Thermo Scientific HyperSep Columns
Application Notebook – Issue 1, April 2011

Removing Uncertainty
by Applying Science to SPE

Pharmaceutical/Biotech • Environmental • Forensics • Food Safety

Thermo SCIENTIFIC

Table of Contents

Introduction

Pharmaceutical/Biotech Applications

Pharmaceutical/Biotech Applications

Table of Contents

Environmental Applications

Environmental Applications

Forensic Applications

Table of Contents

Forensic Applications

Table of Contents

Forensic Applications

Food Safety Applications

Ordering Information

Trademark Information

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.

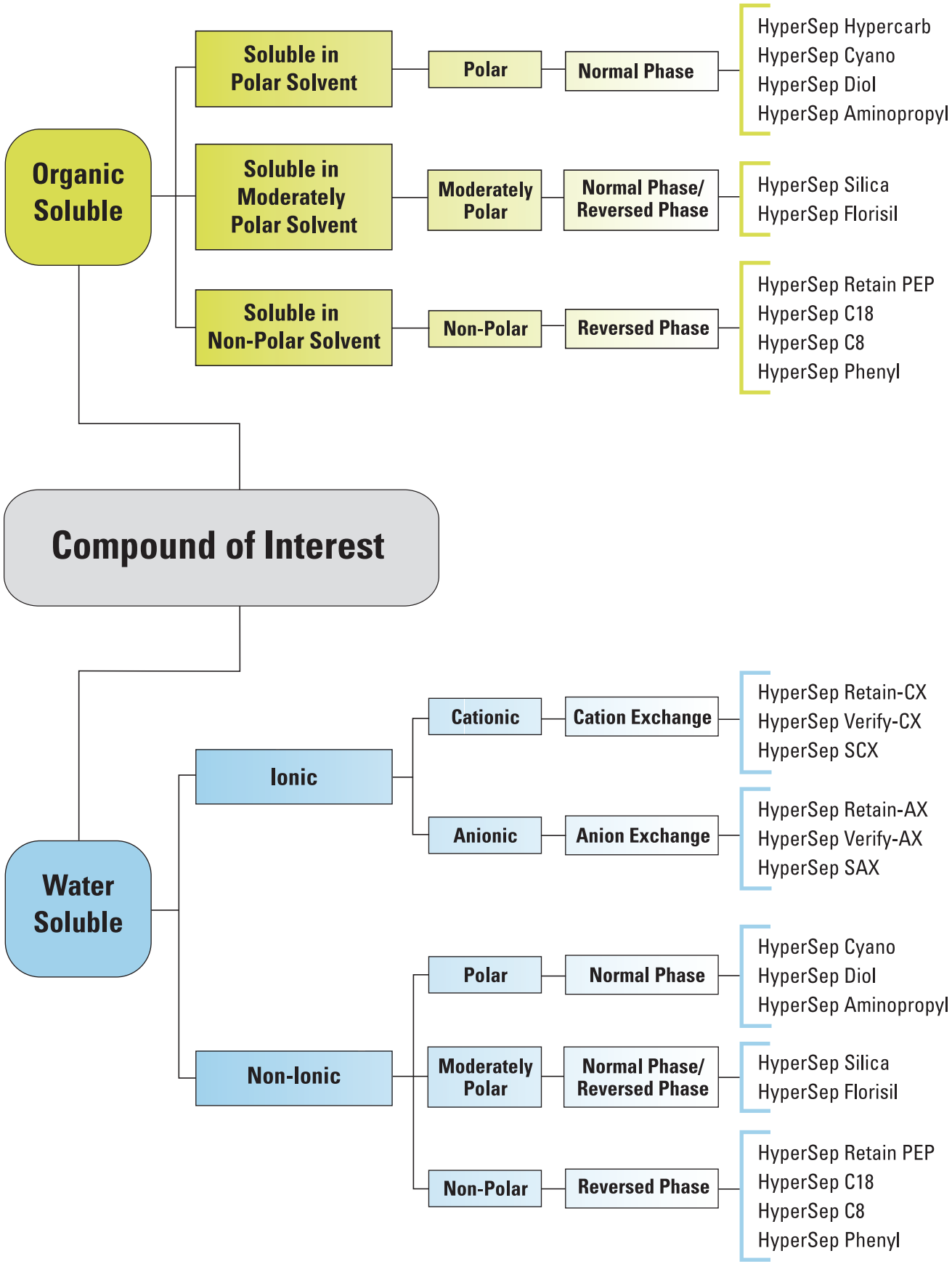
LLE presents additional drawbacks. This process uses large volumes of potentially hazardous solvent and often involves laborious method development. It is a less selective technique for polar compounds.

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

Polymerics

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

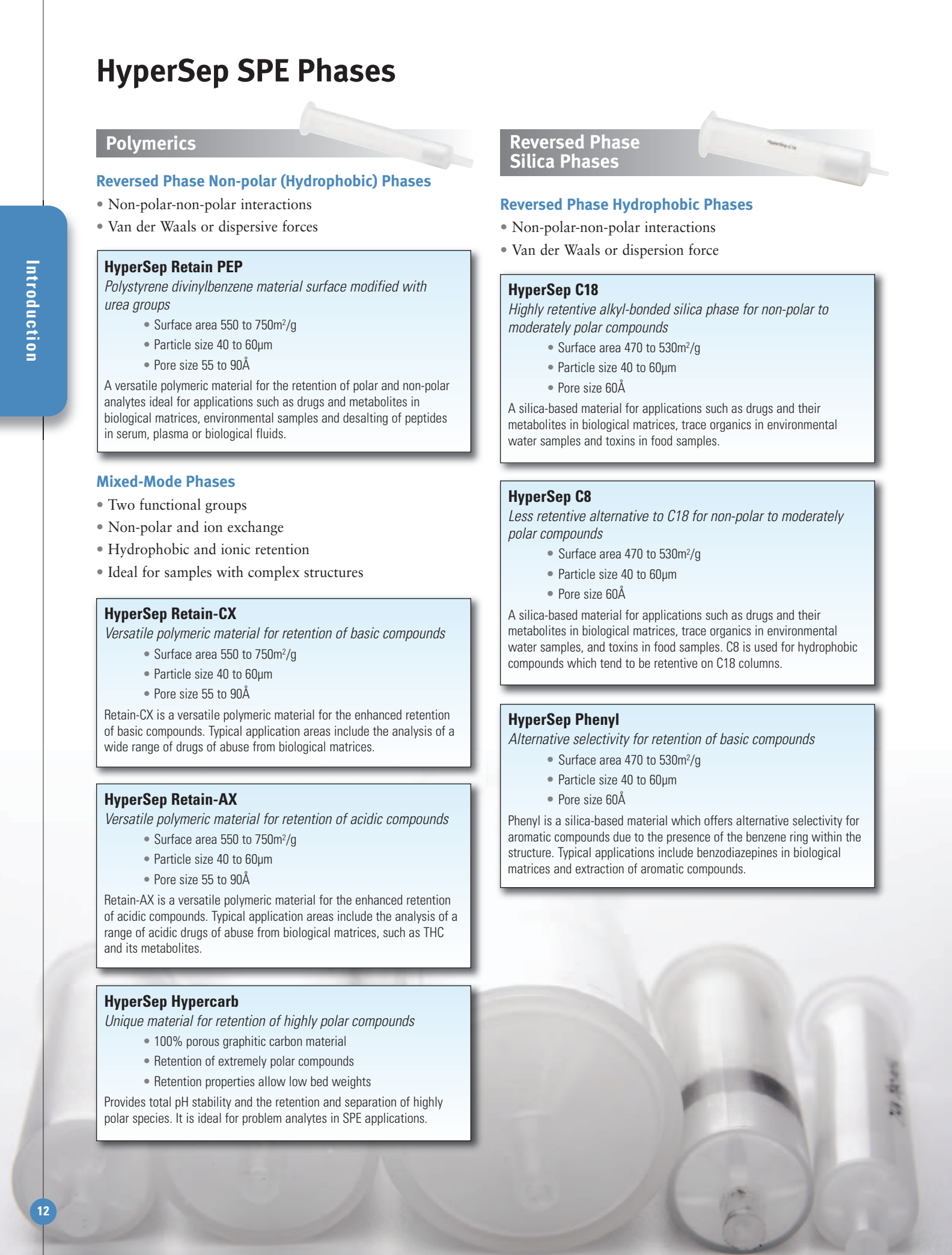
A silica-based material for applications such as drugs and their metabolites in biological matrices, trace organics in environmental water samples, and toxins in food samples. C8 is used for hydrophobic compounds which tend to be retentive on C18 columns.

