test tube
- Add internal standard*
- Vortex and let sit for 10 minutes at room temperature
- Add 0.5mL of glacial acetic acid and mix/vortex for 10 seconds

Condition Verify-CX Extraction Column

- 200µL of CH₃OH
- 200µL of DI H₂O
- 200µL of 100mM HCl

Apply Sample

- Load sample at 1mL/minute (do not exceed this flow rate)

Wash Column

- 500µL of DI H₂O
- 500µL of 0.2N HCl
- 500µL of 100mM HCl/acetonitrile (70:30)
- Dry column (1 minute at >10"Hg)

Elute THC

- 800µL of hexane/ethyl acetate (75:25)
- Collect eluate at 1mL/minute (do not exceed this rate)

Dry Eluate

- Evaporate to full dryness at <40°C under a stream of nitrogen

Derivatize

- Add 25µL of BSTFA (with 1% TMCS)** and 25µL of ethyl acetate
- Overlay with nitrogen and cap
- Vortex then react for 30 minutes at 70°C
- Remove from heat and allow to cool

NOTE: Do not evaporate BSTFA solution

Quantitate

- Inject 2µL onto gas chromatograph
- Monitor the following ions:

Compound	Primary Ion**	Secondary	Tertiary
THC-TMS	371	386	303
THC D3-TMS*	374	389	318

Sample is from either a neat sample capillary tube collection, or eluted off the cotton pad of a swab collection device with oral fluid THC buffer

* Suggested internal standard

** Quantitation ion

*** Part number TS-38831 (10 x 1mL ampules)

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

THC in Oral Fluid

Using 60mg 6mL HyperSep Retain-CX Extraction Column (Part Number: 60107-308)

Sample Preparation

- Add 100 to 500µL of neat oral fluid sample to a clean tube
- Add internal standard*
- Mix/vortex and let sit for 10 minutes at room temperature
- Add 500µL of glacial acetic acid
- Mix/vortex for 10 seconds

Condition HyperSep Retain-CX Extraction Column

- 1 x 200µL CH₃OH
- 1 x 200µL DI H₂O
- 1 x 200µL 100mM HCl

Apply Sample

- Do not exceed 1mL/minute

Wash Column

- 1 x 500µL DI H₂O
- 1 x 500µL 0.2 N HCl
- 1 x 500µL 100mM HCl/ACN (70:30)
- Dry column (1 minute at >10"Hg)

Elute

- 1 x 800µL ethyl acetate/hexane (25:75)
- Do not exceed 1mL/minute
- Evaporate at <40°C under a stream of N₂

Derivatize

- Add 25µL BSTFA (with 1% TMCS)** and 25µL ethyl acetate
- Overlay with N₂ and cap
- Mix/vortex
- React 30 minutes at 70°C
- Remove from heat and allow to cool

NOTE: Do not evaporate BSTFA solution

Analysis

- Inject 2µL onto GC/MS
- Monitor the following ions:

Compound	Primary Ion	Secondary	Tertiary
THC-TMS	371	386	303
THC-D3-TMS*	374	389	318

* Suggested internal standard: THC-D3-TMS

** Part number TS-38831

Recommended GC Column

Part Number

TraceGOLD TG-35MS 30m x 0.25mm x 0.25µm	26094-1420
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Therapeutic and Abused Drugs in Urine, Blood, Plasma/Serum for Acid/Neutral and Basic Drugs for GC or GC/MS Confirmations

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

Urine:

To 2mL of urine add internal standard(s) and 1mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

Sample pH should be 6.0 ± 0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Centrifuge as appropriate

Serum, Plasma or Whole Blood:

To 1mL of sample add internal standard(s), 1mL of phosphate buffer (pH 6.0) and 4mL of DI H₂O

Mix/vortex and let stand for 5 minutes

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Add 2mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

Sample pH should be 6.0 ± 0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0) then aspirate

NOTE: Aspirate at <3 " Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

1mL of 100mM acetic acid

Dry column (5 minutes at >10 " Hg)

2mL of hexane

Elute Acid and Neutral Drugs (Fraction 1)

3mL of hexane/ethyl acetate (50:50);

Collect eluate at <2 mL/minute

Dry Eluate

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

Reconstitute with 100 μ L ethyl acetate

Quantitate Acid and Neutral Drugs

Inject 1 to 2 μ L onto gas chromatograph

Wash Column

3mL of CH₃OH then aspirate

Dry column (5 minutes at >10 " Hg)

Elute Basic Drugs (Fraction 2)

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at $<40^{\circ}\text{C}$ using an appropriate evaporator. Take care not to overheat or over evaporate. Certain compounds are heat labile, such as the amphetamines and phencyclidine.

Reconstitute with 100 μ L of ethyl acetate

Quantitate Basic Drugs

Inject 1 to 2 μ L onto gas chromatograph

Recommended GC Columns

Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 μ m	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 μ m	26AC497P

NOTES:

- (1) Fraction 1 (acids and neutrals) and fraction 2 (bases) can be combined together
- (2) A keeper solvent such as DMF can be used to prevent the volatilization of amphetamines and phencyclidine; use 30 to 50 μ L 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. Evaporate fraction 2 to approximately 100 μ L, then add 1 drop of the solution. Continue to evaporate to dryness.

Tricyclic Antidepressants in Serum or Plasma for HPLC Analysis

Using 200mg 10mL HyperSep Verify-CX Extraction Column (Part Number: 60108-742)

Prepare Sample

To 1mL of serum or plasma add internal standard* and
2mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

Centrifuge for 10 minutes at 2,000rpm and discard pellet

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic
sodium phosphate

Condition Verify-CX Extraction Column

3mL of CH₃OH then aspirate

3mL of DI H₂O then aspirate

1mL of 100mM phosphate buffer (pH 6.0)

NOTE: Aspirate at <3 "Hg to prevent sorbent drying

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

1mL of 100mM acetic acid

3mL of CH₃OH

Dry column (5 minutes at >10" Hg)

Elute Tricyclic Antidepressants

3mL of CH₂Cl₂/IPA/NH₄OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at <40°C

Reconstitute

Reconstitute with 200µL of ethyl acetate/DI H₂O (1:3)

Mix/vortex vigorously for 30 seconds

Analysis

Column temperature: 30°C

Mobile phase: A: 0.1% formic acid

B: ACN + 0.1% formic acid

Gradient: 30 to 50% B in 15 minutes

Flow rate: 1mL/minute

Detection: UV at 254nm

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 150 x 4.6mm

25005-154630

Determination of Beta Agonist Drugs Residue in Animal Tissues

Using 150mg 6mL HyperSep Retain-CX (Part Number: 60107-311)

Sample Preparation

- Extract 20g from liver sample using CAN
- Dry and spike with appropriate standard (10nmol) e.g. clenbuterol hydrochloride, salbutamol, cimaterol, ractopamine etc
- Prepare solutions at 1, 2, 5, 10 and 100µg/L

Condition HyperSep Retain-CX Extraction Column

- 1 x 5mL CH₃OH
- 1 x 5mL H₂O (mmol/L HCl)

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 1 x 5mL H₂O
- 1 x 5mL CH₃OH
- Dry column using N₂

Elute Melamine

- 1 x 5mL of CH₃OH containing 4% NH₄OH
- Collect eluate at 1 to 2mL/minute
- Dry under nitrogen

Derivatize

- Heat the glass tube with stopper in oven at 50°C for a moment to remove water
- Add 100µL toluene and 100µL BSTFA* (with 1% TMCS)*
- Mix/vortex
- Overlay with nitrogen and cap
- Mix/vortex
- Heat at 80°C for 1 hour
- Cool and add 300µL of toluene

* Part number TS-38831

Analysis

- Inject 5 on to GC/MS

Recommended GC Column

Part Number

TraceGOLD TG-5SiIMS 30m x 0.25m x 0.25µm

26096-1420

Multiresidue Analysis in Cereal Grains for LC/MS/MS or GC/MS

Using QuEChERS Methodology (Part Number: 60105-211 and 60105-220)