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.

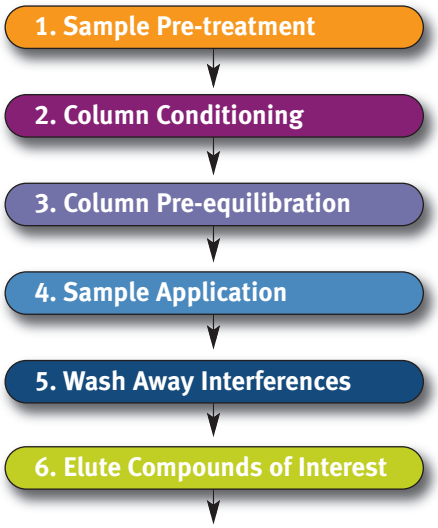
HyperSep Verify-AX

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-equilibriate 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
	Water	Yes
Polar	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

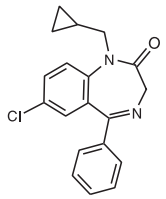
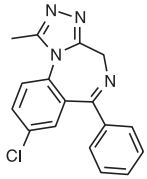
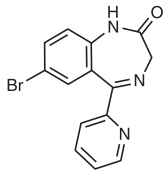
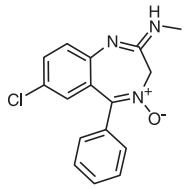
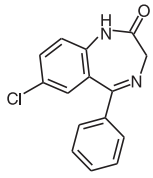
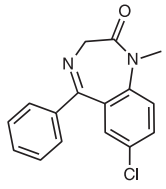
For Ion-Exchange Interactions

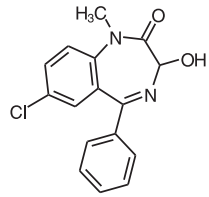
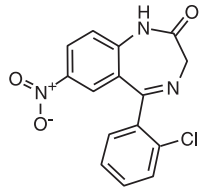
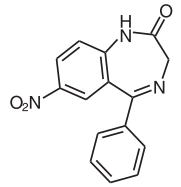
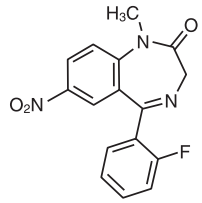
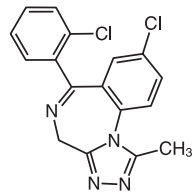
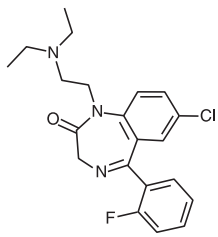
- pH and pKa are important considerations
- Acidic compounds are extracted from a sample solution at least 2 pH units above the pKa of the analyte
- Basic compounds are extracted from a sample solution 2 or more pH units below the pKa of the analyte

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
Prazepam	**3.0**	
Alprazolam	**2.4**	
Bromazepam	**11.0 2.9**	
Chlordiazepoxide HCl	**4.8**	
Nordiazepam	**3.5**	
Diazepam	**3.3**	

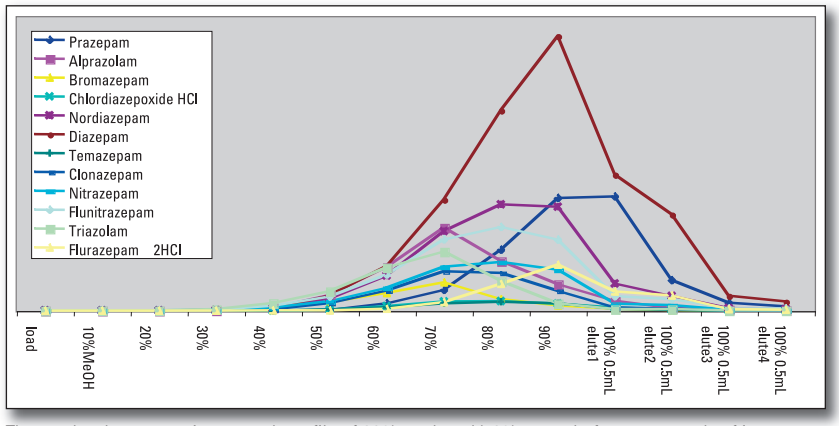
Compound	pKa Value	Structure
Temazepam	**1.3**	
Clonazepam	**10.5 (1-position), 1.5 (4-position)**	
Nitrazepam	**2.5**	
Flunitrazepam	**1.8**	
Triazolam	**1.5**	
Flurazepam	**8.2 1.9**	

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



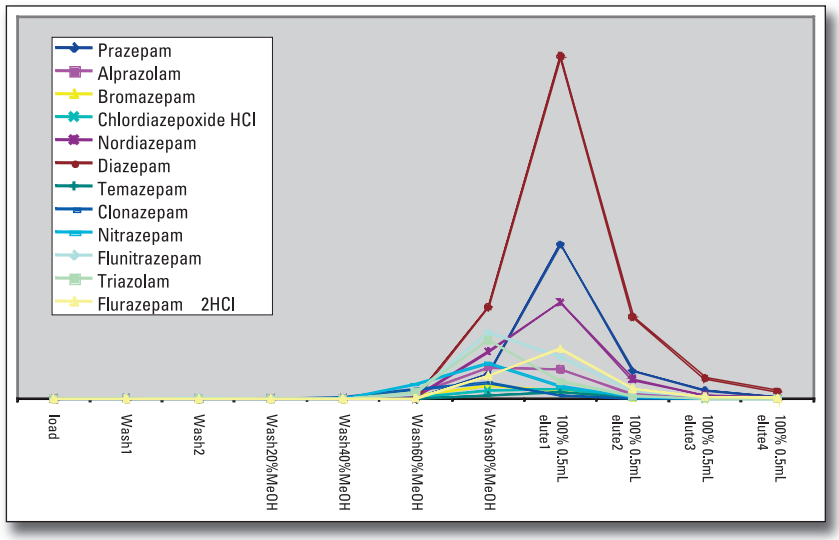
The results show an optimum wash profile of 30% methanol/70% water before compounds of interest start to be eluted. An elution volume of 1.5mL in three aliquots of 500µL eluted off the compounds of interest to give a high recovery level.