Pesticide Standards

- Prepare individual pesticide stock solutions (2,000 to 5,000µg/mL) in ethyl acetate or ACN and store at -18°C
- Prepare two composite pesticide stock solutions, MIX-1 and MIX-2 at 10µg/mL in ACN
- Add 0.1% acetic acid to prevents degradation of base-sensitive analytes in ACN

Isotopically Labeled Internal Standards

- Prepare at 5µg/mL in acetone
- atrazine (ethylamine-d5)
- carbofuran (ring-13C6)
- dimethoate (o,o-dimethyl-d6)
- 2,4-DDT (ring-13C6)
- HCH (13C6)
- parathion (diethyl-d10)

QC Working Solution

- trans-permethrin (phenoxy-13C6) (1 and 5µg/mL in acetone)

Sample Preparation

- Thoroughly homogenize a sample of grain products using a laboratory mill to a flourlike consistency
- Place appropriate weight* of sample into the 50mL centrifuge tube (60105-211)
- Add 10mL of deionized water (15mL for rice) and 10mL of ACN
- Add 200µL of ISTD standard solution
- Mix/vortex tube to disperse sample and standard for 1 hour using a wrist action shaker
- Centrifuge at rcf >3,000 for 10 minutes

Sample Cleanup

- Transfer a 1mL aliquot to a 2mL tube (60105-220)
- Mix/vortex for 30 seconds
- Centrifuge for 5 minutes
- Transfer 300µL of the supernatant into the chamber of a filter vial and add 30µL 1µg/mL QC solution
- Mix thoroughly
- Change to Transfer 125µL of extract into a vial and cap and store overnight at 250°C
- Press the 0.2µm polyvinylidene fluoride (PVDF) filter of the Mini-UniPrep to filter the extract for the LC/MS/MS analysis
- Add 30µL of QC standard solution
- Sample is now ready for analysis

Analysis LC or GC/MS

- Injection appropriate volume on to LC/MS or GC/MS

* Corn 2.5g, Oat 3.5g, Rice 5.0g, Wheat 5.0g

Cyanuric Acid and Melamine in Food Materials Multiresidue Analysis in Cereal Grains for LC/MS/MS or GC/MS

Using 200mg 6mL HyperSep Verify-CX and HyperSep Retain-AX Extraction Columns

(Part Number: 60108-722 and 60107-412)

Sample Preparation

To 1 to 5g of sample add 10 to 25mL of CH₃CN/DI H₂O (50:50)
Shake for 5 minutes
Centrifuge
Transfer 5mL of supernatant to clean glass screw top tube
Add 1mL of 100mM HCl
Add 1mL of CH₂Cl₂
Shake for 5 minutes
Centrifuge
Transfer upper layer to clean glass tube
Add 2mL of DI H₂O to CH₂Cl₂
Shake for 5 minutes
Centrifuge
Add upper layer to previous aqueous portion
Apply to Conditioned HyperSep Verify-CX column

Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O

NOTE: Aspirate at <3 "Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute
Collect effluent for use with HyperSep Retain-AX SPE

Wash Column

1 x 1mL DI H₂O
Collect wash for use with HyperSep Retain-AX
Remove collection tubes from manifold and go to HyperSep Retain-AX section
1 x 3mL 100mM HCl
1 x 1mL CH₃OH
Dry column (5 minutes at >10" Hg)

Elute Melamine

Insert fresh collection tubes into manifold
1 x 2mL of CH₃OH containing 5% NH₄OH
1 x 3mL of CH₃OH containing 5% NH₄OH
Collect eluate at 1 to 2mL/minute

Evaporate

Evaporate eluates under a gentle stream of nitrogen <40°C

Reconstitute

Reconstitute sample in 1,000µL of CH₃CN
Add external standard*
Inject 5µL

HyperSep Retain-AX Extraction Procedure

Adjust solution from wash and elution steps to pH 7**

Condition HyperSep Retain-AX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O

NOTE: Aspirate at <3 "Hg to prevent sorbent drying out

Apply Sample

Load sample which has been pH adjusted at 1 to 2mL/minute

Wash Column

1 x 3mL DI H₂O
1 x 1mL CH₃OH
Dry column

Elute Cyanuric Acid

Insert fresh collection tubes into manifold
1 x 3mL of CH₃OH containing 1% HCl
1 x 2mL of CH₃OH containing 1% HCl
Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°C

Analysis

To sample add 100µL of mobile phase
Add external standard*
Inject 5µL onto LC/MS
Flow rate: 0.50mL/minute
Column temperature: ambient
Mobile phase:

Time	% ACN	%0.1% Formic Acid
0	90	10
3	20	80
3.5	90	10
10	90	10

Compound	MRM Transition
Melamine	127.1/85.1
2, 4 Diamino 6-hydroxy*	127.1/67.0
pyrimidineCyanuric Acid	127.8/84.9

* Suggested external standard: 2, 4 Diamino 6-hydroxy

** Adjust pH with 100 to 200µL of 5% (v/v) (aq) NH₄OH

Recommended HPLC Column

Hypersil GOLD HILIC 3µm, 150 x 2.1mm

Part Number

26503-152130

Determination of Melamine in Egg

Using 60mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-303)

Sample Preparation

To 10mg of melamine add 100mL ACN 10mM/L citric acid +10mM/L heptane sulfonic acid (sodium salt buffer solution (pH 3.0))

Prepare standard solutions; 1mg/L, 5mg/L, 10mg/L, 15mg/L, 20mg/L with water and filter solutions with a 0.45µm syringe filter (F2513-1)

Add 1g of egg sample into a 10mL-centrifuge tube

Prepare 1.0mg/kg, 2.0mg/kg, 10.0mg/kg sample respectively by adding 10µL, 20µL, or 100µL of melamine standard in to the 10mL centrifuge tube

Add 10mL of 1% trichloroacetic acid and 2mL of 5% lead acetate and shake

Ultrasound for 20 minutes

Centrifugate at 8,000rpm for 10 minutes

Condition HyperSep Retain-CX Extraction Column

1 x 3mL CH₃OH

1 x 3mL H₂O

Apply Sample

Load supernate into extraction column

Wash Column

1 x 3mL H₂O

1 x 3mL CH₃OH

Elute Melamine

1 x 5mL of CH₃OH containing 5% NH₄OH

Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen 50°C

Analysis

Reconstitute sample in CH₃CN

Inject 5µL on to LC/MS

Recommended HPLC Column

Part Number

Hypersil GOLD HILIC 3µm, 150 x 2.1mm

26503-152130

Melamine in Animal Feed

Using 60mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-303)

Sample Preparation

To 5g of animal feeds, add 50mL of 0.1% trichloroacetic acid aqueous solution and internal standard if desired

Vortex for 1 minute, then add 2mL of 2% lead acetate aqueous solution. Sonicate for 20 minutes, then transfer a portion of the mixture to a 10mL centrifuge tube.

Centrifuge at 8,000rpm for 10 minutes, then take 3mL of the upper layer solution for SPE clean-up.