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



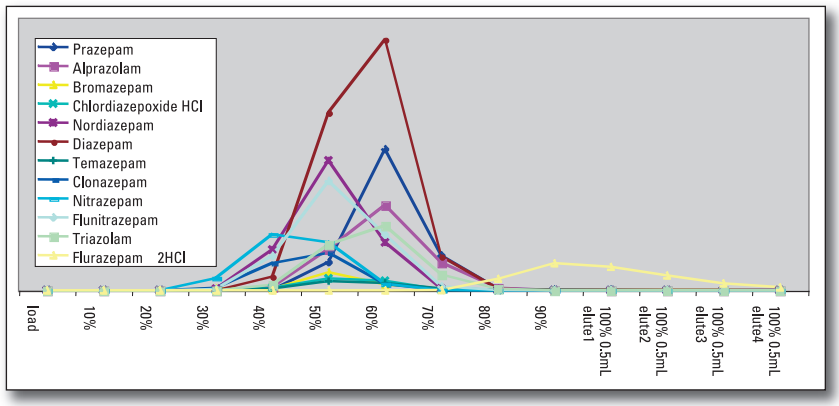
The results show an optimum wash profile of 5% ammonia in 50% methanol/50% water before compounds of interest start to be eluted. An elution volume of 1.5mL in three aliquots of 500µL eluted off the compounds of interest to give a high recovery level.

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% H_3PO_4 (sample 50% serum and 1% H_3PO_4)

Condition Retain PEP Extraction Column

2mL of methanol

2mL of DI H_2O

Apply Sample

Load 2mL sample solutions

Wash Column

2mL of methanol/DI H_2O (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% H_3PO_4 (sample 50% serum and 1% H_3PO_4)

Condition Retain-CX Extraction Column

2mL of methanol

2mL of DI H_2O

Apply Sample

Load 2mL sample solutions

Wash Column

2mL of methanol/DI H_2O (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 CH_3OH

1 x 2mL H_2O water 2mL

Apply Sample

Load 2mL of sample

Wash Column

1 x 2mL 5% CH_3OH

Elute Sample

1 x 2mL CH_3OH

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 H_2O

Apply Sample

Load 4mL samples at 1-2mL/minute

Wash Column

1mL of DI H_2O

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 KH_2PO_4 (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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

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 – ß-Glucuronidase Hydrolysis

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

ß-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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

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 50µL ethyl acetate and 50µL 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 2µL 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
Desmethylflunitrazepam-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 0.25µm	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 H_2O

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

Apply Sample

Load sample by gravity

Wash Sample

1 x 3mL DI H_2O

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 0.25m x 0.25µm	26098-1420

Benzodiazepine Screen: Blood, Serum, Urine and Tissue for GC-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 50µL acetonitrile and 50µL BSTFA with 1% TCMS**

Heat for 30 minutes at 70°C

Remove from heat source to cool

Inject 1µL 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 0.25m x 0.25µm	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 50µL 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: 5µL on to MS triple quad

Compound	MRM Transition
Alprazolam	309.1/281.2
Alprazolam-D5*	314.1/286.2
Alphahydroxyalaprazolam	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 3µm, 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 H_2O

5mL of 30mmol/L of chloric acid

Apply Sample

Load 2mL samples

Wash Column

5mL of DI H_2O

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 CH_3OH

1 x 3mL DI H_2O

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/NH_4OH mix, then add CH_2Cl_2 (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 0.25m 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 CH_3OH

1 x 3mL DI H_2O

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/NH_4OH mix, then add CH_2Cl_2 (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 0.25m 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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

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 H_2O

Apply Sample

Load 10mL samples at 1 to 2mL/minute

Wash Column

1mL of methanol/DI H_2O (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 H_2O

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: ß-Glucuronidase Hydrolysis

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

ß-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 CH_3OH

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% NH_4OH

Collect eluate at 1 to 2mL/minute

NOTE: *Prepare fresh daily*

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50µL ethyl acetate and 50µL 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 2µL 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 0.25m x 0.25µm	26098-1420

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 CH_3CN/DI H_2O (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 CH_2Cl_2
Shake for 5 minutes
Centrifuge
Transfer upper layer to clean glass tube
Add 2mL of DI H_2O to CH_2Cl_2
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 CH_3OH
1 x 3mL DI H_2O

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 H_2O
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 CH_3OH
Dry column (5 minutes at >10"Hg)

Elute Melamine

Insert fresh collection tubes into manifold
1 x 2mL of CH_3OH containing 5% NH_4OH
1 x 3mL of CH_3OH containing 5% NH_4OH
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 CH_3CN
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 CH_3OH
1 x 3mL DI H_2O

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 H_2O
1 x 1mL CH_3OH
Dry column (just enough to remove residual solvent)

Elute Cyanuric Acid

Insert fresh collection tubes into manifold
1 x 3mL of CH_3OH containing 1% HCl
1 x 2mL of CH_3OH 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)NH_4OH*

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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

Dry column (10 minutes at >10mm Hg)

Elute Steroids

1 x 3mL of CH_3OH

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 $NaHCO_3/Na_2CO_3$

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 50µL of MSTFA/NH_4l/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 2µL 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 0.25µm	26098-1420

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 H_2O

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 CH_3OH

1 x 3mL H_2O

Apply Sample

Load spiked serum sample onto SPE cartridge

Wash Column

1 x 3mL H_2O

1 x 3mL 5% CH_3OH

Elution

1 x 3mL CH_3OH

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 CH_3OH
1 x 3mL DI H_2O
1 x 1mL 100mM HCL

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

Apply Sample

Load at 1 tomL/minute

Wash Column

1 x 3mL DI H_2O
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% NH_4OH in CH_3OH

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 0.25m 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 CH_3OH
1 x 3mL DI H_2O
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 H_2O
1 x 3mL ethyl acetate
1 x 3mL hexane
Dry column (10 minutes at >10"Hg)

Elute Gabapentin

1 x 3mL CH_3OH containing 2% NH_4OH
Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°
Dissolve residue in 100µL CH_3OH
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 H_2O

Apply Sample

Load 2mL samples

Wash Column

2mL of DI H_2O

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 H_2O

Apply Sample

Load 10mL samples at 1 to 2mL/minute

Wash Column

1mL of DI H_2O

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 CH_3OH

1 x 3mL DI H_2O

Apply Sample

Decant supernatant onto SPE column

Wash Column

1 x 10mL of DI H_2O

Dry with vacuum for 3 minutes

1 x 5mL of CH_3OH

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 0.25m 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 H_2O

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 KH_2PO_4 (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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

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 CH_3OH

Dry column, (5 minutes at >10"Hg)

Elute Continine and Nicotine

Replace rack of collection tubes

1 x 3mL CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1mL/minute

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 H_2O add internal standard*
Add 1mL blood
Add 8mL of DI H_2O
Mix/vortex and centrifuge as appropriate

Condition HyperSep Cyano Extraction Column

1 x 3mL CH_3OH
1 x 3mL DI H_2O

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 CH_3OH
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 CH_3OH
1 x 1mL H_2O

Apply Sample

Load 0.5mL of sample

Wash Column

1 x 1mL 1%CH_4O_3S
1mL CH_3OH

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 H_2O

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 ß-Glucuronidase solution

ß-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 CH_3OH

1 x 3mL DI H_2O

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 H_2O then aspirate

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

1 x 3mL CH_3OH 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 proprionic anhydride pyridine

Make this solution fresh daily

Mix/vortex

React for 60 minutes at 60°C in a heater block

Remove from heat source to cool

Evaporate to dryness at <40°C

Reconstitute the residue with 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

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 H_2O

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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

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

1 x 3mL CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Opiates

1 x 3mL CH_2Cl_2/IPA/NH_4OH (76:20:4)

Collect eluate at 1 to 2mL/minute

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 100µL ethyl acetate and 100µL BSTFA (with 1% TMCS)**

Overlay with N_2 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-Desmethyltramadol TMS	393	378	303
N-Desmethyltramadol 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 ß-Glucuronidase solution. (ß-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±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 H_2O

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 CH_2Cl_2/IPA/NH_4OH (78/20/2), collect eluate at 1 to 2mL/min

Evaporate eluate to dryness at <40°C

Derivatization

Add 50µL ethyl acetate and 50µL 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 2µL 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 0.25µm	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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

1 x 3mL 100mM acetic acid

1 x 3mL CH_3OH

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 100µL CH_3OH

Injection Volume: 5µL 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 3µm, 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 H_2O

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 H_2O

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 H_2O 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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

1 x 1mL 100mM acetic acid

1 x 3mL CH_3OH

Dry column (5 minutes at >10"Hg)

Elute

1 x 3mL CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1mL/minute

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

Dry Eluate

Evaporate to dryness at <40°C

Quantitate

Reconstitute with 200µL acetonitrile:DI H_2O (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% CH_3CN

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 $ZnSO_4$ to a centrifuge tube

Mix/vortex

Add 500mL methanol and internal standards*

Mix/vortex

Centrifuge

Transfer supernate to a clean tube, add 500mL DI H_2O

Mix/vortex

Condition HyperSep Retain PEP Extraction Column

1 x 2mL CH_3OH

1 x 2mL DI H_2O

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 H_2O

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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

1 x 1mL 100mM acetic acid

1 x 3mL CH_3OH

Dry column (5 minutes at >10"Hg)

Elute

1 x 3mL CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1mL/minute or use gravity flow

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

Dry Eluate

Evaporate to dryness at <40°C

Quantitate

Reconstitute with 200µL ethyl acetate/DI H_2O (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 H_2O

Apply Sample

Load 4mL samples at 1 to 2mL/minute

Wash Column

1mL of DI H_2O

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 H_2O at concentrations of 200, 20 and 2ppm

Condition Retain PEP Extraction Column

3mL of methanol

3mL of DI H_2O

Apply Sample

Load 3mL samples at 1 to 2mL/minute

Wash Column

1mL of DI H_2O

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 H_2O

Apply Sample

Load 4mL samples at 1 to 2mL/minute

Wash Column

1mL of DI H_2O

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 H_2SO_4

Condition Retain PEP Extraction Column

3mL of tetrahydrofuran

5mL of methanol

5mL of DI H_2O

Apply Sample

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

Wash Column

5mL of DI H_2O

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 H_2SO_4

Condition HyperSep Retain PEP Extraction Column

1 x 5mL THF

1 x 5mL CH_3OH

1 x 5mL H_2O

NOTE: *Do not allow the cartridge to dry out*

Apply Sample

Load 1 x 500mL H_2O

Elute at 5mL/minute

Wash Column

1 x 5mL H_2O

Dry column (20 minutes under N_2)

Elute

1 x 3mL THF

Concentrate sample to 3mL under N_2

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 H_2O

Apply Sample

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

Wash Column

10mL of DI H_2O 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 H_2O

Adjust pH to 2 with H_2SO_4

Condition HyperSep Retain PEP Extraction Column

1 x 5mL CH_3OH

1 x 5mL H_2O

NOTE: *Do not allow the cartridge to dry out*

Apply Sample

Load 1 x 500mL H_2O

Elute at 5mL/minute

Wash Column

1 x 5mL H_2O

1 x 1mL CH_3OH

Elute

1 x 3mL THF

Concentrate sample to 3mL under N_2

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 H_2SO_4

Condition HyperSep Retain PEP Extraction Column

1 x 5mL CH_3OH

1 x 5mL H_2O

NOTE: *Do not allow the cartridge to dry out*

Apply Sample

Load 1 x 500mL H_2O

Elute at 5mL/minute

Wash Column

1 x 5mL H_2O

1 x 1mL CH_3OH

Dry column (20 minutes under N_2)

Elute

1 x 3mL THF

Concentrate sample to 3mL under N_2

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 H_2SO_4

Condition Retain PEP Extraction Column

3mL of tetrahydrofuran

5mL of methanol

5mL of DI H_2O

Apply Sample

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

Wash Column

5mL of DI H_2O

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 $MgCl_2$ (final conc. 10mg/L)

Condition HyperSep C18 Extraction Column

1 x 1mL 1:1 EtAc/CH_3Cl_2

1 x 10mL CH_3OH

1 x 10mL H_2O

Apply Sample

Add 1 x 5mL CH_3OH 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 CH_3Cl_2

1 x 3mL EtAc/CH_3Cl_2

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 I 30m x 0.25m 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/CH_3OH

1 x 30mL H_2O

1 x 3mL H_2O to top of cartridge

Apply Sample

Load sample at 10 to 15mL/minute

Wash Column

1 x 5mL H_2O

Dry column

Elute

Insert fresh collection tubes into manifold

1 x 15mL of ammonium acetate/CH_3OH

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/CH_3OH

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) $CaCl_2$

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 CH_3OH

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 H_2O

1 x 50mL DI H_2O

Nitramines, Nitroaromatics

1 x 15mL ACN

1 x 30mL DI H_2O

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 H_2O

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 H_2O adjusted to pH 6

Condition HyperSep Verify-AX Extraction Column

1 x 5mL CH_3OH

1 x 10mL DI H_2O

Apply Sample

Load sample at 1 to 3mL/minute

Wash Column

1 x 10mL DI H_2O

Dry column (10 minutes at >10"Hg)

Elute

1 x 4mL of 1 mol/L HCl/CH_3OH (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 0.25m 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 CH_3OH

1 x 3mL H_2O

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 H_2O

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 H_2O for analysis

Base

1 x 3mL of triethylamine to the column

Flow through at 1 to 3mL/minute

Dilute eluant with H_2O 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-Methylenedianaline 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 H_2O

Apply Sample

Load sample at 1mL/minute

Wash Column

1mL of DI H_2O

Elute 4'4-Methylenedianaline

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 H_2O

Condition Retain-AX Extraction Column

1mL of methanol

1mL of DI H_2O

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

Nitroanalines 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 H_2O

Apply Sample

Load 3mL samples at 1 to 2mL/minute

Wash Column

1mL of 0.1% ammonia aqueous solution

Elute Nitroanalines

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 H_2O

Apply Sample

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

Wash Column

5mL of DI H_2O

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 CH_3OH 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 CH_3OH