Condition Retain-CX Extraction Column

3mL of methanol

3mL of DI H₂O

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H₂O

3mL of methanol

Dry column (5 to 10 minutes at >10"Hg/full flow for positive pressure manifold)

Elute Melamine

5mL of 5% ammonia in methanol

Dry Eluate and Reconstitute

Evaporate to full dryness at 50°C under a stream of nitrogen

Reconstitute the sample to 2mL with 20% methanol aqueous solution

Melamine in Food Materials

Using 200mg 3mL HyperSep Retain-CX (Part Number: 60107-304)

Sample Preparation

To 1 to 5g of sample add 10 to 25mL of CH₃CN/DI H₂O (50:50)
Shake for 5 minutes
Centrifuge
Transfer 5mL of supernatant to clean glass screw top tube
Add 1mL of 100mM HCl
Add 1mL of CH₂Cl₂
Shake for 5 minutes
Centrifuge
Transfer upper layer to clean glass tube
Add 2mL of DI H₂O to CH₂Cl₂
Shake for 5 minutes
Centrifuge
Add upper layer to previous aqueous portion
Apply to Conditioned SPE column

Condition HyperSep Retain-CX Extraction Column

1 x 3mL CH₃OH
1 x 3mL DI H₂O

NOTE: Aspirate at <3 "Hg to prevent sorbent drying out

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL 100mM HCl
1 x 1mL CH₃OH
Dry column (5 minutes at >10"Hg)

Elute Melamine

1 x 2mL of CH₃OH containing 5% NH₄OH
1 x 3mL of CH₃OH containing 5% NH₄OH
Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°C

Analysis

Reconstitute sample in 1,000µL of CH₃CN
Add external standard*
Inject 5µL on to LC/MS
Instrument: Thermo Scientific TSQ Triple Quadrupole
Flow rate: 0.50mL/minute
Mobile phase:

Time:	% ACN %	0.1% Formic Acid
0	90	10
3	20	80
3.5	90	10
10	90	10

Compound	MRM Transition
Melamine	127.1/85.1
2, 4 Diamino 6-hydroxy Pyrimidine*	127.1/67.0

* Suggested internal standard: 2, 4 Diamino 6-hydroxy Pyrimidine

Recommended HPLC Column	Part Number
Hypersil GOLD HILIC 3µm, 150 x 2.1mm	26503-152130

Melamine in Milk Products

Using 60mg 3mL HyperSep Retain-CX Extraction Column (Part Number: 60107-303)

Sample Preparation

Weigh 5g of milk powder (or measure 10mL of milk) into a 250mL flask
Add 50mL of 1% trichloroacetic acid (TCA)
Mix/vortex
Add 2mL of 2% lead acetate/water solution into the mixture then sonicate for 20 minutes
Transfer part of the final mixture into a 10mL centrifuge tube
Centrifuge for 10 minutes at 8,000rpm

Condition Retain-CX Extraction Column

3mL of methanol
3mL of DI H₂O

Apply Sample

Load 6mL of the sample extract onto the Retain-CX SPE product at 1 to 2mL/minute

Wash Column

3mL of DI H₂O
3mL of methanol
Dry column (5 to 10 minutes at >10"Hg/full flow for positive pressure manifold)

Elute Melamine

5mL of 5% ammonia/methanol
Collect eluate at 1 to 2mL/min

Dry Eluate and Reconstitute

Evaporate to dryness at <50°C using nitrogen
Reconstitute sample using 2mL of mobile phase

Determination of Nitrofurans in Milk

Using 60mg 3mL HyperSep Retain PEP (Part Number: 60107-203)

Sample Preparation

- To 15mL of milk add
- 1 x 2mL trichloroacetic acid
- 1 x 1mL H₂O
- Centrifuge at 4,000rpm
- Remove supernatant for analysis

Condition HyperSep Retain PEP Extraction Column

- 1 x 5mL CH₃OH
- 1 x 5mL H₂O

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 1 x 10mL H₂O

Elute Nitrofurans

- 1 x 5mL ethylacetate of 5mL Collect eluate at 1 to 2mL/minute

Evaporation

- Evaporate eluates under a gentle stream of nitrogen <40°C

Analysis

- Reconstitute sample in 1,000µL of CH₃CN
- Inject 5µL on to LC/MS

Recommended HPLC Column

Part Number

Hypersil GOLD 5µm, 100 x 2.1mm

25005-102130

Determination of Nitroimidazole Drugs and Metabolites in Royal Jelly

Using 60mg 3mL HyperSep Retain-CX (Part Number: 60107-303)

Sample Preparation

- Prepare a 100µg/mL deuterated Norfloxacin (NOR-D5) standard in MeOH
- Dilute with MeOH to a concentration of 1µg/mL
- Add 5g of sample to a 50mL centrifuge tube
- Add 50µL of internal standards: Metronidazole (MNZ), Dimetridazole (DMZ) and related metabolites (2-hydroxymethyl, 1-methyl-5-nitroimidazole (HMMNI)), Iprnidazole (IPZ) and related metabolite (2-(2-hydroxy isopropyl)-1-methyl-5-nitroimidazole (IPZOH)), and Ronidazole (RNZ)
- 10mL of 0.5 mol/L sodium hydroxide solution
- Mix for 15 s to dissolve the sample
- Add 10mL of ethyl acetate and mix for 30 seconds
- Centrifugate at 2,500 r/minute for 3 minutes
- Transfer the supernatant ethyl acetate layer to a 50mL glass test tube
- Add 10mL of ethyl acetate again and repeat the extraction procedures
- Combine the ethyl acetates and evaporate to dryness by rotary evaporator in water bath at 40°C
- Dissolve the residue with 5mL of ACN containing 10% formic acid

Condition HyperSep Retain-CX Extraction Column

- 1 x 3mL CH₃OH
- 1 X 3mL H₂O

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 1 x 3mL H₂O
- Dry column (5 minutes at >10"Hg)

Elute Nitroimidazole

- 1 x 3mL of CH₃OH
- Collect eluate at 1 to 2mL/minute and evaporate to dryness

Analysis

- Inject 25µL on to LC/MS
- Flow rate: 0.20mL/minute
- Mobile phase: methanol (A), 5mmol/L ammonium acetate (B)

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 2.1mm

25003-152130

Extraction of Pesticides From Pigmented Fruit or Vegetables

Using QuEChERS Methodology (Part Number: 60105-216, 60105-218 and 60105-221)

Sample Preparation

- Add 15g of homogenized and hydrated tomato product (>80% moisture) to a centrifuge tube 60105-216
- Add 15mL ACN including internal standard
- Mix/vortex for 30 seconds
- Centrifuge for 2 minutes at 3450 rcf
- Draw 1 or 6mL of supernatant for clean-up

Clean-Up

- For 1mL of supernatant, use product 60105-221
- For 6mL of supernatant, use product 60105-218
- Add supernatant to centrifuge tube and shake vigorously for 1 minute
- Centrifuge for 2 minutes at 3450 rcf

Analysis

GC/MS:

- Transfer an aliquot of supernatant from step 2 to a centrifuge tube
- Add TPP solution and 1mL of toluene
- Evaporate using nitrogen at 50°C to approximately 0.3 to 0.6mL
- Bring to 1mL final volume with toluene
- Inject 8µL on to GC/MS

LC/MS:

- Transfer 0.25mL of supernatant from step 2 to an LC vial
- Add TPP solution and 0.86mL of 6.7mM formic acid
- Analyze by LC/MS

Determination of Quinolone Residues in Honey

Using 60mg 3mL HyperSep Retain-CX (Part Number: 60107-303)

Sample Preparation

- Add 5g of sample to a 50mL centrifuge tube
- Make up Quinolone standard solution to a concentration 1µg/mL in 0.1mol/L sodium hydroxide solution
- Quinolone Standards: Enrofloxacin (ENR), Ciprofloxacin (CIP), Norfloxacin (NOR), Ofloxacin(OFL), Flumequine(FLU), Oxolinic acid(OXO), Difloxacin HCl (DIF), Sarafloxacin HCl(SAR), Sparfloxacin (SPA), Danofloxacin (DAN), Fleroxacin (FLE), Marbofloxacin (MAR), Enoxacin (ENO), Orbifloxacin (ORB), Pipemidic acid (PIP), Pefloxacin (PEF), Lomefloxacin (LOM), Cinoxacin (CIN), Nalidixic acid (NAL))

- Add 50µL internal standard solution to 5mL of 0.1mol/L sodium hydroxide solution