NOTE: *Do not allow the cartridge to dry out*

Apply Sample

Load 1 x 10mL H_2O

Elute at 5mL/minute

Wash Column

1 x 10mL H_2O

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 CH_3OH

Condition HyperSep C18 Extraction Column

1 x 10mL methylene Chloride

1 x 10mL acetone

1 x 10 CH_3OH

1 x 20mL DI H_2O

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 CH_3Cl

Dry the extract by passing it through anhydrous sodium sulfate

Rinse the collection device with CH_3Cl

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 0.25m 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 H_2O

Apply Sample

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

Wash Column

20mL of DI H_2O

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-hexachlorocychexane	4,4'-DDE
Lindane	Dieldrin
β-hexachlorocychexane	Endrin
Heptachlor	4,4'-DDD
δ-hexachlorocychexane	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 H_2O

Adjust pH to 2 with 6N HCl

Condition HyperSep Retain PEP Extraction Column

1 x 3mL CH_3Cl

1 x 3mL CH_3OH

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 H_2O

Dry column (1 minute at >10"Hg)

Elute

1 x 10mL CH_3Cl

1 x 3mL CH_3Cl

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 CH_3Cl

Analyze the extract with GC/MS

Recommended GC Column	Part Number
TraceGOLD TG-5MS 30m x 0.25m 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 H_2O

Condition HyperSep Retain PEP Extraction Column

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

1 x 3mL CH_3OH

1 x 3mL H_2O

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 H_2O

Dry column (20 minutes at >10"Hg)

Elute

1 x 2mL CH_3OH

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 H_2O to form a slurry
Mix for 15 minutes
Adjust pH to 2 using 50% aq H_2SO_4
Filter sample through previously acidified filter media

Condition HyperSep C18 Extraction Column

1 x 5mL CH_3OH
1 x 5mL DI H_2O

NOTE: *Do not allow the cartridge to dry out*

Apply Sample

Load sample at 10mL/minute

Elute Phenoxyacetic acid Herbicides

2 x 5mL CH_3OH
Collect at 1 to 2mL/minute
Evaporate to dryness at <40°C using N_2

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
MCPP	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 H_2O

Apply Sample

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

Wash Column

20mL of DI H_2O

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

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 H_2O

Condition HyperSep Retain PEP Extraction Column

1 x 5mL IPA

1 x 5mL H_2O

NOTE: *Do not allow the cartridge to dry out*

Apply Sample

Load 1L of water sample at 20mL/minute

Wash Column

1 x 5mL (H_2O 300mL+CH_3OH 700mL+Na2HPO_4 2.1g+KH_2PO_4 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

Polynuclear Aromatic Hydrocarbons in Pond Water

Using 500mg 3mL HyperSep NH_2 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 NH_2 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 H_2O

Apply Sample

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

Wash Column

20mL of DI H_2O

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 H_2O

Apply Sample

Load 4mL samples at 1 to 2mL/minute

Wash Column

1mL of methanol/DI H_2O (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 phosphorate buffer solution acid (pH 2.5)

Condition Retain PEP Extraction Column

5mL of methanol

5mL of phosphorate buffer (pH 2.5)

Apply Sample

Load 10mL samples at 1 to 2mL/minute

Wash Column

3mL of phosphorate buffer (pH 2.5)

Elute Thiourea Herbicides

5mL of ACN/phosphorate 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

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 CH_3OH
1 x 3mL DI H_2O
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 H_2O
1 x 2mL 100mM acetate buffer (pH 4.5)
1 x 3mL CH_3OH
Dry column (10 minutes at >10"Hg)

Elute

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

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

Evaporate to dryness at <40°C

Derivatize

Add 50µL ethyl acetate
Mix/vortex
Add 50µL BSTFA (with 1% TMCS)**
Overlay with N_2 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-5SilMS 30m x 0.25m x 0.25µm	26096-1420

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Amphetamines

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 N_2 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 CH_3OH

1 x 200µL DI H_2O

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

Apply Sample

Do not exceed 1mL/minute

Wash Column

1 x 500µL DI H_2O

1 x 500µL 100mM acetic acid

1 x 500µL CH_3OH

Dry column (5 minutes at >10"Hg)

Elute

1 x 800µL CH_2Cl_2/IPA/NH_4OH (70:26:4)

Do not exceed 1mL/minute

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 N_2

Derivatize

For Amphetamines:*

Add 50µL PFPA (PFAA)

Mix/vortex

Overlay with N_2 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 CH_3OH

200µL of DI H_2O

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 H_2O

500µL of 100mM acetic acid

500µL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Compounds

800µL of CH_2Cl_2/IPA/NH_4OH (70:26:4)

Collect eluate at 1mL/minute (do not exceed this rate)

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 vulgate 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±0.5 with approximately 700µL of 1.0N NaOH

Condition Verify-CX Extraction Column

3mL of CH_3OH then aspirate

3mL of DI H_2O 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) CH_3OH in DI H_2O

Dry column (5 minutes at >10"Hg)

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

Elute Anabolic Steroids

Option a: 3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2); collect eluate at 1 to 2mL/minute

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

Option b: 3mL CH_2Cl_2/IPA (80:20)

Option c: 3mL ethyl acetate

Option d: 3mL CH_3OH

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

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

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 MSTFA solution*

Quantitate

Inject 1 to 2µL onto gas chromatograph

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

Compound	Primary Ion*	Secondary	Tertiary	Other
Testosterone-TMS	432	301	209	
19-noretiocholanone-TMS	405	315	225	
Oxymethalone	640	52	462	370,143
Dehydroepiandosteronw-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-hydroxyetiocholanone	504	417		
17-α-epitestosterone-TMS	432	341	327	209
Stanazolol	472	381	342	149

** 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

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 CH_3OH

1 x 3mL H_2O

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 H_2O

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 1% acetic acid

3mL of CH_3OH

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 TMPAH***

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 DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL of methanol

Dry column (5 minutes at >10"Hg)

Elute Bases

2mL of CH_4OH/NH_4OH (98:2)

Collect eluate at 1 to 2mL/minute

NOTE: *Prepare elution solvent daily*

Extract

To the eluate add 2.0mL of DI H_2O and 500µL 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°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 ß-glucuronidase solution

ß-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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

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 50µL ethyl acetate and 50µL 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 2µL 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 desalkyflurazepam and hydroxyethylflurazepam will extract with high recovery.

Recommended GC Column	Part Number
TraceGOLD TG-5SilMS 30m x 0.25m x 0.25µm	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 2µL 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
Desmethylflunitrazepam-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 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	26AC497P

Benzodiazepines in Serum or Plasma

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

Sample Preparation

To 1mL H_2O 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 CH_3OH
1 x 3mL DI H_2O
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 H_2O
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 CH_3OH
1 x 3mL DI H_2O
1 x 3mL 0.1M phosphate buffer (pH 6.0)

Apply Sample

Load sample by gravity

Wash Column

1 x 3mL DI H_2O
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-5SilMS 30m x 0.25m 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 CH_3OH

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 50µL ACN and 50µL BSTFA with 1% TCMS

Heat for 30 minutes at 70°C

Remove from heat source to cool

Analysis

Inject 1µL 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-5SilMS 30m x 0.25m x 0.25µm	26096-1420

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 CH_3OH
1 x 3mL DI H_2O
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

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH then aspirate
3mL of DI H_2O 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 CH_2Cl_2/IPA/NH_4OH (78:20:2)
Collect eluate at 1 to 2mL/minute

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH

1 x 3mL DI H_2O

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 CH_3OH/NH_4OH (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-5SilMS 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 CH_3OH
1 x 3mL DI H_2O
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 H_2O
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-5SilMS 30m x 0.25m x 0.25µm	26096-1420

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/minute

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 conjulated 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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 CH_2Cl_2/IPA/NH_4OH (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 50µL of methanol

Quantitate

Inject 5µL 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 3µm, 50 x 2.1mm	25903-054630

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 CH_3OH then aspirate
3mL of DI H_2O 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 H_2O
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 CH_3OH
1 x 3mL DI H_2O
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 H_2O
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 0.25m 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 ≈ 3.0)

Condition Verify-CX Extraction Column

3mL of CH_3OH

3mL of DI H_2O

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 H_2O

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°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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 H_2O
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 0.25m x 0.25µm	26094-1420

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

2mL of 100mM HCl

Dry column (5 minutes at >10"Hg)

3mL of hexane

Elute Carisoprodol/Meprobamate

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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% NH_4OH

Collect eluate at 1 to 2mL/minute

NOTE: *Prepare elution solution fresh daily*

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50µL ethyl acetate and 50µL 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 2µL 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 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

1 x 3mL 100mM HCl

1 x 3mL CH_3OH 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 CH_3OH

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 CH_3OH

1 x 200µL DI H_2O

1 x 200µL 100mM HCl

Apply Sample

Do not exceed 1mL/minute

Wash Column

1 x 500µL DI H_2O

1 x 500µL 100mM HCl acid

1 x 500µL CH_3OH/DI H_2O (50:50)

Dry column (5 minutes at >10"Hg)

Elute

1 x 800µL CH_2Cl_2/IPA/NH_4OH (70:26:4)

Do not exceed 1mL/minute

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

Evaporate at <40°C under a stream of N_2

Derivatize

Fluoroalkylate:

Add 100µL PFPA (PFAA) or HFIP

Overlay with N_2 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 N_2 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

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 CH_3OH

200µL of DI H_2O

200µL of 0.1N HCl

Apply Sample

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

Wash Column

500µL of DI H_2O

500µL of 100mM HCl

500µL of CH_3OH/DI H_2O (50:50)

Dry column (5 minutes at >10"Hg)

Elute Compounds

800µL of CH_2Cl_2/IPA/NH_4OH (70:26:4)

Collect eluate at 1mL/minute (do not exceed this rate)

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 H_2O

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 CH_3OH

1 x 3mL DI H_2O

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 H_2O

1 x 2mL 100mM HCl

1 x 3mL CH_3OH

Dry column (5 minutes at >10"Hg)

Elute

1 x 3mL CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/minute

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

2mL of 100mM HCl

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Cocaine and Benzoylecgonine

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50µL ethyl acetate and 50µL 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 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 CH_3OH

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

2mL of 100mM HCl

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Cocaine and Benzoylecgonine

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 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 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 for GC/MS: D_3-Cocaine and D_3-Benzoylecgonine*

*** 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 or air or nitrogen to 200µL
Add 2mL distilled H_2O (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/NH_4OH
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-Hyroxy- 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 0.25m 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 CH_3OH
1 x 3mL DI H_2O
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 H_2O
1 x 3mL 100mM acetic acid
1 x 3mL CH_3OH
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 CH_3OH, 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

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 CH_3OH

3mL of DI H_2O

1mL of 100mM phosphate buffer (pH 6.0)

Apply Sample

Pour supernatant onto column

Allow to flow via gravity

Wash Column

3mL of DI H_2O

Dry column (10 minutes at >10"Hg)

Elute Steroids

3mL of CH_3OH

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 $NaHCO_3/Na_2CO_3$

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 50µL of MSTFA***/NH_4/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 2µL 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 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	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 CH_3OH
1 x 3mL DI H_2O
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 H_2O
1 x 3mL 100mM acetic acid
1 x 3mL CH_3OH
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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Fentanyls

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH

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 CH_3OH (pH 6)

Add internal standard*

Insert oral swab into CH_3OH and mix for 1 minute,

Add 100µL of CH_3OH

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 CH_3OH

1 x 3mL H_2O

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 H_2O

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

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Fentanyl/Norfentanyl

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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% NH_4OH

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent daily*

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 50µL of ethyl acetate and 50µL MTBSTFA (with 1% TBDMCS)***

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 MTBSTFA solution*

Quantitate

Inject 1 to 2µL 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 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of ethyl acetate

3mL hexane

Dry column (5 minutes at >10"Hg or until column is dry)

Elute Gabapentin

1mL of 2% NH_4OH in CH_3OH

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 H_2O

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 N_2 at 700C

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 CH_3OH

1 x 3mL of DI H_2O

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 CH_3OH/NH_4OH (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 N_2

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 0.25m x 0.25µm	26094-1420

Gamma-Hydroxybutyrate (GHB) in Urine without Conversion to Gamma-Butrylactone (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 CH_3OH

1 x 3mL DI H_2O

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 CH_3OH/NH_4OH (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 N_2

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 N_2

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 0.25m x 0.25µm	26094-1420

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetic acid

3mL CH_3OH

Dry column (5 minutes at >10"Hg or until column is dry)

Elute Ketamine

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/minute using minimal vacuum

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 H_2O 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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetic acid

3mL CH_3OH

Dry column (5 minutes at >10"Hg)

Elute LSD

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/minute using minimal vacuum

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 20µL ethyl acetate and 20µL BSTFA (with 1% TMCS)***

Overlay with N_2 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 DI H_2O 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 H_2O

3mL of 100mM acetic acid

3mL CH_3OH

Dry column (5 minutes at >10"Hg)

Elute LSD

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/minute using minimal vacuum

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

Dry Eluate

Evaporate to dryness at <40°C

Derivatize

Add 20µL ethyl acetate and 20µL 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 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

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 CH_3OH
1 x 3mL DI H_2O
1 x 1mL 100mM phosphate buffer (pH 6)

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL DI H_2O
1 x 3mL 100mM acetic acid
1 x 3mL CH_3OH
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 CH_3OH
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 CH_3OH
1 x 3mL DI H_2O
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 H_2O
1 x 3mL 100mM acetic acid
1 x 3mL CH_3OH
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 CH_3OH
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
Normeperdidine	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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL CH_3OH

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Methadone

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Nicotine and Continine

Replace rack of collection tubes

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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

** D_3-Continine and D_4-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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetate buffer (pH 4.5)

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Opiates

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 N_2 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-desmethyltramadol TMS	393	378	303
N-desmethyltramadol 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 D_6-codeine and D_6-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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetate buffer (pH 4.5)

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Opiates

3mL of CH_2Cl_2/IPA/NH_4OH (84:12:4)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetate buffer (pH 4.5)

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Opiates

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 N_2 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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetic acid

3mL of CH_3OH

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 CH_3OH

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 H_2O

1 x 1mL 100mM acetic acid

1 x 1mL CH_3OH

Dry column (3 minutes at >10"Hg)

Elute

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

Collect eluate at 1 to 2mL/min

Add 1 drop 1% HCl in CH_3OH 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-5SilMS 30m x 0.25m 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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetic acid