Mix/vortex

Condition HyperSep Retain-CX Extraction Column

- 1 x 5mL CH₃OH
- 1 X 3mL H₂O

Apply Sample

- Load sample at 1 to 2mL/minute

Wash Column

- 1 x 3mL H₂O
- 1 x 3mL of CH₃OH

Elute Quinolone

- 1 x 3mL of CH₃OH containing 5% formic acid
- Collect eluate at 1 to 2mL/minute and evaporate to dryness

Analysis

- Inject 25µL on to LC/MS
- Flow rate: 0.20mL/minute
- Mobile phase: methanol + water containing 0.1% formic acid

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 2.1mm

25003-152130

Trichothecene Analysis (A and B) In Wheat and Corn

Using QuEChERS Methodology (Part Number: 60105-211 and 60105-219)

Sample Preparation

Thoroughly homogenize a sample of grain products using a laboratory mill

Weigh 5g of sample into the 50mL centrifuge tube

Add 10mL of methanol:ACN (85:15) into 50mL centrifuge tube

Shake to disperse solvent

Add the contents of the 60105-211 pouch containing 4g anhydrous magnesium sulfate, 1g sodium chloride to the centrifuge tube

Mix/vortex for 1 minute then centrifuge at 4,000rpm for 10 minutes

Sample Cleanup

Transfer a 1mL aliquot to a 2mL 60105-219 tube (150mg anhydrous magnesium sulfate and 50mg PSA)

Shake for 1 minute

Centrifuge for 10 minutes at 4,000rpm

Filter extract through a 0.45µm filter into an LC injection vial if supernatant is not clear

Sample is now ready for analysis

Analysis

Mass spectrometer detection with atmospheric pressure ionization (API) configured for electrospray positive ion mode

Flow rate 0.5mL/minute

Mobile phase:

Time:	1% Methanol%	1% Formic Acid%
0	60	40
10	10	90
25	10	90

Mass Ions for Mycotoxins [Na+M]

Ion	m/z
NIV	355
DON	319
DAS	389
HT2	447
T2	489

Recommended HPLC Column

Part Number

Betasil C18 250mm x 4.6mm x 5µm

70105-254630

Pesticide Analysis in Wine

Using QuEChERS Methodology (Part Number: 60105-205 and 60105-211)

Sample Preparation

Add 20mL ACN and internal standard fluconazole (250µL) to 60105-211 Quantitatively add 20.0mL of wine
Shake for approximately 2 minutes
Centrifuge at 4,500rpm for 5 minutes (use refrigerated centrifuge if available)
Transfer 9.0mL of top layer and add to 60105-205
Mix/vortex tube for approximately 10 seconds
Open tube and add 3.0mL of toluene and shake for 1 minute
Centrifuge the tube for 5 minutes at 4,500rpm
Quantitatively transfer 2.0mL of supernatant to a glass centrifuge tube
Evaporate to dryness at <40°C using N₂
Add 500µL of ACN 25µL of benzanilide (2.0µg/L) surrogate standard for QC and 500µL of 20mM ammonium acetate in 1% ACN to the dried extract
Mix/vortex for approximately 5 seconds and filter into autosampler vial using 17mm, 0.2µm nylon membrane syringe filter (F2513-2) attached to a disposable syringe

Analysis

Inject 3µL onto appropriate LC/MS system
Monitor the following transitions:

Compound	MRM Transition
Acephate	184.0/143.0
Acetamiprid	223.4/126.1
Acibenzolar S-methyl	211.1/136.0
Aldicarb	208.1/116.0
Aldicarb sulfone	240.0/222.9
Aldicarb sulfoxide	224.2/206.9
Atrazine	215.9/173.85
Avermectin B1b	876.6/553.4
Avermectin B1a	890.7/567.5
Azoxystrobin	404.0/372.1
Benalaxyl	326.1/148.1
Benfuracarb	411.2/190.0
Benzanilide	198.1/105.1
Bifenazate	301.3/170.2
Bitertanol	338.2/99.1
Buprofezin	306.3/201.2
Carbaryl	202.1/145.1
Carbendazim	192.0/160.0
Carbofuran	222.1/123.1
Chloroxuron	291.0/72.2
Cyprodinil	226.1/93.0
Cyromazine	167.2/85.1
Diclobutrazol	328.1/70.2
Dimethoate	230.1/199.0
Dimethomorph	388.0/301.1
Dimoxystrobin	327.1/206
Dinotefuran	203.5/14.0
Diuron	233.0/72.1

Compound

MRM Transition

Ethofumesate	286.9/258.9
Famoxadone	373.2/282
Fenamidone	312.2/236.2
Fenbuconazole	337.1/125.0
Fenhexamid	301.9/261.9
Fenpropimorph	304.4/147.1
Fluconazole	307.2/220
Fludioxinil	247.0/180.0
Furathiocarb	383.2/195.1
Hexaconazole	314.0/70.2
Imazalil	297.1/159.0
Imidacloprid	256.1/175.0
Ipconazole	334.1/70.2
Iprovalicarb	321.2/119.0
Kresoxim-methyl	314.1/116.0
Mepanipyrim	224.4/77.3
Metaxyl	280.1/220.1
Methamidophos	142.0/94.0
Methomyl	163.0/88.0
Methoxyfenozide	369.5/149.0
Mevinphos	225.1/192.8
Myclobutanil	289.1/70.2
Omethoate	214.1/183.0
Oxadixyl	279.1/219.1
Piperonyl butoxide	356.2/177.0
Prochloraz	376.1/308.0
Propamocarb	189.1/102.1
Propargite	368.1/231.0
Propiconazole	342.0/159.0
Propoxur	210.0/111.0
Pyraclostrobin	388.0/194.0
Pyridaben	365.3/309.1
Pyrimethanil	200.1/107.0
Quinoxifen	307.8/196.8
Rotenone	395.3/213.2
Simazine	202.2/131.4
Spinosyn A	732.6/142.2
Spinosyn D	746.6/142.2
Spiroxamine	298.2/144.0
Tebuconazole	308.2/70.2
Thiabendazole	202.0/175.0
Triadimefon	294.0/197.1
Trifloxystrobin	409.0/186.0
Triflumizole	346.0/278.1
Vamidothion	288.1/146.0
Zoxamide	336.0/187.0

Recommended HPLC Column

Part Number

Hypersil GOLD 3µm, 150 x 2.1mm 25003-152130

Ordering Information

HyperSep SPE Formats

Thermo Scientific offers a comprehensive selection of SPE products that have been developed for rapid, effective and economical sample preparation. HyperSep SPE products are available in a range of formats, including columns, 96-well plates and microscale products.

HyperSep SPE Columns

Ideal for lower throughput and larger volume samples

- Column volumes from 1mL to 75mL
- Bed weights ranging from 25mg to 10g
- Compatible with manifold systems

HyperSep-96 Well Plates

Designed for high throughput and low volume samples

- 96 individual wells in a single base plate
- Available pre-assembled or available to purchase separately for customization

HyperSep MEPS Products

Saves hours in sample preparation – extraction to injection in a single process

- Miniaturized SPE in a syringe barrel
- Process sample volumes as low as 3.6µL
- Designed for manual or automated use

HyperSep Online SPE Products

Targeted sample preparation and pre-concentration online

- Compatible with conventional HPLC systems
- Available in a range of formats
- Inject sample directly onto HPLC column

HyperSep Hypercarb

HyperSep Hypercarb SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
25mg	1mL	60106-304	50 Pack
50mg	1mL	60106-303	50 Pack
100mg	1mL	60106-302	30 Pack
200mg	3mL	60106-301	30 Pack
500mg	6mL	60106-402	20 Pack
1g	6mL	60106-403	10 Pack
2g	15mL	60106-404	10 Pack

HyperSep-96 Hypercarb Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60302-601	100 Pack
25mg	1mL	60302-602	100 Pack
50mg	1mL	60302-603	100 Pack
WELL PLATES			
10mg	1mL	60302-606	1 Each
25mg	1mL	60302-607	1 Each
50mg	1mL	60302-608	1 Each

HyperSep Retain PEP

HyperSep Retain PEP SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
30mg	1mL	60107-201	100 Pack
30mg	3mL	60107-202	50 Pack
60mg	3mL	60107-203	50 Pack
60mg	6mL	60107-208	30 Pack
100mg	6mL	60107-207	30 Pack
150mg	6mL	60107-211	30 Pack
200mg	3mL	60107-204	50 Pack
200mg	6mL	60107-212	30 Pack
500mg	3mL	60107-205	50 Pack
500mg	6mL	60107-206	30 Pack
1g	25mL	60107-215	20 Pack
2g	25mL	60107-214	20 Pack

HyperSep-96 Retain PEP Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
5mg	1mL	60303-201	100 Pack
10mg	1mL	60303-202	100 Pack
30mg	1mL	60303-203	100 Pack
60mg	1mL	60303-204	100 Pack
WELL PLATES			
5mg	1mL	60303-205	1 Each
10mg	1mL	60303-206	1 Each
30mg	1mL	60303-207	1 Each
60mg	1mL	60303-208	1 Each

HyperSep Retain-CX

HyperSep Retain-CX SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
30mg	1mL	60107-301	100 Pack
30mg	3mL	60107-302	50 Pack
60mg	3mL	60107-303	50 Pack
60mg	6mL	60107-308	30 Pack
100mg	6mL	60107-307	30 Pack
150mg	6mL	60107-311	30 Pack
200mg	3mL	60107-304	50 Pack
200mg	6mL	60107-314	30 Pack
500mg	3mL	60107-305	50 Pack
500mg	6mL	60107-306	30 Pack
1g	25mL	60107-315	20 Pack
2g	25mL	60107-312	20 Pack

HyperSep-96 Retain-CX Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
5mg	1mL	60303-301	100 Pack
10mg	1mL	60303-302	100 Pack
30mg	1mL	60303-303	100 Pack
60mg	1mL	60303-304	100 Pack
WELL PLATES			
5mg	1mL	60303-305	1 Each
10mg	1mL	60303-306	1 Each
30mg	1mL	60303-307	1 Each
60mg	1mL	60303-308	1 Each

HyperSep Retain-AX

HyperSep Retain-AX SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
30mg	1mL	60107-401	100 Pack
30mg	3mL	60107-402	50 Pack
60mg	3mL	60107-403	50 Pack
60mg	6mL	60107-408	30 Pack
100mg	6mL	60107-407	30 Pack
150mg	6mL	60107-411	30 Pack
200mg	3mL	60107-404	50 Pack
200mg	6mL	60107-412	30 Pack
500mg	3mL	60107-405	50 Pack
500mg	6mL	60107-406	30 Pack
1g	25mL	60107-415	20 Pack
2g	25mL	60107-414	20 Pack

HyperSep-96 Retain-AX Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
5mg	1mL	60303-401	100 Pack
10mg	1mL	60303-402	100 Pack
30mg	1mL	60303-403	100 Pack
60mg	1mL	60303-404	100 Pack
WELL PLATES			
5mg	1mL	60303-405	1 Each
10mg	1mL	60303-406	1 Each
30mg	1mL	60303-407	1 Each
60mg	1mL	60303-408	1 Each

HyperSep C18

HyperSep C18 SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-390	100 Pack
100mg	1mL	60108-302	100 Pack
200mg	3mL	60108-303	50 Pack
500mg	3mL	60108-304	50 Pack
500mg	6mL	60108-305	30 Pack
1g	6mL	60108-301	30 Pack
2g	15mL	60108-701	20 Pack
5g	25mL	60108-702	20 Pack
10g	75mL	60108-703	10 Pack

HyperSep-96 C18 Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-421	100 Pack
25mg	1mL	60300-422	100 Pack
50mg	1mL	60300-423	100 Pack
100mg	1mL	60300-524	100 Pack
WELL PLATES			
10mg	1mL	60300-425	1 Each
25mg	1mL	60300-426	1 Each
50mg	1mL	60300-427	1 Each
100mg	1mL	60300-428	1 Each

HyperSep C8

HyperSep C8 SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-391	100 Pack
100mg	1mL	60108-392	100 Pack
200mg	3mL	60108-393	50 Pack
500mg	3mL	60108-309	50 Pack
500mg	6mL	60108-394	30 Pack
1g	6mL	60108-427	30 Pack
2g	15mL	60108-704	20 Pack
5g	25mL	60108-705	20 Pack
10g	75mL	60108-706	10 Pack

HyperSep-96 C8 Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-441	100 Pack
25mg	1mL	60300-442	100 Pack
50mg	1mL	60300-443	100 Pack
100mg	1mL	60300-444	100 Pack
WELL PLATES			
10mg	1mL	60300-445	1 Each
25mg	1mL	60300-446	1 Each
50mg	1mL	60300-447	1 Each
100mg	1mL	60300-448	1 Each

HyperSep Phenyl

HyperSep Phenyl SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-516	100 Pack
100mg	1mL	60108-386	100 Pack
200mg	3mL	60108-387	50 Pack
500mg	3mL	60108-388	50 Pack
500mg	6mL	60108-389	30 Pack
1g	6mL	60108-517	30 Pack
2g	15mL	60108-707	20 Pack
5g	25mL	60108-708	20 Pack
10g	75mL	60108-709	10 Pack

HyperSep-96 Phenyl Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-681	100 Pack
25mg	1mL	60300-682	100 Pack
50mg	1mL	60300-683	100 Pack
100mg	1mL	60300-684	100 Pack
WELL PLATES			
10mg	1mL	60300-685	1 Each
25mg	1mL	60300-686	1 Each
50mg	1mL	60300-687	1 Each
100mg	1mL	60300-688	1 Each

HyperSep Silica

HyperSep Silica SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-409	100 Pack
100mg	1mL	60108-317	100 Pack
200mg	3mL	60108-410	50 Pack
500mg	3mL	60108-315	50 Pack
500mg	6mL	60108-411	30 Pack
1g	6mL	60108-426	30 Pack
2g	15mL	60108-710	20 Pack
5g	25mL	60108-711	20 Pack
10g	75mL	60108-712	10 Pack

HyperSep-96 Silica Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-481	100 Pack
25mg	1mL	60300-482	100 Pack
50mg	1mL	60300-483	100 Pack
100mg	1mL	60300-484	100 Pack
WELL PLATES			
10mg	1mL	60300-485	1 Each
25mg	1mL	60300-486	1 Each
50mg	1mL	60300-487	1 Each
100mg	1mL	60300-488	1 Each

HyperSep SAX Strong Anion Exchanger

HyperSep SAX SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-418	100 Pack
100mg	1mL	60108-360	100 Pack
200mg	3mL	60108-417	50 Pack
500mg	3mL	60108-419	50 Pack
500mg	6mL	60108-434	30 Pack
1g	6mL	60108-521	30 Pack
2g	15mL	60108-713	20 Pack
5g	25mL	60108-714	20 Pack
10g	75mL	60108-715	10 Pack

HyperSep-96 SAX Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-561	100 Pack
25mg	1mL	60300-562	100 Pack
50mg	1mL	60300-563	100 Pack
100mg	1mL	60300-564	100 Pack
WELL PLATES			
10mg	1mL	60300-565	1 Each
25mg	1mL	60300-566	1 Each
50mg	1mL	60300-567	1 Each
100mg	1mL	60300-568	1 Each

HyperSep SCX Strong Cation Exchanger

HyperSep SCX SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-420	100 Pack
100mg	1mL	60108-421	100 Pack
200mg	3mL	60108-422	50 Pack
500mg	3mL	60108-423	50 Pack
500mg	6mL	60108-520	30 Pack
1g	6mL	60108-433	30 Pack
2g	15mL	60108-716	20 Pack
5g	25mL	60108-717	20 Pack
10g	75mL	60108-718	10 Pack

HyperSep-96 SCX Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-581	100 Pack
25mg	1mL	60300-582	100 Pack
50mg	1mL	60300-583	100 Pack
100mg	1mL	60300-584	100 Pack
WELL PLATES			
10mg	1mL	60300-585	1 Each
25mg	1mL	60300-586	1 Each
50mg	1mL	60300-587	1 Each
100mg	1mL	60300-588	1 Each

HyperSep Verify-AX

HyperSep Verify-AX SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
130mg	1mL	60108-727	100 Pack
200mg	6mL	60108-730	50 Pack
300mg	3mL	60108-728	50 Pack
500mg	3mL	60108-729	50 Pack
500mg	6mL	60108-731	30 Pack
1g	6mL	60108-732	30 Pack

HyperSep-96 Verify-AX Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-809	100 Pack
25mg	1mL	60300-810	100 Pack
50mg	1mL	60300-811	100 Pack
100mg	1mL	60300-812	100 Pack
WELL PLATES			
10mg	1mL	60300-813	1 Each
25mg	1mL	60300-814	1 Each
50mg	1mL	60300-815	1 Each
100mg	1mL	60300-816	1 Each

HyperSep Verify-CX

HyperSep Verify-CX SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
130mg	1mL	60108-719	100 Pack
200mg	6mL	60108-722	50 Pack
300mg	3mL	60108-720	50 Pack
500mg	3mL	60108-721	50 Pack
500mg	6mL	60108-723	30 Pack
10g	10mL	60108-742	50 Pack
1g	6mL	60108-724	30 Pack

HyperSep-96 Verify-CX Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-801	100 Pack
25mg	1mL	60300-802	100 Pack
50mg	1mL	60300-803	100 Pack
100mg	1mL	60300-804	100 Pack
WELL PLATES			
10mg	1mL	60300-805	1 Each
25mg	1mL	60300-806	1 Each
50mg	1mL	60300-807	1 Each
100mg	1mL	60300-808	1 Each

HyperSep Florisil

HyperSep Florisil SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-402	100 Pack
100mg	1mL	60108-403	100 Pack
200mg	3mL	60108-404	50 Pack
500mg	3mL	60108-405	50 Pack
500mg	6mL	60108-500	30 Pack
1g	6mL	60108-431	30 Pack
2g	15mL	60108-735	20 Pack
5g	25mL	60108-736	20 Pack
10g	75mL	60108-737	10 Pack

HyperSep-96 Florisil Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-721	100 Pack
25mg	1mL	60300-722	100 Pack
50mg	1mL	60300-723	100 Pack
100mg	1mL	60300-724	100 Pack
WELL PLATES			
10mg	1mL	60300-725	1 Each
25mg	1mL	60300-726	1 Each
50mg	1mL	60300-727	1 Each
100mg	1mL	60300-728	1 Each

HyperSep Aminopropyl

HyperSep Aminopropyl SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-424	100 Pack
100mg	1mL	60108-364	100 Pack
200mg	3mL	60108-425	50 Pack
500mg	3mL	60108-518	50 Pack
500mg	6mL	60108-519	30 Pack
1g	6mL	60108-432	30 Pack
2g	15mL	60108-738	20 Pack
5g	25mL	60108-739	20 Pack
10g	75mL	60108-740	10 Pack

HyperSep-96 Aminopropyl Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-501	100 Pack
25mg	1mL	60300-502	100 Pack
50mg	1mL	60300-503	100 Pack
100mg	1mL	60300-504	100 Pack
WELL PLATES			
10mg	1mL	60300-505	1 Each
25mg	1mL	60300-506	1 Each
50mg	1mL	60300-507	1 Each
100mg	1mL	60300-508	1 Each

HyperSep Cyano

HyperSep Cyano SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-746	100 Pack
100mg	1mL	60108-745	100 Pack
200mg	3mL	60108-747	50 Pack
500mg	3mL	60108-748	50 Pack
500mg	6mL	60108-749	30 Pack
1g	6mL	60108-750	30 Pack
2g	15mL	60108-751	20 Pack
5g	25mL	60108-752	20 Pack
10g	75mL	60108-753	10 Pack

HyperSep-96 Cyano Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-817	100 Pack
25mg	1mL	60300-818	100 Pack
50mg	1mL	60300-819	100 Pack
100mg	1mL	60300-820	100 Pack
WELL PLATES			
10mg	1mL	60300-821	1 Each
25mg	1mL	60300-822	1 Each
50mg	1mL	60300-823	1 Each
100mg	1mL	60300-824	1 Each

HyperSep Diol

HyperSep Diol SPE Columns

Bed Weight	Volume	Cat. No.	Quantity
50mg	1mL	60108-571	100 Pack
100mg	1mL	60108-572	100 Pack
200mg	3mL	60108-573	50 Pack
500mg	3mL	60108-574	50 Pack
500mg	6mL	60108-575	30 Pack
1g	6mL	60108-576	30 Pack
2g	15mL	60108-755	20 Pack
5g	25mL	60108-756	20 Pack
10g	75mL	60108-757	10 Pack

HyperSep-96 Diol Well Plates and Individual Wells

Bed Weight	Volume	Cat. No.	Quantity
INDIVIDUAL WELLS			
10mg	1mL	60300-635	100 Pack
25mg	1mL	60300-636	100 Pack
50mg	1mL	60300-637	100 Pack
100mg	1mL	60300-638	100 Pack
WELL PLATES			
10mg	1mL	60300-630	1 Each
25mg	1mL	60300-631	1 Each
50mg	1mL	60300-632	1 Each
100mg	1mL	60300-633	1 Each

HyperSep MEPS Products

MEPS Syringes and Components

Description	Cat. No.	Quantity
THERMO SCIENTIFIC, CTC ANALYTICS, HTA AND VARIAN 8400 SYSTEMS		
100µL removable needle MEPS syringe	60308-101	1 Each
Replacement plunger assembly for 100µL MEPS syringe	60308-102	1 Each
250µL removable needle MEPS syringe	60308-103	1 Each
Replacement plunger assembly for 250µL MEPS syringe	60308-104	1 Each
CTC ANALYTICS ONLY		
250µL removable needle MEPS syringe	60308-105	1 Each
Replacement plunger assembly for 250µL CTC-compatible syringe	60308-106	1 Each

MEPS For GC: Thermo Scientific, CTC Analytics, HTA and Varian 8400 Systems*

Description	Cat. No.	Quantity
HyperSep Retain PEP MEPS	60308-201	5 Pack
HyperSep Retain-CX MEPS	60308-202	5 Pack
HyperSep Retain-AX MEPS	60308-203	5 Pack
HyperSep Hypercarb MEPS	60308-204	5 Pack
HyperSep Verify-CX MEPS	60308-205	5 Pack
HyperSep Verify-AX MEPS	60308-206	5 Pack
HyperSep C18 MEPS	60308-207	5 Pack
HyperSep Silica MEPS	60308-208	5 Pack
HyperSep MEPS Development Kit for GC Applications	60308-209	5 Pack

Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18

MEPS For GC: CTC Analytics using 250µL Syringes

Description	Cat. No.	Quantity
HyperSep Retain PEP MEPS	60308-301	5 Pack
HyperSep Retain-CX MEPS	60308-302	5 Pack
HyperSep Retain-AX MEPS	60308-303	5 Pack
HyperSep Hypercarb MEPS	60308-304	5 Pack
HyperSep Verify-CX MEPS	60308-305	5 Pack
HyperSep Verify-AX MEPS	60308-306	5 Pack
HyperSep C18 MEPS	60308-307	5 Pack
HyperSep Silica MEPS	60308-308	5 Pack
HyperSep MEPS Development Kit for GC Applications	60308-309	5 Pack

Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18

MEPS For LC: Thermo Scientific, CTC Analytics, HTA and Varian 8400 Systems*

Description	Cat. No.	Quantity
HyperSep Retain PEP MEPS	60308-401	5 Pack
HyperSep Retain-CX MEPS	60308-402	5 Pack
HyperSep Retain-AX MEPS	60308-403	5 Pack
HyperSep Hypercarb MEPS	60308-404	5 Pack
HyperSep Verify-CX MEPS	60308-405	5 Pack
HyperSep Verify-AX MEPS	60308-406	5 Pack
HyperSep C18 MEPS	60308-407	5 Pack
HyperSep Silica MEPS	60308-408	5 Pack
HyperSep MEPS Development Kit for LC applications	60308-409	5 Pack

Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18

MEPS For LC Applications: CTC Analytics using 250µL Syringes

Description	Cat. No.	Quantity
HyperSep Retain PEP MEPS	60308-501	5 Pack
HyperSep Retain-CX MEPS	60308-502	5 Pack
HyperSep Retain-AX MEPS	60308-503	5 Pack
HyperSep Hypercarb MEPS	60308-504	5 Pack
HyperSep Verify-CX MEPS	60308-505	5 Pack
HyperSep Verify-AX MEPS	60308-506	5 Pack
HyperSep C18 MEPS	60308-507	5 Pack
HyperSep Silica MEPS	60308-508	5 Pack
HyperSep MEPS Development Kit for LC applications	60308-509	5 Pack

Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18

* For use with 100µL and 250µL MEPS syringes

HyperSep Online SPE

HyperSep Javelin Direct-Connect Online SPE Columns

I.D.	Length	Retain PEP	Retain-CX	Retain-AX	Hypercarb	Quantity
2.1mm	10mm	60310-201	60310-301	60310-401	60310-501	4 Pack
3.0mm	10mm	60310-202	60310-302	60310-402	60310-502	4 Pack

HyperSep UNIGUARD Direct-Connect Online SPE Cartridges

I.D.	Length	Retain PEP	Retain-CX	Retain-AX	Hypercarb	Quantity
2.1mm	10mm	60311-201	60311-301	60311-401	60311-501	4 Pack
3.0mm	10mm	60311-202	60311-302	60311-402	60311-502	4 Pack

HyperSep HPLC Columns for Online SPE

I.D.	Length	Retain PEP	Retain-CX	Retain-AX	Hypercarb	Quantity
2.1mm	20mm	60312-201	60312-301	60312-401	60312-501	1 Each
3.0mm	20mm	60312-202	60312-302	60312-402	60312-502	1 Each

HyperSep Vacuum Manifolds



HyperSep Universal Vacuum Manifold

Accommodates both SPE columns and 96-well plates; system supplied with manifold, base/gauge, flask and stopper, tubing and spigots

Description	Cat. No.	Quantity
Universal vacuum manifold	60104-230	1 Each
Vacuum pump, European plug	60104-241	1 Each
Vacuum pump, North American plug	60104-243	1 Each
Plugs for 24-position extraction plate	60104-234	24 Pack
Plugs for 48-position extraction plate	60104-235	48 Pack

HyperSep-96 Well Plate Manifold

Accommodates 96-well plates; system includes base, lid and waste collection tray

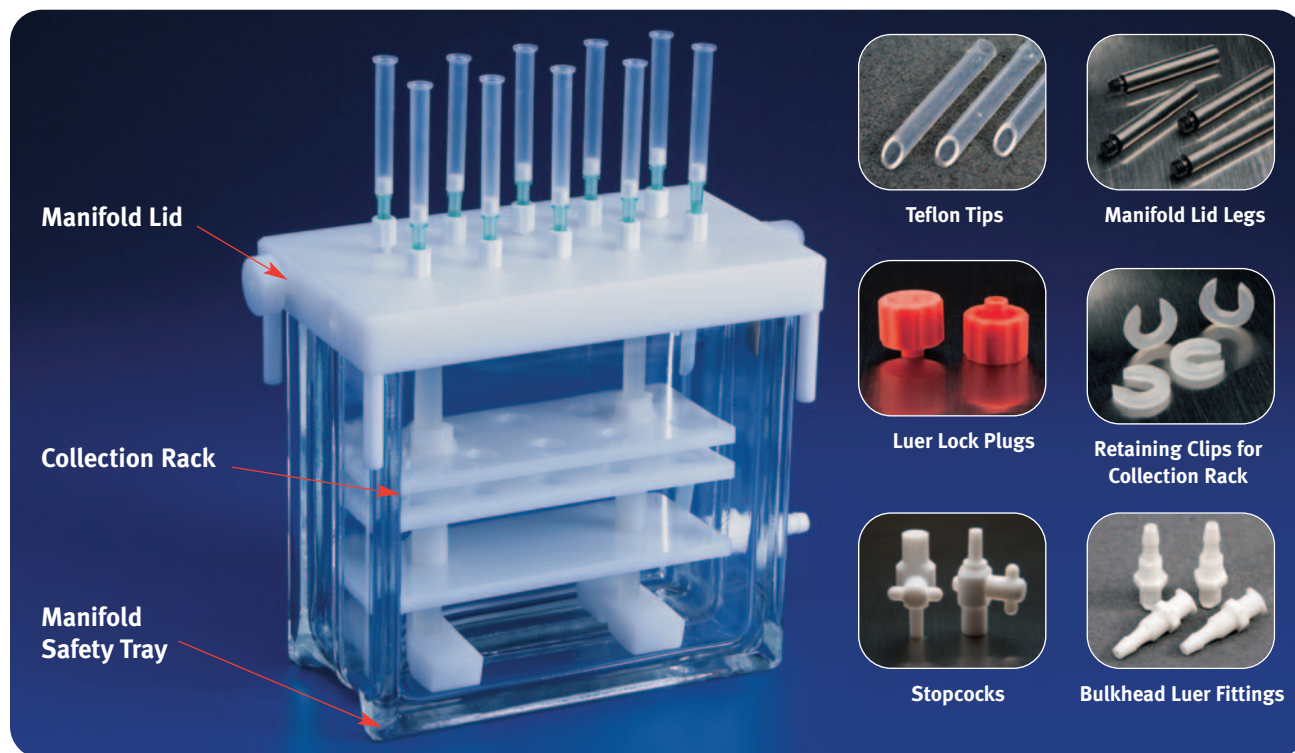
Description	Cat. No.	Quantity
HyperSep-96 vacuum manifold	60103-351	1 Each
Vacuum pump, European plug	60104-241	1 Each
Vacuum pump, North American plug	60104-243	1 Each

ACCESSORIES

Base plate for HyperSep-96 well plate	60300-301	1 Each
Base plate for HyperSep-96 well plate	60300-303	5 Pack
Sample collection plate, 1mL	60300-402	50 Pack
Sample collection plate, 2mL	60300-403	50 Pack
Adaptors for 1mL, 3mL and 6mL SPE columns	60104-259	15 Pack
Empty 1mL wells	60300-318	100 Pack
Empty 1mL wells, fritted	60300-311	100 Pack



HyperSep Glass Block Vacuum Manifolds



16-Port Vacuum Manifold

- Glass block, Corian® manifold lid, cover gasket, vacuum gauge and valve assembly, 16 Teflon® tips, adjustable collection rack, bulkhead Luer fittings, 16 plugs and manifold safety tray

24-Port Vacuum Manifold

- Glass Block, Corian manifold lid, cover gasket, vacuum gauge and valve assembly, 24 Teflon tips, adjustable collection rack, bulkhead Luer fittings, 24 plugs and manifold safety tray



Vacuum Gauge and Valve Assembly

Description	Cat. No.	Quantity
16-port vacuum manifold	60104-232	1 Each
24-port vacuum manifold	60104-233	1 Each
Vacuum pump, European plug	60104-241	1 Each
Vacuum pump, North American plug	60104-243	1 Each
REPLACEMENT PARTS		
Vacuum gauge	60104-240	1 Each
Stopcocks for 16-port vacuum manifold	60104-242	16 Pack
Stopcocks for 24-port vacuum manifold	60104-244	24 Pack
TFE tips for vacuum manifold	60104-245	12 Pack
Vacuum gauge and valve assembly	60104-261	1 Each
Lid for 16-port glass block manifold	60104-262	1 Each
Lid for 24-port glass block manifold	60104-248	1 Each
Gasket for 16-port manifold	60104-249	1 Each
Gasket for 24-port manifold	60104-250	1 Each
Collection rack for 16-port vacuum manifold	60104-251	1 Each
Collection rack for 24-port vacuum manifold	60104-252	1 Each
Glass block for 16-port vacuum manifold	60104-253	1 Each
Glass block for 24-port vacuum manifold	60104-254	1 Each
Manifold safety tray	60104-260	1 Each
Retaining clips for collection rack	60104-255	12 Pack
Bulkhead Luer fittings	60104-256	12 Pack
Manifold lid legs	60104-257	4 Pack
Luer lock plugs	60104-258	12 Pack

HyperSep Dispersive SPE Products – QuEChERS

Thermo Scientific QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) Dispersive SPE Products offer a convenient and effective approach for determining pesticide residues in fruit, vegetables and other foods.

The QuEChERS method offers the advantages of high recoveries, accurate results and high sample throughput, combined with the associated cost savings with lower solvent and labour requirements. Yet the procedure is robust and rugged.

- Quick and easy way to determine pesticide residues in fruit and vegetables
- Simple procedure with no automation required
- Determine wide range of pesticide types
- High recoveries and accurate results
- High sample throughput



HyperSep Dispersive SPE Products – QuEChERS

QuEChERS Extraction Products

Description	Capacity	Cat. No.	Quantity
6g MgSO ₄ , 1.5g Sodium Acetate	50mL	60105-210	250
4g MgSO ₄ , 1g sodium Chloride	50mL	60105-211	250
1.5g Sodium Chloride, 1.5g Sodium Citrate Tribasic Dihydrate, 750mg Sodium citrate Dibasic	50mL	60105-212	250
4g Anhydrous Magnesium Sulfate, 1g Sodium Chloride, 1g Sodium Citrate Tribasic Dihydrate, 500mg Sodium citrate Dibasic	50mL	60105-216	250

QuEChERS Clean-Up Products

Description	Capacity	Cat. No.	Quantity
6g Anhydrous Magnesium Sulfate, 1.5g Anhydrous Sodium Acetate	50mL	60105-310	25
4g Anhydrous Magnesium Sulfate, 1g NaCl	50mL	60105-311	25
6g Anhydrous Magnesium Sulfate, 1.5g Sodium Chloride, 1.5g Sodium Citrate Tribasic Dihydrate, 750mg Sodium citrate Dibasic	50mL	60105-312	25
4g Anhydrous Magnesium Sulfate, 1g Sodium Chloride, 1g Sodium Citrate Tribasic Dihydrate, 500mg Sodium citrate Dibasic	50mL	60105-316	25
Centrifuge Tubes with 900mg MgSO ₄ , 300mg PSA and 150mg Carbon	15mL	60105-205	50
900mg MgSO ₄ , 300mg PSA and 150mg C18	15mL	60105-206	50
750mg MgSO ₄ , 250mg PSA, 250mg endcapped C18 and 250 Carbon	15mL	60105-213	50
900mg Anhydrous MgSO ₄ , 300mg PSA	15mL	60105-214	50
900mg Anhydrous MgSO ₄ , 150mg PSA	15mL	60105-215	50
900mg MgSO ₄ , 150mg PSA and 45mg Carbon	15mL	60105-217	50
900mg MgSO ₄ , 150mg PSA and 15mg Carbon	15mL	60105-218	50
1200mg MgSO ₄ , 400mg PSA	15mL	60105-224	50
1200mg MgSO ₄ , 400mg PSA and 400mg C18	15mL	60105-225	50

QuEChERS Clean-Up Products *Continued*

Description	Capacity	Cat. No.	Quantity
1200mg MgSO ₄ , 400mg PSA, 400mg C18 and 400mg Carbon	15mL	60105-226	50
900mg MgSO ₄ , 150mg PSA and 150mg C18	15mL	60105-227	50
150mg MgSO ₄ , 50mg PSA and 50mg Carbon	15mL	60105-230	50
150mg MgSO ₄ , 300mg PSA and 150mg Chlorofiltr	15mL	60105-231	50
900mg Anhydrous MgSO ₄ , 300mg PSA and 150mg Carbon	15mL	60105-305	10
900mg Anhydrous MgSO ₄ , 300mg PSA and 150mg C18	15mL	60105-306	10
150mg MgSO ₄ , 50mg PSA, 50mg Carbon	15mL	60105-313	10
900mg Anhydrous MgSO ₄ , 300mg PSA	15mL	60105-314	10
900mg Anhydrous MgSO ₄ , 150mg PSA	15mL	60105-315	10
900mg MgSO ₄ , 150mg PSA and 45mg Carbon	15mL	60105-317	10
900mg MgSO ₄ , 150mg PSA and 15mg Carbon	15mL	60105-318	10
1200mg MgSO ₄ , 400mg PSA	15mL	60105-324	10
1200mg MgSO ₄ , 400mg PSA and 400mg C18	15mL	60105-325	10
1200mg MgSO ₄ , 400mg PSA, 400mg C18 and 400mg Carbon	15mL	60105-326	10
900mg MgSO ₄ , 150mg PSA and 150mg C18	15mL	60105-327	10
150mg MgSO ₄ , 50mg PSA and 50mg Carbon	15mL	60105-330	10
150mg MgSO ₄ , 300mg PSA and 150mg Chlorofiltr	15mL	60105-331	10
400mg PSA on bottom, 200mg Carbon on top	6mL	60105-207	30
500mg PSA on bottom, 250mg Carbon on top	6mL	60105-208	30
Columns with 500mg PSA on bottom, 500mg Carbon on top	6mL	60105-209	30
400mg PSA on bottom, 200mg Carbon on Top, with Teflon Frit	6mL	60105-307	10
500mg PSA on bottom, 250mg Carbon on Top, with Teflon Frit	6mL	60105-308	10
500mg PSA on bottom, 500mg Carbon on Top, with Teflon Frit	6mL	60105-309	10
150mg MgSO ₄ , 50mg PSA and 50mg Carbon	2mL	60105-202	100
150mg MgSO ₄ , 50mg PSA	2mL	60105-203	100
150mg MgSO ₄ , 50mg PSA and 50mg C18	2mL	60105-204	100
150mg MgSO ₄ , 25mg PSA	2mL	60105-219	100
150mg MgSO ₄ , 25mg PSA and 25mg C18	2mL	60105-220	100
150mg MgSO ₄ , 25mg PSA and 2.5mg Carbon	2mL	60105-221	100
150mg MgSO ₄ , 25mg PSA and 7.5mg Carbon	2mL	60105-222	100
150mg MgSO ₄ , 50mg PSA, 50mg C18 and 50mg Carbon	2mL	60105-223	100
150mg Anhydrous MgSO ₄ , 50mg PSA and 50mg Carbon	2mL	60105-302	10
150mg Anhydrous MgSO ₄ , 50mg PSA	2mL	60105-303	10
150mg Anhydrous MgSO ₄ , 50mg PSA and 50mg C18	2mL	60105-304	10
150mg MgSO ₄ , 25mg PSA	2mL	60105-319	10
50mg MgSO ₄ , 25mg PSA and 25mg C18	2mL	60105-320	10
2mL Centrifuge Tube with 150mg MgSO ₄ , 25mg PSA and 2.5mg Carbon	2mL	60105-321	10
150mg MgSO ₄ , 25mg PSA and 7.5mg Carbon	2mL	60105-322	10
150mg MgSO ₄ , 50mg PSA, 50mg C18 and 50mg Carbon	2mL	60105-323	10



Resources

for Chromatographers

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