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Phencyclidine

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetic acid

3mL of CH_3OH

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 CH_3OH

Analysis

Inject 5µL of sample onto LC/MS/MS system

Monitor the following ions:

Compound	MRM Transition
Phencyclidine	244.3/86.1
Phencyclidine-D5*	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

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetic acid

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Propoxyphene

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

3mL of 100mM acetic acid

3mL of CH_3OH

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 CH_2Cl_2/IPA/NH_4OH (78:20:2, v/v/v)

Collect eluate at 1 to 2mL/min

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

Evaporate

Evaporate to half volume under a stream of nitrogen at <40°C

Add 100µL of 0.1% HCl in CH_3OH

Mix/vortex

Continue evaporation to dryness at <40°C

Reconstitute

Reconstitute sample in 100µL of CH_3OH

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 CH_3OH

Dry column (3 minutes at >10"Hg)

Elute Psilocin

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at <35°C

Derivatize

Add 50µL of ethyl acetate

Mix/vortex

Add 50µL of MSTFA***

React at 70°C for 30 minutes

Remove from heat

NOTE: *Do not evaporate MSTFA solution*

Quantitate

Inject 1 to 2µL 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 0.25µm	26AF130P
TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33µm	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 CH_3OH then aspirate

3mL of DI H_2O 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 CH_3OH

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 H_2O (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 CH_3OH then aspirate
3mL of DI H_2O 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 H_2O
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 CH_3OH
Dry column (5 minutes at >10"Hg)

Elute Basic Drugs

Replace collection tubes from elution of acidic and neutral drugs
3mL of CH_2Cl_2/IPA/NH_4OH (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/NH_4OH mix, then add CH_2Cl_2 (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 Desmethylsertraline 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 H_2O 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 CH_3OH then aspirate
3mL of DI H_2O 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 H_2O
1mL of 100mM acetic acid
3mL of CH_3OH
Dry column (5 minutes at >10"Hg)

Elute Sertraline and Desmethylsertraline

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)
Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at <40°C

Reconstitute

Reconstitute with 200µL of ethyl acetate/DI H_2O (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% CH_3CN
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 ß-glucuronidase solution
(ß-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 CH_3OH
1 x 3mL DI H_2O
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 H_2O
1 x 3mL 100mM acetic acid
1 x 3mL CH_3OH
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 CH_3OH
Inject 5µL 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 3µm, 100 x 2.1mm	25403-102130

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute SMA

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Concentrate Eluate

Add 30µL silylation grade DMF*** to eluate

Evaporate to 30µL at <40°C

Alternate Drying Procedure – Fluoroacylate PFPA (PFAA) Derivative

Add 50µL PFPA (PFAA)****

Overlay with N_2 and cap

Improved derivatization by addition of 50µL PFPOH†

React for 20 minutes at 70°C

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:

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
Methylenedioxyamphetamine	135	162	325
Methylenedioxymethamphetamine	204	162	339

** Suggested internal standards for GC/MS: D_5-amphetamine and D_5-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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute SMA

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute SMA

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

NOTE: *Prepare elution solvent fresh daily; add IPA/NH_4OH mix, then add CH_2Cl_2 (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 CH_3OH

3mL of DI H_2O

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 H_2O

3mL of hexane

Dry column (5 minutes at >10"Hg)

Elute Analytes

1mL of CH_3OH

Dry Eluate

Evaporate to full dryness at <40°C under a stream of nitrogen

Reconstitute

Add 200µL of CH_3OH

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 CH_3OH
200µL of DI H_2O
200µL of 100mM HCl

Apply Sample

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

Wash Column

500µL of DI H_2O
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 CH_3OH
1 x 200µL DI H_2O
1 x 200µL 100mM HCl

Apply Sample

Do not exceed 1mL/minute

Wash Column

1 x 500µL DI H_2O
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 N_2

Derivatize

Add 25µL BSTFA (with 1% TMCS)** and 25µL ethyl acetate
Overlay with N_2 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 0.25m x 0.25µm	26094-1420

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 H_2O

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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

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 <2mL/minute

Dry Eluate

Evaporate to dryness at <40°C

Reconstitute with 100µL ethyl acetate

Quantitate Acid and Neutral Drugs

Inject 1 to 2µL onto gas chromatograph

Wash Column

3mL of CH_3OH then aspirate

Dry column (5 minutes at >10"Hg)

Elute Basic Drugs (Fraction 2)

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at <40°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 100µL of ethyl acetate

Quantitate Basic Drugs

Inject 1 to 2µL onto gas chromatograph

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

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 50µL of high purity DMF in the sample (fraction 2) before evaporation*

(3) *A 1% HCl in CH_3OH solution has been used to prevent volatization by the formation of the hydrochloric salt of the drugs. Evaporate fraction 2 to approximately 100µL, 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 CH_3OH then aspirate

3mL of DI H_2O 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 H_2O

1mL of 100mM acetic acid

3mL of CH_3OH

Dry column (5 minutes at >10"Hg)

Elute Tricyclic Antidepressants

3mL of CH_2Cl_2/IPA/NH_4OH (78:20:2)

Collect eluate at 1 to 2mL/min

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

Dry Eluate

Evaporate to dryness at <40°C

Reconstitute

Reconstitute with 200µL of ethyl acetate/DI H_2O (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 CH_3OH

1 x 5mL H_2O (mmol/L HCl)

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 5mL H_2O

1 x 5mL CH_3OH

Dry column using N_2

Elute Melamine

1 x 5mL of CH_3OH containing 4% NH_4OH

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-5SilMS 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 polyvinylidine 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 CH_3CN/DI H_2O (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 CH_2Cl_2
Shake for 5 minutes
Centrifuge
Transfer upper layer to clean glass tube
Add 2mL of DI H_2O to CH_2Cl_2
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 CH_3OH
1 x 3mL DI H_2O

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 H_2O
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 CH_3OH
Dry column (5 minutes at >10"Hg)

Elute Melamine

Insert fresh collection tubes into manifold
1 x 2mL of CH_3OH containing 5% NH_4OH
1 x 3mL of CH_3OH containing 5% NH_4OH
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 CH_3CN
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 CH_3OH
1 x 3mL DI H_2O

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 H_2O
1 x 1mL CH_3OH
Dry column

Elute Cyanuric Acid

Insert fresh collection tubes into manifold
1 x 3mL of CH_3OH containing 1% HCl
1 x 2mL of CH_3OH 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) NH_4OH*

Recommended HPLC Column	Part Number
Hypersil GOLD HILIC 3µm, 150 x 2.1mm	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 CH_3OH

1 x 3mL H_2O

Apply Sample

Load supernate into extraction column

Wash Column

1 x 3mL H_2O

1 x 3mL CH_3OH

Elute Melamine

1 x 5mL of CH_3OH containing 5% NH_4OH

Collect eluate at 1 to 2mL/minute

Evaporation

Evaporate eluates under a gentle stream of nitrogen 50°C

Analysis

Reconstitute sample in CH_3CN

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 H_2O

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

3mL of DI H_2O

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 CH_3CN/DI H_2O (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 CH_2Cl_2
Shake for 5 minutes
Centrifuge
Transfer upper layer to clean glass tube
Add 2mL of DI H_2O to CH_2Cl_2
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 CH_3OH
1 x 3mL DI H_2O

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 CH_3OH
Dry column (5 minutes at >10"Hg)

Elute Melamine

1 x 2mL of CH_3OH containing 5% NH_4OH
1 x 3mL of CH_3OH containing 5% NH_4OH
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 CH_3CN
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 H_2O

Apply Sample

Load 6mL of the sample extract onto the Retain-CX SPE product at 1 to 2mL/minute

Wash Column

3mL of DI H_2O
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 H_2O

Centrifuge at 4,000rpm

Remove supernatant for analysis

Condition HyperSep Retain PEP Extraction Column

1 x 5mL CH_3OH

1 x 5mL H_2O

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 10mL H_2O

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 CH_3CN

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)), Ipronidazole (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 CH_3OH

1 X 3mL H_2O

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL H_2O

Dry column (5 minutes at >10"Hg)

Elute Nitroimidazole

1 x 3mL of CH_3OH

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), Fleroxcain (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 CH_3OH

1 X 3mL H_2O

Apply Sample

Load sample at 1 to 2mL/minute

Wash Column

1 x 3mL H_2O

1 x 3mL of CH_3OH

Elute Quinolone

1 x 3mL of CH_3OH 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 N_2

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
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
Metalaxyl	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
Quinoxyfen	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
Triadmimefon	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 *Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18*	60308-209	5 Pack

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 *Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18*	60308-309	5 Pack

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 *Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18*	60308-409	5 Pack

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 *Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18*	60308-509	5 Pack

** 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

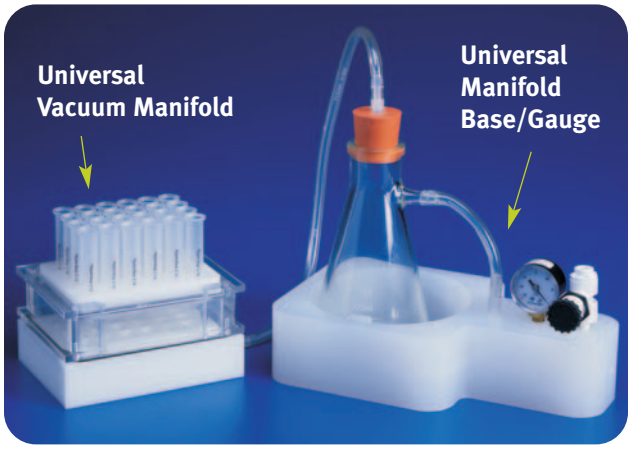
HyperSep UNIGUARD Direct-Connect Online SPE Cartridges

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

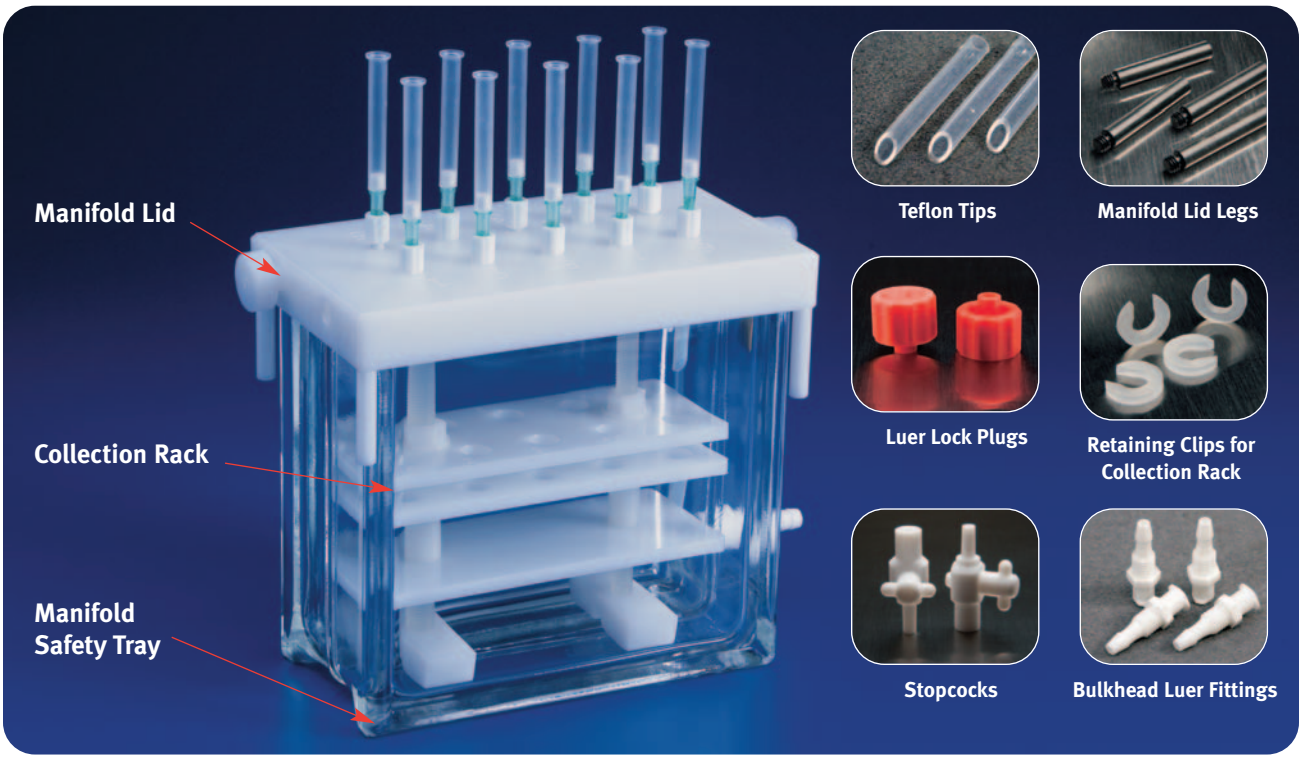
Description	Cat. No.	Quantity
Universal vacuum manifold	60104-230	1 Each
Vacuum pump, European plug	60104-241	1 Each
Vacuum pump, North American plug	60104-243	1 Each
Plugs for 24-position extraction plate	60104-234	24 Pack
Plugs for 48-position extraction plate	60104-235	48 Pack

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 MgSO4, 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

Thermo Scientific Chromatography Columns and Consumables Catalog

This extensive catalog offers 540 pages of proven chromatography tools and product selection guides. Available online, with a robust search tool and optimized for your iPad™. Visit **www.thermoscientific.com/catalog**

Chromatography Resource Center

Our web-based resource center provides technical support, applications, technical tips and literature to help move your separations forward. Visit **www.thermoscientific.com/chromatography**

USA and Canada +1 800 332 3331
Australia 1300 735 292 (free call domestic)
China +86-21-68654588
+86-10-84193588
France +33 (0)1 60 92 48 34
Germany +49 (0) 2423 9431 20
+49 (0) 2423 9431 21
India 1 800 22 8374 (toll-free)
+91 22 6716 2200
Japan +81 3 5826 1615
Switzerland +41 56 618 41 11
United Kingdom +44 (0) 1928 534 110
New Zealand 0800 933 966 (free call domestic)
All Other Enquiries +44 (0) 1928 534 050

Technical Support
North America +1 800 332 3331
Outside North America +44 (0) 1928 534 440

BRCCSSPEAPBOOK 0311

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific