

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



# Removing Uncertainty

by Applying Science to SPE

Pharmaceutical/Biotech • Environmental • Forensics • Food Safety

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# 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.

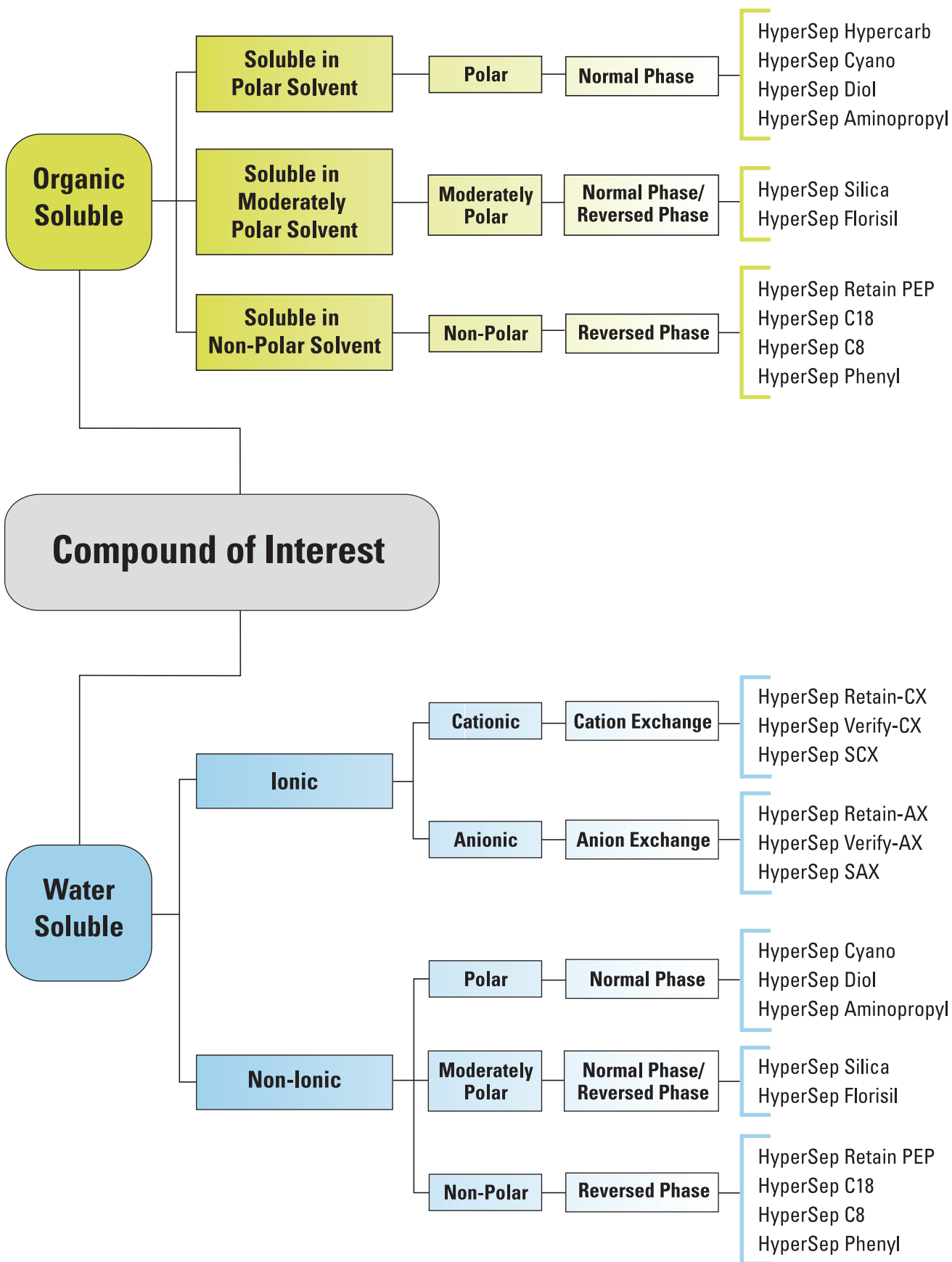
LLE presents additional drawbacks. This process uses large volumes of potentially hazardous solvent and often involves laborious method development. It is a less selective technique for polar compounds.

## 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<sub>a</sub> etc.*
  - *Solubility log P*

# SPE Phase Selection



# HyperSep SPE Phases

## Polymeric



### Reversed Phase Non-polar (Hydrophobic) Phases

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

#### HyperSep Retain PEP

*Polystyrene divinylbenzene material surface modified with urea groups*

- Surface area 550 to 750m<sup>2</sup>/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 750m<sup>2</sup>/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 750m<sup>2</sup>/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 530m<sup>2</sup>/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 530m<sup>2</sup>/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 530m<sup>2</sup>/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 530m<sup>2</sup>/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 289m<sup>2</sup>/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 530m<sup>2</sup>/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 530m<sup>2</sup>/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 530m<sup>2</sup>/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 530m<sup>2</sup>/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 530m<sup>2</sup>/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 530m<sup>2</sup>/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.

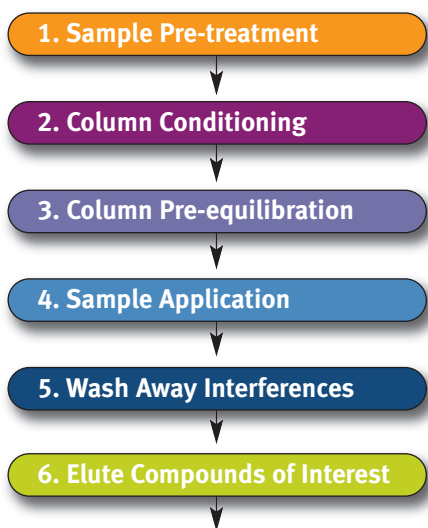
#### HyperSep Verify-AX

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

- Surface area 470 to 530m<sup>2</sup>/g
- Particle size 40 to 60µm
- Pore size 60Å

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

# SPE Procedure – Six Steps for Clean Extract



## 1 Sample Pre-treatment

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

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

### Sample Matrix Sample Pre-Treatment

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

## 2 Column Conditioning

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

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

## 3 Column Pre-equilibration

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

## 4 Sample Application

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

## 5 Wash Away Interferences

Remove impurities bound less strongly than the compounds of interest.

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

## 6 Elute Compounds of Interest


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

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



# Solvent Selection in SPE

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

| Polarity   | Solvent              | Miscibility with Water |
|--|----------------------|------------------------|
| <b>Nonpolar</b><br><br><b>Polar</b> | 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                    |
|  | Acetic Acid          | Yes                    |

## Method Development Optimization in SPE

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

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

### Factors that influence levels of recovery are:

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

### Application Examples

#### 1) Optimization of Load Step

##### *For Reversed-Phase Interactions*

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

##### *For Normal-Phase Interactions*

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

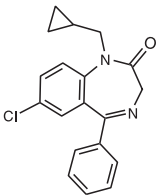
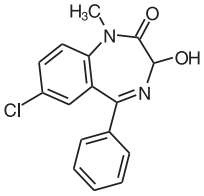
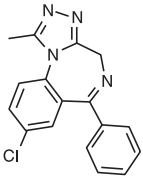
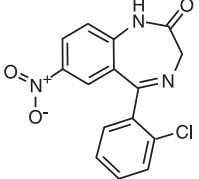
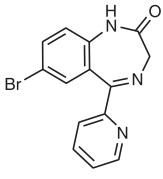
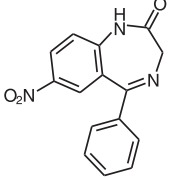
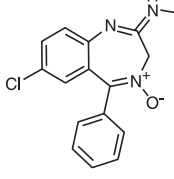
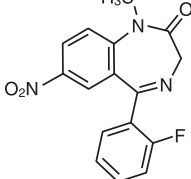
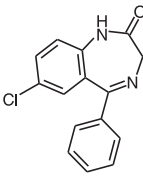
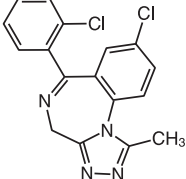
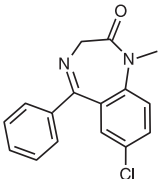
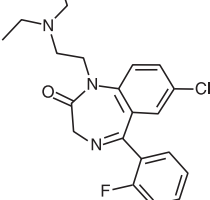
##### *For Ion-Exchange Interactions*

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

## 2) Optimization of Wash and Elution Steps

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

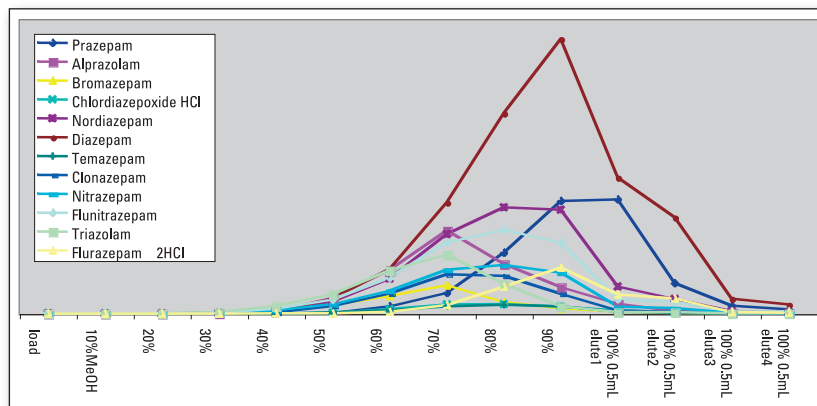
For all phases, the following compounds were investigated:

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

**HyperSep Retain PEP**

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

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

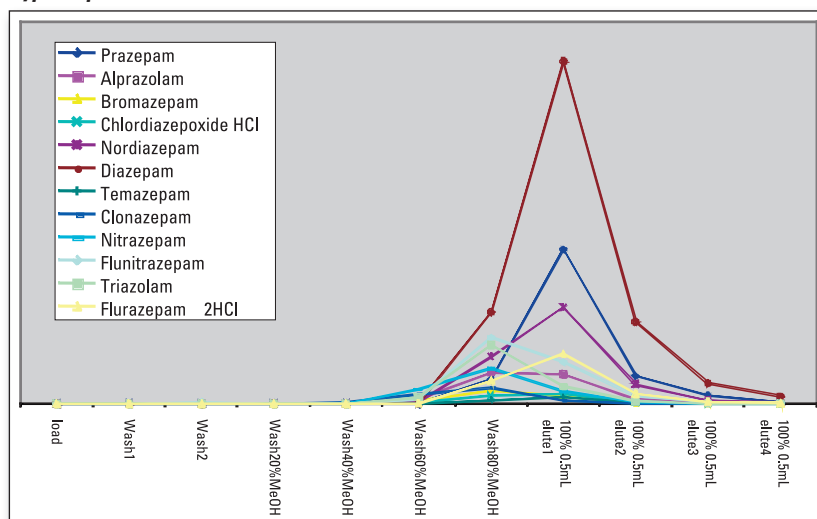
**HyperSep Retain PEP Wash/Elution Profile**

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

**HyperSep Retain-CX**

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

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

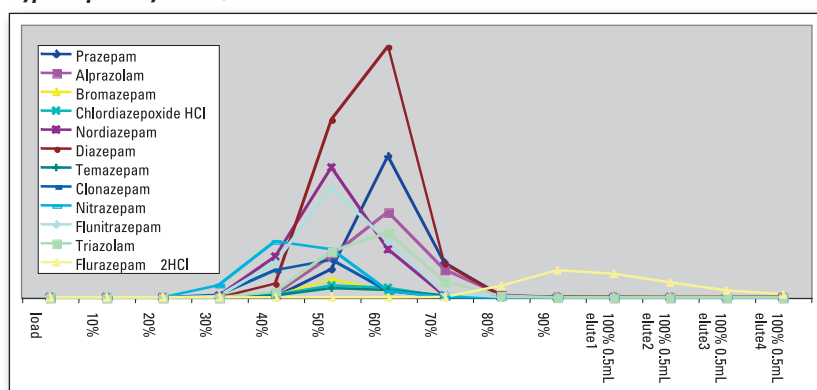
**HyperSep Retain-CX Wash/Elution Profile**

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

**HyperSep Phenyl**

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

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

**HyperSep Phenyl Wash/Elution Profile**

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

**Conclusion**

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

## Acetaminophen in Calf Serum

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

### Sample Preparation

- Dilute 10mg of acetaminophen in 100mL of water to give a 100ppm solution
- Mix 25mL of acetaminophen (100ppm) solution in a 50mL flask and dilute to volume with water (standard solution 50ppm)
- Mix 25mL of acetaminophen (100ppm) solution in a 50mL flask and dilute to volume with serum and 1% H<sub>3</sub>PO<sub>4</sub> (sample 50% serum and 1% H<sub>3</sub>PO<sub>4</sub>)

### Condition Retain PEP Extraction Column

- 2mL of methanol
- 2mL of DI H<sub>2</sub>O

### Apply Sample

- Load 2mL sample solutions

### Wash Column

- 2mL of methanol/DI H<sub>2</sub>O (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<sub>3</sub>PO<sub>4</sub> (sample 50% serum and 1% H<sub>3</sub>PO<sub>4</sub>)

### Condition Retain-CX Extraction Column

- 2mL of methanol
- 2mL of DI H<sub>2</sub>O

### Apply Sample

- Load 2mL sample solutions

### Wash Column

- 2mL of methanol/DI H<sub>2</sub>O (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<sub>3</sub>OH
- 1 x 2mL H<sub>2</sub>O water 2mL

### Apply Sample

- Load 2mL of sample

### Wash Column

- 1 x 2mL 5% CH<sub>3</sub>OH

### Elute Sample

- 1 x 2mL CH<sub>3</sub>OH

### 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<sub>2</sub>O

## Apply Sample

Load 4mL samples at 1-2mL/minute

## Wash Column

1mL of DI H<sub>2</sub>O

## 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<sub>2</sub>PO<sub>4</sub> (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<sub>3</sub>OH

1 x 3mL DI H<sub>2</sub>O

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<sub>2</sub>O

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 – $\beta$ -Glucuronidase Hydrolysis

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

$\beta$ -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<sub>3</sub>OH

1 x 3mL DI H<sub>2</sub>O

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<sub>2</sub>O

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

Dry column (5 minutes at >10" Hg)

1 x 2mL hexane

## Elute Benzodiazepines

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

## Dry Eluate

Evaporate to dryness at <40°C

## Derivatize

Add 50 $\mu$ L ethyl acetate and 50 $\mu$ L BSTFA\*\*  
(with 1% TMCS)\*\*

Overlay with Nitrogen and cap

Mix/vortex

React 20 minutes at 70°C

Remove from heat source to cool

**NOTE:** Do not evaporate BSTFA solution

## Quantitate

Inject 1 to 2 $\mu$ L onto GC/MS

For mass spectrometry monitor the following ions:

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

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

\*\* Part number TS-38831

\*\*\* Quantitation ion

## Recommended GC Column

## Part Number

TraceGOLD TG-5MS 30m x 0.25m x 0.25 $\mu$ m

26098-1420



# Benzodiazepines in Whole Blood for GC or GC/MS Confirmations

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

## Prepare Sample

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

Add 5mL of pH 6 buffer

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

## Condition HyperSep Diol Extraction Column

1 x 3mL ethyl acetate

1 x 3mL MeOH

1 x 3mL DI H<sub>2</sub>O

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

## Apply Sample

Load sample by gravity

## Wash Sample

1 x 3mL DI H<sub>2</sub>O

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    |
| Alphahydroxyalprazolam     | 325.1/242.9    |
| Alphahydroxyalprazolam-D5* | 330.1/302.2    |
| Chlordiazepoxide           | 300.1/227.0    |
| Diazepam                   | 285.5/192.5    |
| Diazepam*                  | 292.2/198.2    |
| Lorazepam                  | 321.1/275.1    |
| Lorazepam-D4*              | 325.1/279.0    |
| Nordiazepam                | 271.1/140.1    |
| Nordiazepam-D5*            | 275.6/140.1    |
| Oxazepam                   | 287.1/241.1    |
| Oxazepam-D5*               | 290.2/198.2    |
| Temazepam                  | 301.1/255.1    |
| Temazepam-D5*              | 306.1//260.1   |

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

## Recommended HPLC Column

| 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<sub>2</sub>O  
5mL of 30mmol/L of chloric acid

## Apply Sample

Load 2mL samples

## Wash Column

5mL of DI H<sub>2</sub>O  
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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 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<sub>2</sub>O

### Apply Sample

Load 10mL samples at 1 to 2mL/minute

### Wash Column

1mL of methanol/DI H<sub>2</sub>O (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<sub>2</sub>O

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: $\beta$ -Glucuronidase Hydrolysis

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

$\beta$ -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<sub>3</sub>OH

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<sub>4</sub>OH

Collect eluate at 1 to 2mL/minute

**NOTE:** Prepare fresh daily

## Dry Eluate

Evaporate to dryness at <40°C

## Derivatize

Add 50 $\mu$ L ethyl acetate and 50 $\mu$ L MTBSTFA (with 1%  
TBDMCS)\*\*

Mix/vortex

React 20 minutes at 90°C

Remove from heat source to cool

**NOTE:** Do not evaporate MTBSTFA solution

## Analysis

Inject 1 to 2 $\mu$ L of sample onto GC/MS

For mass spectrometry monitor the following ions:

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

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

\*\* Part number TS-48927

\*\*\* Quantitation ion

## Recommended GC Column

Part Number

TraceGOLD TG-5MS 30m x 0.25m x 0.25 $\mu$ m 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<sub>3</sub>CN/DI H<sub>2</sub>O (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<sub>2</sub>Cl<sub>2</sub>  
Shake for 5 minutes  
Centrifuge  
Transfer upper layer to clean glass tube  
Add 2mL of DI H<sub>2</sub>O to CH<sub>2</sub>Cl<sub>2</sub>  
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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O

**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<sub>2</sub>O  
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<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute Melamine

Insert fresh collection tubes into manifold  
1 x 2mL of CH<sub>3</sub>OH containing 5% NH<sub>4</sub>OH  
1 x 3mL of CH<sub>3</sub>OH containing 5% NH<sub>4</sub>OH  
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<sub>3</sub>CN  
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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O

**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<sub>2</sub>O  
1 x 1mL CH<sub>3</sub>OH  
Dry column (just enough to remove residual solvent)

## Elute Cyanuric Acid

Insert fresh collection tubes into manifold  
1 x 3mL of CH<sub>3</sub>OH containing 1% HCl  
1 x 2mL of CH<sub>3</sub>OH 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<sub>4</sub>OH

## 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<sub>3</sub>OH

1 x 3mL DI H<sub>2</sub>O

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<sub>2</sub>O

Dry column (10 minutes at >10mm Hg)

## Elute Steroids

1 x 3mL of CH<sub>3</sub>OH

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  $\beta$ -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<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub>

## Additional Clean-up

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

## Derivatize

Add 50 $\mu$ L of MSTFA/NH<sub>4</sub>I/dithioerythritol  
(1,000:2:5, V/W/W) and incubate at 70°C for  
20 minutes

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

## Quantitate

Inject 1 to 2 $\mu$ L onto GC/MS

For mass spectrometry monitor the following ions:

| Compound                         | Primary Ion** | Secondary |
|----------------------------------|---------------|-----------|
| Testosterone                     | 432           | 417       |
| Epitestosterone                  | 432           | 417       |
| DHEA                             | 432           | 417       |
| 16 $\alpha$ Hydroxytestosterone* | 520           | 259       |

\* Suggested internal standard at 20ng/mL: 16  $\alpha$  Hydroxytestosterone

\*\* Quantitation ion

## Recommended GC Column

|   | Part Number |
|---|-------------|
| TraceGOLD TG-5MS 30m x 0.25m x 0.25 $\mu$ m | 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<sub>2</sub>O

### 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<sub>3</sub>OH
- 1 x 3mL H<sub>2</sub>O

### Apply Sample

- Load spiked serum sample onto SPE cartridge

### Wash Column

- 1 x 3mL H<sub>2</sub>O
- 1 x 3mL 5% CH<sub>3</sub>OH

### Elution

- 1 x 3mL CH<sub>3</sub>OH

### 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 500 $\mu$ L of 20% acetic acid add internal standard\*  
Mix/vortex  
Add 500 $\mu$ L of blood, plasma/serum  
Mix/vortex  
Centrifuge as appropriate

## Condition HyperSep C18 Extraction Column

1 x 3mL CH<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O  
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<sub>4</sub>OH in CH<sub>3</sub>OH

## Dry Eluate

Evaporate to dryness at <40°C

## Derivatization

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

## Quantitate

Inject 1 to 2 $\mu$ L 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 0.25 $\mu$ m 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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O  
1 x 3mL ethyl acetate  
1 x 3mL hexane  
Dry column (10 minutes at >10" Hg)

## Elute Gabapentin

1 x 3mL CH<sub>3</sub>OH containing 2% NH<sub>4</sub>OH  
Collect eluate at 1 to 2mL/minute

## Evaporation

Evaporate eluates under a gentle stream of nitrogen <40°  
Dissolve residue in 100 $\mu$ L CH<sub>3</sub>OH  
Inject 5 $\mu$ L of sample on to LC/MS

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

## Recommended HPLC Column

## Part Number

Hypersil GOLD 3 $\mu$ m, 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<sub>2</sub>O

### Apply Sample

Load 2mL samples

### Wash Column

2mL of DI H<sub>2</sub>O

### 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<sub>2</sub>O

### Apply Sample

Load 10mL samples at 1 to 2mL/minute

### Wash Column

1mL of DI H<sub>2</sub>O

### 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

Hypersil GOLD aQ 5µm, 150 x 4.6mm

### Part Number

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<sub>3</sub>OH

1 x 3mL DI H<sub>2</sub>O

### Apply Sample

Decant supernatant onto SPE column

### Wash Column

1 x 10mL of DI H<sub>2</sub>O

Dry with vacuum for 3 minutes

1 x 5mL of CH<sub>3</sub>OH

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

TraceGOLD TG-5MS 30m x 0.25m x 0.25µm

### Part Number

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<sub>2</sub>O

## 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<sub>2</sub>PO<sub>4</sub> (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 Cotinine 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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O  
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<sub>3</sub>OH  
Dry column, (5 minutes at >10" Hg)

## Elute Cotinine and Nicotine

Replace rack of collection tubes

1 x 3mL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix,  
then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub>O add internal standard\*  
Add 1mL blood  
Add 8mL of DI H<sub>2</sub>O  
Mix/vortex and centrifuge as appropriate

## Condition HyperSep Cyano Extraction Column

1 x 3mL CH<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O

**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<sub>3</sub>OH  
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<sub>3</sub>OH  
1 x 1mL H<sub>2</sub>O

## Apply Sample

Load 0.5mL of sample

## Wash Column

1 x 1mL 1%CH<sub>4</sub>O<sub>3</sub>S  
1mL CH<sub>3</sub>OH

## 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<sub>2</sub>O  
Mix/vortex  
Heat to 90°C for 40 minutes in a heating block, or an autoclave for 15 minutes on a liquid cycle  
Cool before proceeding  
Centrifuge for 10 minutes at 2,000rpm and discard pellet  
Add 500µL 50% ammonium hydroxide  
Mix/vortex  
Adjust sample pH 5 to 6 by drop wise addition with 50% ammonium hydroxide

## Prepare Sample-enzymatic Hydrolysis of Glucuronides

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

## Condition Clean Extraction Column

1 x 3mL CH<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O then aspirate  
1 x 3mL 100mM acetate buffer (pH 4.5) then aspirate  
1 x 3mL CH<sub>3</sub>OH then aspirate  
Dry column (5 minutes at >10" Hg)

## Elute Opiates

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

## Dry Eluant

Evaporate to dryness at <40°C

## Derivatize

Add 200µL of a 1:1 solution of propionic anhydride pyridine  
Make this solution fresh daily  
Mix/vortex  
React for 60 minutes at 60°C in a heater block  
Remove from heat source to cool  
Evaporate to dryness at <40°C  
Reconstitute the residue with 50µL of ethyl acetate/methanol (70:30)

## Quantitate

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

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

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

\*\* Quantitation ion

## Recommended GC Column

|                                       | Part Number |
|---------------------------------------|-------------|
| TraceGOLD TG-5MS 30m x 0.25m x 0.25µm | 26098-1420  |

# 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<sub>2</sub>O
- 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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 1 x 3mL 100mM acetate buffer (pH 4.5)
- 1 x 3mL CH<sub>3</sub>OH
- Dry column (5 minutes at >10" Hg)

## Elute Opiates

- 1 x 3mL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (76:20:4)
- Collect eluate at 1 to 2mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> 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  $\beta$ -Glucuronidase solution. ( $\beta$ -Glucuronidase solution contains 5,000 F units/mL *Patella Vulgata* in 100mM acetate buffer, pH 5.0). Hydrolyze for 3 hours at 60°C.

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

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

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

## Apply Sample

Load at a rate of 1 to 2mL/min

## Was Column

1 x 1mL DI H<sub>2</sub>O

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<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78/20/2), collect eluate at 1 to 2mL/min

Evaporate eluate to dryness at <40°C

## Derivatization

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

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

**NOTE:** Do not evaporate BSTFA solution

## Analyze

Inject 1 to 2 $\mu$ L onto GC/MS

For mass spectrometry monitor the following ions:

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

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

\*\* Part number TS-38831

## Recommended GC Column

## Part Number

TraceGOLD TG-5MS 30m x 0.25m x 0.25 $\mu$ m 26098-1420

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

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

## Prepare Sample

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

Add 1mL whole blood, serum/plasma or urine

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

Mix/vortex and centrifuge as appropriate

## Condition HyperSep Verify-CX Extraction Column

1 x 3mL CH<sub>3</sub>OH

1 x 3mL DI H<sub>2</sub>O

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<sub>2</sub>O

1 x 3mL 100mM acetic acid

1 x 3mL CH<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

## Elute Paroxetine

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

Collect eluate at 1 to 2mL/minute

## Evaporation

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

Dissolve residue in 100 $\mu$ L CH<sub>3</sub>OH

Injection Volume: 5 $\mu$ L onto LC/MS triple quad

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

\* Suggested internal standard: Paroxetine-D6

## Recommended HPLC Column

## Part Number

Hypersil GOLD C8 3 $\mu$ m, 50 x 4.6mm 25203-054630

## Propranolol in Rat Serum

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

### Sample Preparation

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

### Condition Retain PEP Extraction Column

- 2mL of methanol
- 2mL of DI H<sub>2</sub>O

### 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<sub>2</sub>O
- 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<sub>2</sub>O 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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O  
1 x 1mL 100mM acetic acid  
1 x 3mL CH<sub>3</sub>OH  
Dry column (5 minutes at >10"Hg)

## Elute

1 x 3mL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

## Dry Eluate

Evaporate to dryness at <40°C

## Quantitate

Reconstitute with 200µL acetonitrile:DI H<sub>2</sub>O (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<sub>3</sub>CN  
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<sub>4</sub> 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<sub>2</sub>O  
Mix/vortex

## Condition HyperSep Retain PEP Extraction Column

1 x 2mL CH<sub>3</sub>OH  
1 x 2mL DI H<sub>2</sub>O

**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<sub>2</sub>O  
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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O  
1 x 1mL 100mM acetic acid  
1 x 3mL CH<sub>3</sub>OH  
Dry column (5 minutes at >10"Hg)

## Elute

1 x 3mL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1mL/minute or use gravity flow

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

## Dry Eluate

Evaporate to dryness at <40°C

## Quantitate

Reconstitute with 200µL ethyl acetate/DI H<sub>2</sub>O (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<sub>2</sub>O

### Apply Sample

Load 4mL samples at 1 to 2mL/minute

### Wash Column

1mL of DI H<sub>2</sub>O

### 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<sub>2</sub>O at concentrations of 200, 20 and 2ppm

### Condition Retain PEP Extraction Column

3mL of methanol

3mL of DI H<sub>2</sub>O

### Apply Sample

Load 3mL samples at 1 to 2mL/minute

### Wash Column

1mL of DI H<sub>2</sub>O

### 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 20 $\mu$ L of acetic acid

### Condition Retain PEP Extraction Column

2mL of methanol  
2mL of DI H<sub>2</sub>O

### Apply Sample

Load 4mL samples at 1 to 2mL/minute

### Wash Column

1mL of DI H<sub>2</sub>O

### 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 5 $\mu$ m, 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  $\leq 3$  using 0.5 M H<sub>2</sub>SO<sub>4</sub>

### Condition Retain PEP Extraction Column

3mL of tetrahydrofuran  
5mL of methanol  
5mL of DI H<sub>2</sub>O

### Apply Sample

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

### Wash Column

5mL of DI H<sub>2</sub>O  
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<sub>2</sub>SO<sub>4</sub>

### Condition HyperSep Retain PEP Extraction Column

1 x 5mL THF  
1 x 5mL CH<sub>3</sub>OH  
1 x 5mL H<sub>2</sub>O

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

### Apply Sample

Load 1 x 500mL H<sub>2</sub>O  
Elute at 5mL/minute

### Wash Column

1 x 5mL H<sub>2</sub>O  
Dry column (20 minutes under N<sub>2</sub>)

### Elute

1 x 3mL THF  
Concentrate sample to 3mL under N<sub>2</sub>

### 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<sub>2</sub>O

### Apply Sample

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

### Wash Column

10mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
Adjust pH to 2 with H<sub>2</sub>SO<sub>4</sub>

### Condition HyperSep Retain PEP Extraction Column

1 x 5mL CH<sub>3</sub>OH  
1 x 5mL H<sub>2</sub>O

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

### Apply Sample

Load 1 x 500mL H<sub>2</sub>O  
Elute at 5mL/minute

### Wash Column

1 x 5mL H<sub>2</sub>O  
1 x 1mL CH<sub>3</sub>OH

### Elute

1 x 3mL THF  
Concentrate sample to 3mL under N<sub>2</sub>

### Analysis

Inject onto HPLC

### Recommended HPLC Column

Hypersil GOLD PFP 3µm, 150 x 4.6mm

### Part Number

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<sub>2</sub>SO<sub>4</sub>

### Condition HyperSep Retain PEP Extraction Column

1 x 5mL CH<sub>3</sub>OH  
1 x 5mL H<sub>2</sub>O

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

### Apply Sample

Load 1 x 500mL H<sub>2</sub>O  
Elute at 5mL/minute

### Wash Column

1 x 5mL H<sub>2</sub>O  
1 x 1mL CH<sub>3</sub>OH  
Dry column (20 minutes under N<sub>2</sub>)

### Elute

1 x 3mL THF  
Concentrate sample to 3mL under N<sub>2</sub>

### Analysis

Inject onto HPLC

### Recommended HPLC Column

Hypersil GOLD PFP 3µm, 150 x 4.6mm

### Part Number

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<sub>2</sub>SO<sub>4</sub>

### Condition Retain PEP Extraction Column

3mL of tetrahydrofuran  
5mL of methanol  
5mL of DI H<sub>2</sub>O

### Apply Sample

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

### Wash Column

5mL of DI H<sub>2</sub>O  
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<sub>2</sub> (final conc. 10mg/L)

### Condition HyperSep C18 Extraction Column

1 x 1mL 1:1 EtAc/CH<sub>2</sub>Cl<sub>2</sub>  
1 x 10mL CH<sub>3</sub>OH  
1 x 10mL H<sub>2</sub>O

### Apply Sample

Add 1 x 5mL CH<sub>3</sub>OH 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<sub>2</sub>Cl<sub>2</sub>  
1 x 3mL EtAc/CH<sub>2</sub>Cl<sub>2</sub>

### 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

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

### Part Number

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<sub>3</sub>OH

1 x 30mL H<sub>2</sub>O

1 x 3mL H<sub>2</sub>O to top of cartridge

## Apply Sample

Load sample at 10 to 15mL/minute

## Wash Column

1 x 5mL H<sub>2</sub>O

Dry column

## Elute

Insert fresh collection tubes into manifold

1 x 15mL of ammonium acetate/CH<sub>3</sub>OH

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<sub>3</sub>OH

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<sub>2</sub>
- 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<sub>3</sub>OH

### 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<sub>2</sub>O
- 1 x 50mL DI H<sub>2</sub>O

### Nitramines, Nitroaromatics

- 1 x 15mL ACN
- 1 x 30mL DI H<sub>2</sub>O

## 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<sub>2</sub>O

### 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<sub>2</sub>O adjusted to pH 6

### Condition HyperSep Verify-AX Extraction Column

1 x 5mL CH<sub>3</sub>OH  
1 x 10mL DI H<sub>2</sub>O

### Apply Sample

Load sample at 1 to 3mL/minute

### Wash Column

1 x 10mL DI H<sub>2</sub>O  
Dry column (10 minutes at >10" Hg)

### Elute

1 x 4mL of 1 mol/L HCl/CH<sub>3</sub>OH (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<sub>3</sub>OH

1 x 3mL H<sub>2</sub>O

**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<sub>2</sub>O

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<sub>2</sub>O for analysis

### Base

1 x 3mL of triethylamine to the column

Flow through at 1 to 3mL/minute

Dilute eluant with H<sub>2</sub>O for analysis

### Analysis

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

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

## 4'4-Methylenedianiline in Serum

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

### Prepare Sample

None

### Condition Verify-CX Extraction Column

3mL of methanol

3mL of DI H<sub>2</sub>O

### Apply Sample

Load sample at 1mL/minute

### Wash Column

1mL of DI H<sub>2</sub>O

### Elute 4'4-Methylenedianiline

0.25mL of methanol containing 1 M ammonium hydroxide

### Analysis

Inject 10µL onto HPLC system

Extracted 100 µg/mL sample

Retention time: 3.162 seconds

Mobile phase: methanol/water (50:50)

Flow rate: 1.2mL/min

Injection volume: 10µL

Wavelength: UV 254nm

### Recommended HPLC Column

### Part Number

Hypersil GOLD 5µm, 250 x 4.6mm

25005-254630

# Miticides and Agrochemicals in Honey Bees, Wax and Pollen

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

## Sample Preparation

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

## Clean-Up

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

## Analysis

**LC**  
LC analysis required for neonicotinoids, polar pesticides and their metabolites

## GC

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

## Recommended HPLC Column

## Part Number

Hypersil GOLD 3µm, 150 x 2.1mm

25003-152130

# Nicosulfuron in Solution

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

## Sample Preparation

Prepare a 10ppm solution of nicosulfuron in DI H<sub>2</sub>O

## Condition Retain-AX Extraction Column

1mL of methanol  
1mL of DI H<sub>2</sub>O

## 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<sub>2</sub>O

### 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<sub>2</sub>O

### Apply Sample

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

### Wash Column

5mL of DI H<sub>2</sub>O  
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<sub>3</sub>OH 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<sub>3</sub>OH

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

### Apply Sample

Load 1 x 10mL H<sub>2</sub>O  
Elute at 5mL/minute

### Wash Column

1 x 10mL H<sub>2</sub>O  
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<sub>3</sub>OH

### Condition HyperSep C18 Extraction Column

1 x 10mL methylene Chloride  
1 x 10mL acetone  
1 x 10 CH<sub>3</sub>OH  
1 x 20mL DI H<sub>2</sub>O

**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<sub>3</sub>Cl  
Dry the extract by passing it through anhydrous sodium sulfate  
Rinse the collection device with CH<sub>3</sub>Cl  
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<sub>2</sub>O

### Apply Sample

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

### Wash Column

20mL of DI H<sub>2</sub>O

### Dry Column

Use maximum vacuum pressure for 15 to 30 minutes

### Elute Chlorophenoxy Acid Herbicides

10mL of hexane/acetone (50:50)

### Concentration/Evaporation

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

Evaporate to 500µL under a nitrogen stream at room temperature

### Pesticides Extracted

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

## GC/MS Determination of Phenols in Drinking Water

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

### Sample Preparation

Collect 1L of H<sub>2</sub>O

Adjust pH to 2 with 6N HCl

### Condition HyperSep Retain PEP Extraction Column

1 x 3mL CH<sub>3</sub>Cl

1 x 3mL CH<sub>3</sub>OH

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<sub>2</sub>O

Dry column (1 minute at >10" Hg)

### Elute

1 x 10mL CH<sub>3</sub>Cl

1 x 3mL CH<sub>3</sub>Cl

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<sub>3</sub>Cl

Analyze the extract with GC/MS

### Recommended GC Column

TraceGOLD TG-5MS 30m x 0.25m x 0.25µm

### Part Number

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<sub>2</sub>O

## Condition HyperSep Retain PEP Extraction Column

1 x 3mL methyl tertiary butyl ether (10:90, V/V)  
1 x 3mL CH<sub>3</sub>OH  
1 x 3mL H<sub>2</sub>O

**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<sub>2</sub>O  
Dry column (20 minutes at >10" Hg)

## Elute

1 x 2mL CH<sub>3</sub>OH  
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<sub>2</sub>O to form a slurry
- Mix for 15 minutes
- Adjust pH to 2 using 50% aq H<sub>2</sub>SO<sub>4</sub>
- Filter sample through previously acidified filter media

## Condition HyperSep C18 Extraction Column

- 1 x 5mL CH<sub>3</sub>OH
- 1 x 5mL DI H<sub>2</sub>O

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

## Apply Sample

- Load sample at 10mL/minute

## Elute Phenoxyacetic acid Herbicides

- 2 x 5mL CH<sub>3</sub>OH
- Collect at 1 to 2mL/minute
- Evaporate to dryness at <40°C using N<sub>2</sub>

## 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<sub>2</sub>O

## Apply Sample

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

## Wash Column

- 20mL of DI H<sub>2</sub>O

## 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<sub>2</sub>O

## Condition HyperSep Retain PEP Extraction Column

- 1 x 5mL IPA
- 1 x 5mL H<sub>2</sub>O

**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<sub>2</sub>O 300mL+CH<sub>3</sub>OH 700mL+Na<sub>2</sub>HPO<sub>4</sub> 2.1g+KH<sub>2</sub>PO<sub>4</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>O

### Apply Sample

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

### Wash Column

20mL of DI H<sub>2</sub>O

### 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<sub>2</sub>O

### Apply Sample

Load 4mL samples at 1 to 2mL/minute

### Wash Column

1mL of methanol/DI H<sub>2</sub>O (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

Hypersil GOLD 5µm, 250 x 4.6mm

### Part Number

25005-254630

# Thiourea Herbicides in Soil

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

## Sample Preparation

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

## Condition Retain PEP Extraction Column

5mL of methanol

5mL of phosphate buffer (pH 2.5)

## Apply Sample

Load 10mL samples at 1 to 2mL/minute

## Wash Column

3mL of phosphate buffer (pH 2.5)

## Elute Thiourea Herbicides

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

## Dry Eluate and Reconstitute

Evaporate to full dryness under a stream of nitrogen

Reconstitute the sample to 1mL with methanol

## Analysis

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

Flow: 1mL/minute

Temperature: 30°C

Detection: mass spectrometry

## Results

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

## Recommended HPLC Column

## Part Number

|                                |              |
|--------------------------------|--------------|
| Hypersil GOLD 5µm, 250 x 4.6mm | 25005-254630 |
|--------------------------------|--------------|

# 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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 1 x 2mL 100mM acetate buffer (pH 4.5)
- 1 x 3mL CH<sub>3</sub>OH
- Dry column (10 minutes at >10"Hg)

## Elute

- 1 x 3mL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)
- Collect eluate at 1 to 2mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> and cap
- Mix/vortex
- React 45 minutes at 70°C
- Remove from heat source to cool

**NOTE:** Do not evaporate BSTFA solution

## Analysis

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

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

\* Suggested internal standard for GC/MS:

\*\* Part number TS-38831

\*\*\* Quantitation ion

## Recommended GC Column

Part Number

TraceGOLD TG-5SiIMS 30m x 0.25m 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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

1mL of 100mM acetic acid

3mL of CH<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

## Elute Amphetamines

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> 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 <sub>5</sub> -amphetamine*     | 194           | 92        | 123      |
| Amphetamine                      | 190           | 91        | 118      |
| D <sub>5</sub> -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<sub>3</sub>OH
- 1 x 200µL DI H<sub>2</sub>O
- 1 x 200µL 100mM phosphate buffer (pH=6.0)

## Apply Sample

- Do not exceed 1mL/minute

## Wash Column

- 1 x 500µL DI H<sub>2</sub>O
- 1 x 500µL 100mM acetic acid
- 1 x 500µL CH<sub>3</sub>OH
- Dry column (5 minutes at >10" Hg)

## Elute

- 1 x 800µL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (70:26:4)
- Do not exceed 1mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub>

## Derivatize

### For Amphetamines\*:

- Add 50µL PFPA (PFAA)
- Mix/vortex
- Overlay with N<sub>2</sub> 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<sub>3</sub>OH
- 200µL of DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 500µL of 100mM acetic acid
- 500µL of CH<sub>3</sub>OH
- Dry column (5 minutes at >10" Hg)

## Elute Compounds

- 800µL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (70:26:4)
- Collect eluate at 1mL/minute (do not exceed this rate)

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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 – $\beta$ -Glucuronidase Hydrolysis

To 5mL of urine add suitable internal standards and 2mL of  $\beta$ -Glucuronidase

$\beta$ -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 $\pm$ 0.5 with approximately 700 $\mu$ L of 1.0N NaOH

## Condition Verify-CX Extraction Column

3mL of CH<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>3</sub>OH in DI H<sub>2</sub>O

Dry column (5 minutes at >10" Hg)

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

## Elute Anabolic Steroids

**Option a:** 3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2); collect eluate at 1 to 2mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

**Option b:** 3mL CH<sub>2</sub>Cl<sub>2</sub>/IPA (80:20)

**Option c:** 3mL ethyl acetate

**Option d:** 3mL CH<sub>3</sub>OH

## Dry Eluate

Evaporate to dryness at <40°C

## Derivatize

Add 50 $\mu$ L ethyl acetate and 50 $\mu$ L MSTFA (with 3% trimethylsilyliodide)

Overlay with N<sub>2</sub> and cap

Mix/vortex

React for 20 minutes at 70°C

Remove from heat source to cool

**NOTE:** Do not evaporate MSTFA solution

## Quantitate

Inject 1 to 2 $\mu$ L onto gas chromatograph

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

| Compound                          | Primary Ion* | Secondary | Tertiary | Other   |
|-----------------------------------|--------------|-----------|----------|---------|
| Testosterone-TMS                  | 432          | 301       | 209      |         |
| 19-noretiocholanone-TMS           | 405          | 315       | 225      |         |
| Oxymethalone                      | 640          | 52        | 462      | 370,143 |
| Dehydroepiandrosteronw-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- $\beta$ -hydroxyandosterone   | 522          | 417       | 158      |         |
| Methandienone                     | 409          | 313       | 281      |         |
| 19-norandosterone-2TMS            | 405          | 315       | 225      |         |
| Alpha-hydroxyetiocholanone        | 504          | 417       |          |         |
| 17- $\alpha$ -epitestosterone-TMS | 432          | 341       | 327      | 209     |
| Stanozolol                        | 472          | 381       | 342      | 149     |

\* Quantitation ion

## Recommended GC Columns

### Part Number

|  |          |
|--|----------|
| TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 $\mu$ m  | 26AF130P |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu$ m | 26AC497P |

# Antidepressant/Painkillers in Blood and Urine

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

## Sample Preparation

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

Add 1mL of blood or urine

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

Mix/vortex

Sample pH should be 6.0±0.5

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Mix/vortex

Centrifuge as appropriate

## Condition HyperSep Retain-CX Extraction Column

1 x 3mL CH<sub>3</sub>OH

1 x 3mL H<sub>2</sub>O

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<sub>2</sub>O

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<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O
- 3mL of 1% acetic acid
- 3mL of CH<sub>3</sub>OH
- 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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

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 <sub>5</sub> -butalbital                 | <b>201</b>    | 214       |          |
| Butalbital                                 | <b>196</b>    | 195       | 209      |
| Amobarbital                                | <b>169</b>    | 184       | 185      |
| Pentobarbital                              | <b>169</b>    | 184       | 112      |
| <sup>13</sup> C <sub>4</sub> -secobarbital | <b>200</b>    |           | 185      |
| Secobarbital                               | <b>196</b>    | 195       | 181      |
| D <sub>5</sub> -phenobarbital              | <b>237</b>    | 151       |          |
| Phenobarbital                              | <b>232</b>    | 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  $6.0 \pm 0.5$ ; adjust sample pH accordingly with 100mM monobasic or dibasic sodium phosphate

Centrifuge as appropriate

## Condition Verify-CX Extraction Column

3mL of  $\text{CH}_3\text{OH}$  then aspirate

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

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

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

## Apply Sample

Load sample at 1 to 2mL/minute

## Wash Column

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

1mL of 100mM acetic acid

3mL of methanol

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

## Elute Bases

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

Collect eluate at 1 to 2mL/minute

**NOTE:** Prepare elution solvent daily

## Extract

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

Mix/vortex

Centrifuge at 2,000rpm for 10 minutes

Transfer organic lower layer to a clean test tube

## Evaporate

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

## Quantitate

Reconstitute in mobile phase and inject onto the HPLC system

# Benzodiazepines in Urine

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

## Sample Preparation

- To 2mL of urine add internal standard(s)\* and 1mL of  $\beta$ -glucuronidase solution
- $\beta$ -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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 1 x 2mL 20% ACN in 100mM phosphate buffer (pH= 6.0)
- Dry column (5 minutes at >10" Hg)
- 1 x 2mL hexane

## Elute

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

## Derivatize

- Add 50 $\mu$ L ethyl acetate and 50 $\mu$ L BSTFA\*\* (with 1% TMCS)\*\*
- Overlay with Nitrogen and cap
- Mix/vortex
- React 20 minutes at 70°C
- Remove from heat source to cool

**NOTE:** Do not evaporate BSTFA solution

## Analysis

- Inject 1 to 2 $\mu$ L onto GC/MS
- For mass spectrometry monitor the following ions:

| Generic Name                    | Trade Name | Primary Ion*** | Secondary | Tertiary |
|---------------------------------|------------|----------------|-----------|----------|
| Alprazolam                      | Xanax®     | 308            | 279       | 204      |
| $\alpha$ -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      |
| $\alpha$ -Hydroxytriazolam-TMS  |            | 415            | 417       | 190      |

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

\*\* Part number TS-38831

\*\*\* Quantitation ion

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

## Recommended GC Column

## Part Number

TraceGOLD TG-5SiIMS 30m x 0.25m x 0.25 $\mu$ m 26096-1420

# Benzodiazepines in Urine

## Alternative Derivatization

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

### Derivatize

To 2mL of urine add internal standard(s)\* and 1mL of  $\beta$ -Glucuronidase

$\beta$ -Glucuronidase: 5,000 F units/mL *Patella vulgate* in 100mM acetate buffer (pH 5.0)

Mix/vortex

Hydrolyze for 3 hours at 65°C

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

### Analysis

Inject 1 to 2 $\mu$ L onto GC/MS

For mass spectrometry monitor the following ions:

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

\* Suggested internal standard

\*\* Quantitation ion

### Recommended GC Columns

#### Part Number

|  |          |
|--|----------|
| TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 $\mu$ m  | 26AF130P |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu$ m | 26AC497P |

## Benzodiazepines in Serum or Plasma

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

### Sample Preparation

- To 1mL H<sub>2</sub>O 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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 1 x 3mL 0.1M phosphate buffer (pH 6.0)

### Apply Sample

- Load sample by gravity

### Wash Column

- 1 x 3mL DI H<sub>2</sub>O
- 1 x 3mL 5% ACN in 0.1M phosphate buffer (pH 6.0)
- Dry columns 5 minutes at full vacuum or >10" Hg
- 1 x 3mL Hexane

### Elute

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

### Derivatize

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

**NOTE:** Do not evaporate MTBSTFA solution

### Analysis

- Inject onto GC/MS

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

### Recommended GC Column

### Part Number

TraceGOLD TG-5SiIMS 30m x 0.25m 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<sub>3</sub>OH  
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-5SiIMS 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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
1 x 3mL 100mM acetate buffer (pH 4.7)

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

### Apply Sample

Load at 1 to 2mL/minute

### Wash Column

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

### Elute

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

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1 to 2mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>OH

1 x 3mL DI H<sub>2</sub>O

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<sub>3</sub>OH/NH<sub>4</sub>OH (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

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

# Buprenorphine and Norbuprenorphine in Blood and Urine

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

## Sample Preparation

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

## Enzyme Hydrolysis of Glucuronides

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

## Condition HyperSep Aminopropyl Extraction Column

- 1 x 3mL CH<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 1 x 3mL 100mM acetate buffer (pH=5.0)
- 1 x 3mL methanol
- Dry column (5 to 10 minutes at greater than 10"Hg/full flow for positive pressure manifold)

## Elute

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

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

- Evaporate to dryness at <40°C

## Derivatize

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

**NOTE:** Do not evaporate BSTFA

## Analysis

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

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

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

\*\* Part number TS-38831

## Recommended GC Column

|  | Part Number |
|--|-------------|
| TraceGOLD TG-5SiIMS 30m x 0.25m 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  $6.0 \pm 0.5$

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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

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<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

**NOTE:** Before proceeding, ensure there are no water droplets at the bottom of the collection tube (this may increase drying time and decrease BSTFA derivatizing efficiency)

## Dry Eluate

Evaporate to dryness at  $<40$ °C

## Derivatize

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

React for 20 minutes at 70°C

Remove from heat source to cool

**NOTE:** Do not evaporate BSTFA

## Quantitate

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

For mass spectrometry, monitor the following ions:

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

\* Suggested internal standard

\*\* Suggested quantitation ion

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

## Recommended GC Columns

### Part Number

TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25µm 26AF130P

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

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

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

## Prepare Sample

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

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

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

Mix/vortex and centrifuge as appropriate

*Total (sum of free and conjugated Buprenorphine/Norbuprenorphine)*

To 1mL of acetate buffer (pH 5.0) containing 5,000 F units/mL of  $\beta$ -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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

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<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2, v/v/v)

or

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

Collect eluate at 1 to 2mL/minute

## Evaporate

Evaporate to dryness at <40°C

## Reconstitute

Add 50 $\mu$ L of methanol

## Quantitate

Inject 5 $\mu$ L of sample onto the LC/MS system

For mass spectrometry, monitor the following ions:

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

\* Suggested internal standards

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

Flow rate: 0.35mL/min

## Recommended HPLC Column

## Part Number

Hypersil GOLD Phenyl 3 $\mu$ m, 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<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O  
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  $\approx$  3.0)

## Condition Verify-CX Extraction Column

3mL of CH<sub>3</sub>OH

3mL of DI H<sub>2</sub>O

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<sub>2</sub>O

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 <sub>3</sub> -Carboxy-delta <sub>9</sub> -THC  | 374           | 476       | 491      |
| D <sub>9</sub> -Carboxy-delta <sub>9</sub> -THC* | 380           | 479       | 497      |
| Carboxy-delta <sub>9</sub> -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  $3.0 \pm 0.5$  with approximately  
2.0mL of 100mM sodium acetate buffer (check pH of  
buffer to ensure that the pH value is  $\approx 3.0$ )

## Condition Verify-AX Extraction Column

3mL of CH<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
2mL of 100mM HCl/acetonitrile (95:5)  
Dry column (5 to 10 minutes at  $>10$ "Hg/full flow for  
positive pressure manifold)  
200 $\mu$ L of hexane then aspirate (Additional step to remove  
any residual moisture. Could substitute  
200 $\mu$ L 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 200 $\mu$ L of hexane in  
the wash/dry step (the 200 $\mu$ L 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  $<40^\circ\text{C}$

## Derivatize

Add 50 $\mu$ L of ethyl acetate and 50 $\mu$ L of BSTFA  
(with 1% TMCS)\*\*  
Mix/vortex  
React for 20 minutes at  $70^\circ\text{C}$   
Remove from heat source to cool

**NOTE:** Do not evaporate BSTFA

## Quantitate

Inject 1 to 2 $\mu$ L onto gas chromatograph  
For mass spectrometry, monitor the following ions:

| Compound (TMS)                        | Primary Ion | Secondary | Tertiary |
|---------------------------------------|-------------|-----------|----------|
| D <sub>3</sub> -Carboxy-delta-9-THC*  | 374         | 476       | 491      |
| D <sub>9</sub> -Carboxy-delta-9-THC*  | 380         | 479       | 497      |
| Carboxy-delta-9-THC                   | 371         | 473       | 488      |
| D <sub>3</sub> -Hydroxy-delta-9-THC*  | 374         | 462       | 477      |
| Hydroxy-delta-9-THC                   | 371         | 459       | 474      |
| D <sub>3</sub> -delta-9-THC*          | 374         | 389       |          |
| Delta-9-THC<br>(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 0.25 $\mu$ m  | 26AF130P |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu$ m | 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<sub>2</sub>O
- 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<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O
- 2mL of 100mM HCl
- Dry column (5 minutes at >10" Hg)
- 3mL of hexane

## Elute Carisoprodol/Meprobamate

- 3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)
- Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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 – $\beta$ -Glucuronidase Hydrolysis

To 2mL of urine add internal standard(s)\* and 1mL of  $\beta$ -Glucuronidase

$\beta$ -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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

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<sub>4</sub>OH

Collect eluate at 1 to 2mL/minute

**NOTE:** Prepare elution solution fresh daily

## Dry Eluate

Evaporate to dryness at <40°C

## Derivatize

Add 50 $\mu$ L ethyl acetate and 50 $\mu$ L MTBSTFA (with 1% TBDMCS)\*\*\*

Mix/vortex

React for 20 minutes at 90°C

Remove from heat source to cool

**NOTE:** Do not evaporate MTBSTFA solution

## Quantitate

Inject 1 to 2 $\mu$ L onto gas chromatograph

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

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

\* Suggested internal standard

\*\* Quantitation ion

\*\*\* Part number TS-48927 (10 x 1mL ampules)

## Recommended GC Columns

Part Number

|  |          |
|--|----------|
| TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 $\mu$ m  | 26AF130P |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu$ m | 26AC497P |

# Cocaine and Benzoyllecgonine 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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 1 x 3mL 100mM HCl
- 1 x 3mL CH<sub>3</sub>OH 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<sub>3</sub>OH
- Inject 5µL on to LC/MS

| Compound             | MRM Transition |
|----------------------|----------------|
| Cocaine              | 304.2/182.3    |
| Cocaine-D3*          | 307.2/185.2    |
| Benzoyllecgonine     | 290.1/168.0    |
| Benzoyllecgonine-D8* | 298.2/171.3    |
| Cocaethylene         | 318.2/196.2    |
| Cocaethylene-D8*     | 326.2/204.2    |

\* Internal standards: Cocaine-D3, Benzoyllecgonine-D8, Cocaethylene-D8

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

# Cocaine and Benzoyllecgonine 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<sub>3</sub>OH
- 1 x 200µL DI H<sub>2</sub>O
- 1 x 200µL 100mM HCl

## Apply Sample

- Do not exceed 1mL/minute

## Wash Column

- 1 x 500µL DI H<sub>2</sub>O
- 1 x 500µL 100mM HCl acid
- 1 x 500µL CH<sub>3</sub>OH/DI H<sub>2</sub>O (50:50)
- Dry column (5 minutes at >10" Hg)

## Elute

- 1 x 800µL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (70:26:4)
- Do not exceed 1mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

- Evaporate at <40°C under a stream of N<sub>2</sub>

## Derivatize

### Fluoroalkylate:

- Add 100µL PFPA (PFAA) or HFIP
- Overlay with N<sub>2</sub> 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<sub>2</sub> 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       |
| Benzoyllecgonine-TMS     | 240                       | 256, 361       |
| Benzoyllecgonine-D8-TMS* | 243                       | 259, 369       |

\* Internal standards: Cocaine-D3, Benzoyllecgonine-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 Benzoyllecgonine 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<sub>3</sub>OH
- 200µL of DI H<sub>2</sub>O
- 200µL of 0.1N HCl

## Apply Sample

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

## Wash Column

- 500µL of DI H<sub>2</sub>O
- 500µL of 100mM HCl
- 500µL of CH<sub>3</sub>OH/DI H<sub>2</sub>O (50:50)
- Dry column (5 minutes at >10" Hg)

## Elute Compounds

- 800µL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (70:26:4)
- Collect eluate at 1mL/minute (do not exceed this rate)

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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       |
| Benzoyllecgonine         | 240                       | 256, 361       |
| Benzoyllecgonine-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

| 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 Benzoyllecgonine in Serum, Plasma and Whole Blood

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

## Sample Preparation

4mL of DI H<sub>2</sub>O  
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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O  
1 x 2mL 100mM HCl  
1 x 3mL CH<sub>3</sub>OH  
Dry column (5 minutes at >10 "Hg)

## Elute

1 x 3mL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1 to 2mL/minute

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
2mL of 100mM HCl  
3mL of CH<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute Cocaine and Benzoylecgonine

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> 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 <sub>3</sub> -cocaine*             | 185           | 201       | 306      |
| Cocaine                              | 182           | 198       | 303      |
| D <sub>3</sub> -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<sub>3</sub>OH  
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<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
2mL of 100mM HCl  
3mL of CH<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute Cocaine and Benzoyllecgonine

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix,  
then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> 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 <sub>3</sub> -Cocaine*              | 185           | 201       | 306      |
| Cocaine                               | 182           | 198       | 303      |
| D <sub>3</sub> -Benzoyllecgonine-TMS* | 243           | 259       | 364      |
| Benzoyllecgonine-TMS                  | 240           | 256       | 361      |

\* Suggested internal standards for GC/MS: D<sub>3</sub>-Cocaine and D<sub>3</sub>-Benzoyllecgonine

\*\* Quantitation ion

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

## Recommended GC Columns

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

# Delta 9-THC, Delta 9-Hydroxy THC, Carboxy-Delta-9-THC in Whole Blood

Using 200mg 6mL HyperSep Verify-AX Extraction Column (Part Number: 60108-730)

## Sample Preparation

- 1 to 2mL whole blood
- Add internal standards\* prepared in alcohol
- Add drop-wise 2mL ice cold ACN
- Mix thoroughly and centrifuge
- Decant ACN into a clean tube
- Evaporate ACN under a stream of air or nitrogen to 200µL
- Add 2mL distilled H<sub>2</sub>O (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<sub>4</sub>OH
- 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}**<br>(D3 THC) {T-003}** | THC-OH {H-041}**<br>(D3 THC-OH) {H-027}** | THC-COOH {T-006}**<br>(D9 THC-COOH) {T007}** |
|--------------------|-------------------------------------|---|--|
| BSTFA              | 371, 343, 386<br>(374, 346, 389)    | 371, 459, 474<br>(374, 462, 477)          | 371, 473, 488<br>(380, 479, 497)             |

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

\*\* Ions common to deuterated delta-9 THC and non-deuterated compounds

\*\*\* Part number TS-38831

## Recommended GC Column

## Part Number

TraceGOLD TG-35MS 30m x 0.25m 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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O  
1 x 3mL 100mM acetic acid  
1 x 3mL CH<sub>3</sub>OH  
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<sub>3</sub>OH, 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

| 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<sub>3</sub>OH  
3mL of DI H<sub>2</sub>O  
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<sub>2</sub>O  
Dry column (10 minutes at >10" Hg)

## Elute Steroids

3mL of CH<sub>3</sub>OH  
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  $\beta$ -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<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub>

## Additional Clean-up

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

## Derivatize

Add 50 $\mu$ L of MSTFA\*\*\*/NH<sub>4</sub>/dithioerythritol (1000:2:5, v/w/w) and incubate at 70°C for 20 minutes

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

## Quantitate

Inject 1 to 2 $\mu$ L onto the gas chromatograph  
For mass spectrometry, monitor the following ions:

| Compound                           | Primary Ion** | Secondary |
|------------------------------------|---------------|-----------|
| Testosterone                       | 432           | 417       |
| Epitestosterone                    | 432           | 417       |
| DHEA                               | 432           | 417       |
| 16 $\alpha$ hydroxyl-testosterone* | 520           | 259       |

\* Suggested internal standard at 20ng/mL

\*\* Quantitation ion

\*\*\* Part number TS-48910 (10 x 1mL ampules)

## Recommended GC Columns

|  | Part Number |
|--|-------------|
| TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 $\mu$ m  | 26AF130P    |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu$ m | 26AC497P    |

# Duloxetine in Blood and Urine

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

## Sample Preparation

- 1mL of 100mM phosphate buffer (pH= 6)
- Add internal standard\*
- Add 1mL of blood or urine
- Add 2mL of 100 phosphate buffer (pH= 6)
- Mix/vortex
- Sample pH should be 6.0±0.5
- Adjust pH with 100mM monobasic or dibasic sodium phosphate
- Mix/vortex
- Centrifuge

## Condition HyperSep Verify-CX Extraction Column

- 1 x 3mL CH<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 1 x 3mL 100mM acetic acid
- 1 x 3mL CH<sub>3</sub>OH
- 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<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O
- 1mL of 100mM acetic acid
- 3mL of CH<sub>3</sub>OH
- Dry column (5 minutes at >10" Hg)

## Elute Fentanyls

- 3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)
- Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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 <sub>5</sub> -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

| 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<sub>3</sub>OH  
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<sub>3</sub>OH (pH 6)  
Add internal standard\*  
Insert oral swab into CH<sub>3</sub>OH and mix for 1 minute,  
Add 100µL of CH<sub>3</sub>OH  
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<sub>3</sub>OH  
1 x 3mL H<sub>2</sub>O  
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<sub>2</sub>O  
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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

1mL of 100mM acetic acid

3mL of CH<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

## Elute Fentanyl/Norfentanyl

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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 – $\beta$ -Glucuronidase Hydrolysis

- To 2mL of urine add internal standard(s)\* and 1mL of  $\beta$ -Glucuronidase
- $\beta$ -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<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O
- 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<sub>4</sub>OH
- Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent daily

## Dry Eluate

- Evaporate to dryness at <40°C

## Derivatize

- Add 50 $\mu$ L of ethyl acetate and 50 $\mu$ L MTBSTFA (with 1% TBDMCS)\*\*\*
- Overlay with N<sub>2</sub> and cap
- Mix/vortex
- React for 20 minutes at 70°C
- Remove from heat source to cool

**NOTE:** Do not evaporate MTBSTFA solution

## Quantitate

- Inject 1 to 2 $\mu$ L onto gas chromatograph
- For mass spectrometry, monitor the following ions:

| Compound                  | Primary Ion** | Secondary | Tertiary |
|---------------------------|---------------|-----------|----------|
| Flunitrazepam             | 312           | 286       | 266      |
| 7-aminoflunitrazepam      | 283           | 255       | 254      |
| Desmethylflunitrazepam    | 356           | 357       | 310      |
| D <sub>5</sub> -oxazepam* | 462           | 464       | 463      |

\* Suggested internal standard

\*\* Quantitation ion

\*\*\* Part number TS-48927 (10 x 1mL ampules)

## Recommended GC Columns

## Part Number

|  |          |
|--|----------|
| TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 $\mu$ m  | 26AF130P |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu$ m | 26AC497P |

# Gabapentin in Serum, Plasma or Whole Blood for GC or GC/MS Confirmations

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

## Prepare Sample

To 500 $\mu$ L of blood, plasma or serum, add internal standard\*

Vortex tube and add 500 $\mu$ L of 20% acetic acid and vortex tube again

Centrifuge as appropriate

## Condition C18 Extraction Column

3mL of CH<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

3mL of ethyl acetate

3mL hexane

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

## Elute Gabapentin

1mL of 2% NH<sub>4</sub>OH in CH<sub>3</sub>OH

## Dry Eluate

Evaporate to dryness at <40°C

## Derivatize

Add 50 $\mu$ L of ethyl acetate and 50 $\mu$ L of BSTFA (with 1% TMCS)\*\* or

Add 50 $\mu$ L of MTBSTFA (with 1% TBDMCS)\*\*\* and 50 $\mu$ L ethyl acetate

Cap and heat at 70°C for 30 minutes

Remove and allow to cool

## Quantitate

Inject 1 to 2 $\mu$ L 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 0.25 $\mu$ m  | 26AF130P |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu$ m | 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<sub>2</sub>O

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<sub>2</sub> at 70°C

Reconstitute extracts with 200µL of 100mM Phosphate Buffer (pH 6.0)

Mix/vortex 15 seconds

## Condition HyperSep Retain AX Extraction Column

1 x 3mL of CH<sub>3</sub>OH

1 x 3mL of DI H<sub>2</sub>O

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<sub>3</sub>OH/NH<sub>4</sub>OH (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<sub>2</sub>

## 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-Butyrolactone (GBL)

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

## Sample Preparation

200µL of urine  
Add internal standard\* and 100µL of 100mM phosphate buffer (pH 6.0)  
Mix/vortex

## Condition HyperSep Retain AX Extraction Column

1 x 3mL CH<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>3</sub>OH/NH<sub>4</sub>OH (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<sub>2</sub>

## 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<sub>2</sub>

## 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  $6.0 \pm 0.5$

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

Centrifuge as appropriate

## Condition Verify-CX Extraction Column

3mL of CH<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

3mL of 100mM acetic acid

3mL CH<sub>3</sub>OH

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

## Elute Ketamine

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

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

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

## Dry Eluate

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

Reconstitute with 100 $\mu\text{L}$  ethyl acetate

## Quantitate

Inject 1 to 2 $\mu\text{L}$  onto gas chromatograph

For mass spectrometry, monitor the following ions:

| Compound                  | Primary Ion** | Secondary | Tertiary |
|---------------------------|---------------|-----------|----------|
| D <sub>4</sub> -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 0.25 $\mu\text{m}$  | 26AF130P |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu\text{m}$ | 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<sub>2</sub>O 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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

3mL of 100mM acetic acid

3mL CH<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

## Elute LSD

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

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

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> 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 <sub>3</sub> -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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

3mL of 100mM acetic acid

3mL CH<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

## Elute LSD

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

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

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> 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 <sub>3</sub> -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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 1 x 1mL 100mM phosphate buffer (pH 6)

## Apply Sample

- Load sample at 1 to 2mL/minute

## Wash Column

- 1 x 3mL DI H<sub>2</sub>O
- 1 x 3mL 100mM acetic acid
- 1 x 3mL CH<sub>3</sub>OH
- 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<sub>3</sub>OH
- 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<sub>3</sub>OH
- 1 x 3mL DI H<sub>2</sub>O
- 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<sub>2</sub>O
- 1 x 3mL 100mM acetic acid
- 1 x 3mL CH<sub>3</sub>OH
- 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<sub>3</sub>OH
- Inject 5µL onto LC/MS
- Mobile phase:

| Time % | ACN % | 0.1% Formic Acid |
|--------|-------|------------------|
| 0      | 90    | 10               |
| 5      | 30    | 70               |
| 6      | 90    | 10               |
| 10     | 90    | 10               |

- Flow rate: 0.35mL/minute
- Column temperature: ambient
- Detector: Thermo Scientific TSQ Triple Quadrupole

| Compound          | MRM Transition |
|-------------------|----------------|
| Meperidine        | 248.2/220.0    |
| Meperidine-D4*    | 252.2/224.1    |
| Normeperidine     | 234.1/160.0    |
| Normeperidine-D4* | 238.1/164.0    |

\* Suggested internal standard: Meperidine-D4 and Normeperidine-D4

- Recovery: >90% (n=10)
- LOD: 10ng/mL

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

# Methadone/EDDP in Urine, Blood, Plasma/Serum and Tissue for GC or GC/MS Confirmations

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

## Prepare Sample

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

## Condition Verify-CX Extraction Column

- 3mL of CH<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O
- 1mL of 100mM acetic acid
- 3mL CH<sub>3</sub>OH
- 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<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O
- 1mL of 100mM acetic acid
- 3mL CH<sub>3</sub>OH
- Dry column (5 minutes at >10" Hg)

## Elute Methadone

- 3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)
- Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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 <sub>9</sub> -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<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O
- 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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

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<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

## Elute Nicotine and Continine

Replace rack of collection tubes

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>-Continine and D<sub>4</sub>-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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

3mL of 100mM acetate buffer (pH 4.5)

3mL of CH<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

## Elute Opiates

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> 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 <sub>4</sub> -meperidine*             | 251           | 222       | 250      |
| Meperidine                              | 247           | 218       | 246      |
| D <sub>4</sub> -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 <sub>3</sub> -codeine TMS*            | 374           | 359       | 346      |
| D <sub>6</sub> -codeine TMS*            | 377           | 349       | 316      |
| Codeine TMS                             | 371           | 356       | 343      |
| Norcodeine TMS                          | 429           | 414       | 356      |
| Dihydrocodeine TMS                      | 373           | 315       | 358      |
| D <sub>3</sub> -morphine TMS*           | 432           | 417       | 404      |
| D <sub>6</sub> -morphine TMS*           | 435           | 420       | 404      |
| Morphine TMS                            | 429           | 414       | 401      |
| Nomorphine TMS                          | 487           | 472       | 414      |
| Diacetylmorphine                        | 369           | 327       | 268      |
| D <sub>3</sub> -hydrocodone Oxime TMS   | 389           | 300       | 374      |
| D <sub>6</sub> -hydrocodone Oxime TMS*  | 392           | 303       | 377      |
| Hydrocodone Oxime TMS                   | 386           | 297       | 371      |
| D <sub>3</sub> -hydromorphone Oxime TMS | 447           | 432       | 358      |
| Hydromorphone Oxime TMS                 | 444           | 429       | 355      |
| D <sub>3</sub> -oxycodone Oxime TMS     | 477           | 462       | 420      |
| D <sub>6</sub> -oxycodone Oxime TMS*    | 480           | 465       | 420      |
| Oxycodone Oxime TMS                     | 474           | 459       | 417      |
| D <sub>3</sub> -oxymorphone Oxime TMS   | 535           | 520       | 290      |
| Oxymorphone Oxime TMS                   | 532           | 517       | 287      |

\* Suggested internal standards for GC/MS; suggest trying D<sub>6</sub>-codeine and D<sub>6</sub>-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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

3mL of 100mM acetate buffer (pH 4.5)

3mL of CH<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

## Elute Opiates

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (84:12:4)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
3mL of 100mM acetate buffer (pH 4.5)  
3mL of CH<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute Opiates

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> 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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

3mL of 100mM acetic acid

3mL of CH<sub>3</sub>OH

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<sub>3</sub>OH

## 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<sub>2</sub>O

1 x 1mL 100mM acetic acid

1 x 1mL CH<sub>3</sub>OH

Dry column (3 minutes at >10" Hg)

## Elute

2 x 0.5mL CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78/20/2)

Collect eluate at 1 to 2mL/min

Add 1 drop 1% HCl in CH<sub>3</sub>OH to eluate before evaporating

Evaporate to dryness at <40°C

Reconstitute with 100µL ethyl acetate

## Analysis

Inject 1 to 2µL onto GC/MS

For mass spectrometry monitor the following ions:

| Compound          | Target (Quantitation) Ion | Qualifier Ions |
|-------------------|---------------------------|----------------|
| Phencyclidine     | 200                       | 91, 242        |
| Phencyclidine-D5* | 205                       | 96, 247        |

\* Suggested internal standard: Phencyclidine-D5

## Recommended GC Column

Part Number

|  |            |
|--|------------|
| TraceGOLD TG-5SiIMS 30m x 0.25m 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<sub>3</sub>OH then aspirate  
 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
 3mL of 100mM acetic acid  
 3mL of CH<sub>3</sub>OH  
 Dry column (5 minutes at >10" Hg)

## Elute Phencyclidine

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
 Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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 <sub>5</sub> -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<sub>3</sub>OH then aspirate  
 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
 3mL of 100mM acetic acid  
 3mL of CH<sub>3</sub>OH  
 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<sub>3</sub>OH

## 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<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
3mL of 100mM acetic acid  
3mL of CH<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute Propoxyphene

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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 <sub>5</sub> -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<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O
- 3mL of 100mM acetic acid
- 3mL of CH<sub>3</sub>OH
- 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<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2, v/v/v)
- Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

## Evaporate

- Evaporate to half volume under a stream of nitrogen at <40°C
- Add 100µL of 0.1% HCl in CH<sub>3</sub>OH
- Mix/vortex
- Continue evaporation to dryness at <40°C

## Reconstitute

- Reconstitute sample in 100µL of CH<sub>3</sub>OH

## 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  $\beta$ -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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

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<sub>3</sub>OH

Dry column (3 minutes at >10" Hg)

## Elute Psilocin

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

## Dry Eluate

Evaporate to dryness at <35°C

## Derivatize

Add 50 $\mu$ L of ethyl acetate

Mix/vortex

Add 50 $\mu$ L of MSTFA \*\*\*

React at 70°C for 30 minutes

Remove from heat

**NOTE:** Do not evaporate MSTFA solution

## Quantitate

Inject 1 to 2 $\mu$ L onto gas chromatograph

For mass spectrometry, monitor the following ions:

| Compound          | Primary Ion** | Secondary | Tertiary |
|-------------------|---------------|-----------|----------|
| PSILOCIN-TMS      | 290           | 348       | 73 (291) |
| PSILOCIN-D10-TMS* | 300           | 358       | 83 (301) |

\* Suggested internal standard

\*\* Quantitation ion

\*\*\* Part number TS-48910 (10 x 1mL ampules)

## Recommended GC Columns

### Part Number

|  |          |
|--|----------|
| TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 $\mu$ m  | 26AF130P |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu$ m | 26AC497P |

## Source

Greishaber A., Moore, K. Levine, B. and Smith M. (1999, November). The detection of psilocin in human urine. Presented at the TRI Services Meeting.

# Quetiapine in Urine, Blood, Plasma/Serum and Tissue for LC/PDA Confirmations

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

## Prepare Sample

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

## Condition Verify-CX Extraction Column

- 3mL of CH<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>3</sub>OH
- 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  $<40^{\circ}\text{C}$

## Reconstitute

- Reconstitute sample in 100 $\mu\text{L}$  of 0.1% trifluoroacetic acid (aq)

## Analysis

- Inject 50 $\mu\text{L}$  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 3 $\mu\text{m}$ , 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<sub>2</sub>O (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<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
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<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute Basic Drugs

Replace collection tubes from elution of acidic and neutral drugs  
3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (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<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub>O 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<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
1mL of 100mM acetic acid  
3mL of CH<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute Sertraline and Desmethylsertraline

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)  
Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

## Dry Eluate

Evaporate to dryness at <40°C

## Reconstitute

Reconstitute with 200µL of ethyl acetate/DI H<sub>2</sub>O (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<sub>3</sub>CN  
Flow rate: 2mL/minute

## Recommended HPLC Column

Hypersil GOLD C8 3µm, 150 x 4.6mm

## Part Number

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  $\beta$ -glucuronidase solution  
( $\beta$ -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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O  
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<sub>2</sub>O  
1 x 3mL 100mM acetic acid  
1 x 3mL CH<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute

1 x 3mL ethyl acetate; ACN:ammonia (78:20:2 v/v)  
Collect eluate at 1 to 2mL/minute  
Evaporate eluates to dryness under a gentle stream of nitrogen

## Analysis

Reconstitute sample in 50 $\mu$ L of CH<sub>3</sub>OH  
Inject 5 $\mu$ L onto LC/MS  
Mobile phase:

| Time | % ACN | %0.1% Formic Acid |
|------|-------|-------------------|
| 0    | 20    | 80                |
| 5    | 20    | 80                |

Flow rate: 0.20mL/minute  
Column temperature: ambient  
Detector: Thermo Scientific TSQ Triple Quadrupole

| Compound      | MRM Transition |
|---------------|----------------|
| Psilocin      | 205.2/58.2     |
| Psilocin-D10* | 215.2/68.2     |

\* Suggested internal standard: Psilocin-D10

Recovery >90% (n=10)  
LOD: 10ng/mL

## Recommended HPLC Column

|  | Part Number  |
|--|--------------|
| Hypersil GOLD PFP 3 $\mu$ m, 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<sub>3</sub>OH then aspirate
- 3mL of DI H<sub>2</sub>O 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<sub>2</sub>O
- 1mL of 100mM acetic acid
- 3mL of CH<sub>3</sub>OH
- Dry column (5 minutes at >10" Hg)

### Elute SMA

- 3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)
- Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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<sub>2</sub> 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 <sub>5</sub> -amphetamine*     | 194           | 92        | 123      |
| Amphetamine                      | 190           | 91        | 118      |
| D <sub>5</sub> -methamphetamine* | 208           | 92        | 163      |
| Methamphetamine                  | 204           | 91        | 160      |
| Pseudoephedrine                  | 204           | 160       | 119      |
| Ephedrine                        | 204           | 160       | 119      |
| Phenylephrine                    | 190           | 119       | 267      |
| Methylenedioxymphetamine         | 135           | 162       | 325      |
| Methylenedioxymphetamine         | 204           | 162       | 339      |

\* Suggested internal standards for GC/MS: D<sub>5</sub>-amphetamine and D<sub>5</sub>-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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

1mL of 100mM acetic acid

3mL of CH<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

### Elute SMA

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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 <sub>5</sub> -amphetamine*     | 120           | 197       | 92       |
| D <sub>6</sub> -amphetamine*     | 120           | 198       | 93       |
| D <sub>10</sub> -amphetamine*    | 120           | 202       | 97       |
| D <sub>11</sub> -amphetamine*    | 120           | 203       | 98       |
| Amphetamine                      | 116           | 192       | 91       |
| D <sub>5</sub> -methamphetamine* | 134           | 211       | 92       |
| D <sub>8</sub> -methamphetamine* | 137           | 214       | 92       |
| D <sub>9</sub> -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-Carboethoxyhexafluorobutyl 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<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

1mL of 100mM acetic acid

3mL of CH<sub>3</sub>OH

Dry column (5 minutes at >10" Hg)

### Elute SMA

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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-Carboethoxyhexafluorobutyl 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 <sub>5</sub> -amphetamine*                    | 298           | 270       | 399      |
| Amphetamine                                     | 294           | 266       | 248      |
| D <sub>5</sub> -methamphetamine*                | 312           | 284       | 266      |
| Methamphetamine                                 | 308           | 280       | 262      |
| D <sub>5</sub> -methylenedioxyamphetamine*      | 136           | 434       | 270      |
| Methylenedioxyamphetamine                       | 162           | 429       | 266      |
| D <sub>5</sub> -methylenedioxymethamphetamine*  | 312           | 284       | 266      |
| Methylenedioxymethamphetamine                   | 308           | 280       | 262      |
| D <sub>6</sub> -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<sub>3</sub>OH  
3mL of DI H<sub>2</sub>O  
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<sub>2</sub>O  
3mL of hexane  
Dry column (5 minutes at >10" Hg)

## Elute Analytes

1mL of CH<sub>3</sub>OH

## Dry Eluate

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

## Reconstitute

Add 200µL of CH<sub>3</sub>OH  
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<sub>3</sub>OH then aspirate  
3mL of DI H<sub>2</sub>O 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<sub>2</sub>O  
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<sub>3</sub>OH
- 200µL of DI H<sub>2</sub>O
- 200µL of 100mM HCl

## Apply Sample

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

## Wash Column

- 500µL of DI H<sub>2</sub>O
- 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<sub>3</sub>OH
- 1 x 200µL DI H<sub>2</sub>O
- 1 x 200µL 100mM HCl

## Apply Sample

- Do not exceed 1mL/minute

## Wash Column

- 1 x 500µL DI H<sub>2</sub>O
- 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<sub>2</sub>

## Derivatize

- Add 25µL BSTFA (with 1% TMCS)\*\* and 25µL ethyl acetate
- Overlay with N<sub>2</sub> 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  $6.0 \pm 0.5$

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<sub>2</sub>O

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  $6.0 \pm 0.5$

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

## Condition Verify-CX Extraction Column

3mL of CH<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

1mL of 100mM acetic acid

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

2mL of hexane

## Elute Acid and Neutral Drugs (Fraction 1)

3mL of hexane/ethyl acetate (50:50);

Collect eluate at  $<2$ mL/minute

## Dry Eluate

Evaporate to dryness at  $<40$ °C

Reconstitute with 100 $\mu$ L ethyl acetate

## Quantitate Acid and Neutral Drugs

Inject 1 to 2 $\mu$ L onto gas chromatograph

## Wash Column

3mL of CH<sub>3</sub>OH then aspirate

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

## Elute Basic Drugs (Fraction 2)

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (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 100 $\mu$ L of ethyl acetate

## Quantitate Basic Drugs

Inject 1 to 2 $\mu$ L onto gas chromatograph

## Recommended GC Columns

### Part Number

|  |          |
|--|----------|
| TRACE TR-DoA5 Column, 15m x 0.25mm x 0.25 $\mu$ m  | 26AF130P |
| TRACE TR-DoA35 Column, 15m x 0.20mm x 0.33 $\mu$ m | 26AC497P |

### NOTES:

- (1) Fraction 1 (acids and neutrals) and fraction 2 (bases) can be combined together
- (2) A keeper solvent such as DMF can be used to prevent the volatilization of amphetamines and phencyclidine; use 30 to 50 $\mu$ L of high purity DMF in the sample (fraction 2) before evaporation
- (3) A 1% HCl in CH<sub>3</sub>OH solution has been used to prevent volatilization by the formation of the hydrochloric salt of the drugs. Evaporate fraction 2 to approximately 100 $\mu$ L, then add 1 drop of the solution. Continue to evaporate to dryness.

# Tricyclic Antidepressants in Serum or Plasma for HPLC Analysis

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

## Prepare Sample

To 1mL of serum or plasma add internal standard\* and 2mL of 100mM phosphate buffer (pH 6.0)

Mix/vortex

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

Sample pH should be  $6.0 \pm 0.5$

Adjust pH accordingly with 100mM monobasic or dibasic sodium phosphate

## Condition Verify-CX Extraction Column

3mL of CH<sub>3</sub>OH then aspirate

3mL of DI H<sub>2</sub>O 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<sub>2</sub>O

1mL of 100mM acetic acid

3mL of CH<sub>3</sub>OH

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

## Elute Tricyclic Antidepressants

3mL of CH<sub>2</sub>Cl<sub>2</sub>/IPA/NH<sub>4</sub>OH (78:20:2)

Collect eluate at 1 to 2mL/min

**NOTE:** Prepare elution solvent fresh daily; add IPA/NH<sub>4</sub>OH mix, then add CH<sub>2</sub>Cl<sub>2</sub> (pH 11-12)

## Dry Eluate

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

## Reconstitute

Reconstitute with 200 $\mu\text{L}$  of ethyl acetate/DI H<sub>2</sub>O (1:3)

Mix/vortex vigorously for 30 seconds

## Analysis

Column temperature:  $30^{\circ}\text{C}$

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 5 $\mu\text{m}$ , 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<sub>3</sub>OH  
1 x 5mL H<sub>2</sub>O (mmol/L HCl)

## Apply Sample

Load sample at 1 to 2mL/minute

## Wash Column

1 x 5mL H<sub>2</sub>O  
1 x 5mL CH<sub>3</sub>OH  
Dry column using N<sub>2</sub>

## Elute Melamine

1 x 5mL of CH<sub>3</sub>OH containing 4% NH<sub>4</sub>OH  
Collect eluate at 1 to 2mL/minute  
Dry under nitrogen

## Derivatize

Heat the glass tube with stopper in oven at 50°C for a moment to remove water  
Add 100µL toluene and 100µL BSTFA\* (with 1% TMCS)\*  
Mix/vortex  
Overlay with nitrogen and cap  
Mix/vortex  
Heat at 80°C for 1 hour  
Cool and add 300µL of toluene

\* Part number TS-38831

## Analysis

Inject 5 on to GC/MS

## Recommended GC Column

## Part Number

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

26096-1420

# Multiresidue Analysis in Cereal Grains for LC/MS/MS or GC/MS

Using QuEChERS Methodology (Part Number: 60105-211 and 60105-220)

## Pesticide Standards

Prepare individual pesticide stock solutions (2,000 to 5,000µg/mL) in ethyl acetate or ACN and store at -18°C  
Prepare two composite pesticide stock solutions, MIX-1 and MIX-2 at 10µg/mL in ACN  
Add 0.1% acetic acid to prevents degradation of base-sensitive analytes in ACN

## Isotopically Labeled Internal Standards

Prepare at 5µg/mL in acetone  
atrazine (ethylamine-d5)  
carbofuran (ring-13C6)  
dimethoate (o,o-dimethyl-d6)  
2,4-DDT (ring-13C6)  
-HCH (13C6)  
parathion (diethyl-d10)

## QC Working Solution

trans-permethrin (phenoxy-13C6) (1 and 5µg/mL in acetone)

## Sample Preparation

Thoroughly homogenize a sample of grain products using a laboratory mill to a flourlike consistency  
Place appropriate weight\* of sample into the 50mL centrifuge tube (60105-211)  
Add 10mL of deionized water (15mL for rice) and 10mL of ACN  
Add 200µL of ISTD standard solution  
Mix/vortex tube to disperse sample and standard for 1 hour using a wrist action shaker  
Centrifuge at rcf >3,000 for 10 minutes

## Sample Cleanup

Transfer a 1mL aliquot to a 2mL tube (60105-220)  
Mix/vortex for 30 seconds  
Centrifuge for 5 minutes  
Transfer 300µL of the supernatant into the chamber of a filter vial and add 30µL 1µg/mL QC solution  
Mix thoroughly  
Change to Transfer 125µL of extract into a vial and cap and store overnight at 250°C  
Press the 0.2µm polyvinylidene fluoride (PVDF) filter of the Mini-UniPrep to filter the extract for the LC/MS/MS analysis  
Add 30µL of QC standard solution  
Sample is now ready for analysis

## Analysis LC or GC/MS

Injection appropriate volume on to LC/MS or GC/MS

\* Corn 2.5g, Oat 3.5g, Rice 5.0g, Wheat 5.0g



# Cyanuric Acid and Melamine in Food Materials Multiresidue Analysis in Cereal Grains for LC/MS/MS or GC/MS

Using 200mg 6mL HyperSep Verify-CX and HyperSep Retain-AX Extraction Columns

(Part Number: 60108-722 and 60107-412)

## Sample Preparation

To 1 to 5g of sample add 10 to 25mL of CH<sub>3</sub>CN/DI H<sub>2</sub>O (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<sub>2</sub>Cl<sub>2</sub>  
Shake for 5 minutes  
Centrifuge  
Transfer upper layer to clean glass tube  
Add 2mL of DI H<sub>2</sub>O to CH<sub>2</sub>Cl<sub>2</sub>  
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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O

**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<sub>2</sub>O  
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<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute Melamine

Insert fresh collection tubes into manifold  
1 x 2mL of CH<sub>3</sub>OH containing 5% NH<sub>4</sub>OH  
1 x 3mL of CH<sub>3</sub>OH containing 5% NH<sub>4</sub>OH  
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<sub>3</sub>CN  
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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O

**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<sub>2</sub>O  
1 x 1mL CH<sub>3</sub>OH  
Dry column

## Elute Cyanuric Acid

Insert fresh collection tubes into manifold  
1 x 3mL of CH<sub>3</sub>OH containing 1% HCl  
1 x 2mL of CH<sub>3</sub>OH 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<sub>4</sub>OH

| 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<sub>3</sub>OH  
1 x 3mL H<sub>2</sub>O

### Apply Sample

Load supernate into extraction column

### Wash Column

1 x 3mL H<sub>2</sub>O  
1 x 3mL CH<sub>3</sub>OH

### Elute Melamine

1 x 5mL of CH<sub>3</sub>OH containing 5% NH<sub>4</sub>OH  
Collect eluate at 1 to 2mL/minute

### Evaporation

Evaporate eluates under a gentle stream of nitrogen 50°C

### Analysis

Reconstitute sample in CH<sub>3</sub>CN  
Inject 5µL on to LC/MS

### Recommended HPLC Column

Hypersil GOLD HILIC 3µm, 150 x 2.1mm

### Part Number

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<sub>2</sub>O

### Apply Sample

Load sample at 1 to 2mL/minute

### Wash Column

3mL of DI H<sub>2</sub>O  
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<sub>3</sub>CN/DI H<sub>2</sub>O (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<sub>2</sub>Cl<sub>2</sub>  
Shake for 5 minutes  
Centrifuge  
Transfer upper layer to clean glass tube  
Add 2mL of DI H<sub>2</sub>O to CH<sub>2</sub>Cl<sub>2</sub>  
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<sub>3</sub>OH  
1 x 3mL DI H<sub>2</sub>O

**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<sub>3</sub>OH  
Dry column (5 minutes at >10" Hg)

## Elute Melamine

1 x 2mL of CH<sub>3</sub>OH containing 5% NH<sub>4</sub>OH  
1 x 3mL of CH<sub>3</sub>OH containing 5% NH<sub>4</sub>OH  
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<sub>3</sub>CN  
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<sub>2</sub>O

## 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<sub>2</sub>O  
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<sub>2</sub>O
- Centrifuge at 4,000rpm
- Remove supernatant for analysis

### Condition HyperSep Retain PEP Extraction Column

- 1 x 5mL CH<sub>3</sub>OH
- 1 x 5mL H<sub>2</sub>O

### Apply Sample

- Load sample at 1 to 2mL/minute

### Wash Column

- 1 x 10mL H<sub>2</sub>O

### 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<sub>3</sub>CN
- Inject 5µL on to LC/MS

### Recommended HPLC Column

#### Part Number

Hypersil GOLD 5µm, 100 x 2.1mm

25005-102130

## Determination of Nitroimidazole Drugs and Metabolites in Royal Jelly

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

### Sample Preparation

- Prepare a 100µg/mL deuterated Norfloxacin (NOR-D5) standard in MeOH
- Dilute with MeOH to a concentration of 1µg/mL
- Add 5g of sample to a 50mL centrifuge tube
- Add 50µL of internal standards: Metronidazole (MNZ), Dimetridazole (DMZ) and related metabolites (2-hydroxymethyl, 1-methyl-5-nitroimidazole (HMMNI)), Iprnidazole (IPZ) and related metabolite (2-(2-hydroxy isopropyl)-1-methyl-5-nitroimidazole (IPZOH)), and Ronidazole (RNZ)
- 10mL of 0.5 mol/L sodium hydroxide solution
- Mix for 15 s to dissolve the sample
- Add 10mL of ethyl acetate and mix for 30 seconds
- Centrifugate at 2,500 r/minute for 3 minutes
- Transfer the supernatant ethyl acetate layer to a 50mL glass test tube
- Add 10mL of ethyl acetate again and repeat the extraction procedures
- Combine the ethyl acetates and evaporate to dryness by rotary evaporator in water bath at 40°C
- Dissolve the residue with 5mL of ACN containing 10% formic acid

### Condition HyperSep Retain-CX Extraction Column

- 1 x 3mL CH<sub>3</sub>OH
- 1 X 3mL H<sub>2</sub>O

### Apply Sample

- Load sample at 1 to 2mL/minute

### Wash Column

- 1 x 3mL H<sub>2</sub>O
- Dry column (5 minutes at >10" Hg)

### Elute Nitroimidazole

- 1 x 3mL of CH<sub>3</sub>OH
- Collect eluate at 1 to 2mL/minute and evaporate to dryness

### Analysis

- Inject 25µL on to LC/MS
- Flow rate: 0.20mL/minute
- Mobile phase: methanol (A), 5mmol/L ammonium acetate (B)

### Recommended HPLC Column

#### Part Number

Hypersil GOLD 3µm, 150 x 2.1mm

25003-152130

# Extraction of Pesticides From Pigmented Fruit or Vegetables

Using QuEChERS Methodology (Part Number: 60105-216, 60105-218 and 60105-221)

## Sample Preparation

- Add 15g of homogenized and hydrated tomato product (>80% moisture) to a centrifuge tube 60105-216
- Add 15mL ACN including internal standard
- Mix/vortex for 30 seconds
- Centrifuge for 2 minutes at 3450 rcf
- Draw 1 or 6mL of supernatant for clean-up

## Clean-Up

- For 1mL of supernatant, use product 60105-221
- For 6mL of supernatant, use product 60105-218
- Add supernatant to centrifuge tube and shake vigorously for 1 minute
- Centrifuge for 2 minutes at 3450 rcf

## Analysis

### GC/MS:

- Transfer an aliquot of supernatant from step 2 to a centrifuge tube
- Add TPP solution and 1mL of toluene
- Evaporate using nitrogen at 50°C to approximately 0.3 to 0.6mL
- Bring to 1mL final volume with toluene
- Inject 8µL on to GC/MS

### LC/MS:

- Transfer 0.25mL of supernatant from step 2 to an LC vial
- Add TPP solution and 0.86mL of 6.7mM formic acid
- Analyze by LC/MS

# Determination of Quinolone Residues in Honey

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

## Sample Preparation

- Add 5g of sample to a 50mL centrifuge tube
- Make up Quinolone standard solution to a concentration 1µg/mL in 0.1mol/L sodium hydroxide solution
- Quinolone Standards: Enrofloxacin (ENR), Ciprofloxacin (CIP), Norfloxacin (NOR), Ofloxacin(OFL), Flumequine(FLU), Oxolinic acid(OXO), Difloxacin HCl (DIF), Sarafloxacin HCl(SAR), Sparfloxacin (SPA), Danofloxacin (DAN), Fleroxacin (FLE), Marbofloxacin (MAR), Enoxacin (ENO), Orbifloxacin (ORB), Pipemidic acid (PIP), Pefloxacin (PEF), Lomefloxacin (LOM), Cinoxacin (CIN), Nalidixic acid (NAL))

- Add 50µL internal standard solution to 5mL of 0.1mol/L sodium hydroxide solution

Mix/vortex

## Condition HyperSep Retain-CX Extraction Column

- 1 x 5mL CH<sub>3</sub>OH
- 1 X 3mL H<sub>2</sub>O

## Apply Sample

- Load sample at 1 to 2mL/minute

## Wash Column

- 1 x 3mL H<sub>2</sub>O
- 1 x 3mL of CH<sub>3</sub>OH

## Elute Quinolone

- 1 x 3mL of CH<sub>3</sub>OH 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<sub>2</sub>  
Add 500µL of ACN 25µL of benzanilide (2.0µg/L) surrogate standard for QC and 500µL of 20mM ammonium acetate in 1% ACN to the dried extract  
Mix/vortex for approximately 5 seconds and filter into autosampler vial using 17mm, 0.2µm nylon membrane syringe filter (F2513-2) attached to a disposable syringe

## Analysis

Inject 3µL onto appropriate LC/MS system  
Monitor the following transitions:

| Compound             | MRM Transition |
|----------------------|----------------|
| Acephate             | 184.0/143.0    |
| Acetamiprid          | 223.4/126.1    |
| Acibenzolar S-methyl | 211.1/136.0    |
| Aldicarb             | 208.1/116.0    |
| Aldicarb sulfone     | 240.0/222.9    |
| Aldicarb sulfoxide   | 224.2/206.9    |
| Atrazine             | 215.9/173.85   |
| Avermectin B1b       | 876.6/553.4    |
| Avermectin B1a       | 890.7/567.5    |
| Azoxystrobin         | 404.0/372.1    |
| Benalaxyl            | 326.1/148.1    |
| Benfuracarb          | 411.2/190.0    |
| Benzanilide          | 198.1/105.1    |
| Bifenazate           | 301.3/170.2    |
| Bitertanol           | 338.2/99.1     |
| Buprofezin           | 306.3/201.2    |
| Carbaryl             | 202.1/145.1    |
| Carbendazim          | 192.0/160.0    |
| Carbofuran           | 222.1/123.1    |
| Chloroxuron          | 291.0/72.2     |
| Cyprodinil           | 226.1/93.0     |
| Cyromazine           | 167.2/85.1     |
| Diclobutrazol        | 328.1/70.2     |
| Dimethoate           | 230.1/199.0    |
| Dimethomorph         | 388.0/301.1    |
| Dimoxystrobin        | 327.1/206      |
| Dinotefuran          | 203.5/14.0     |
| Diuron               | 233.0/72.1     |

| Compound           | MRM Transition |
|--------------------|----------------|
| Ethofumesate       | 286.9/258.9    |
| Famoxadone         | 373.2/282      |
| Fenamidone         | 312.2/236.2    |
| Fenbuconazole      | 337.1/125.0    |
| Fenhexamid         | 301.9/261.9    |
| Fenpropimorph      | 304.4/147.1    |
| Fluconazole        | 307.2/220      |
| Fludioxinil        | 247.0/180.0    |
| Furathiocarb       | 383.2/195.1    |
| Hexaconazole       | 314.0/70.2     |
| Imazalil           | 297.1/159.0    |
| Imidacloprid       | 256.1/175.0    |
| Ipconazole         | 334.1/70.2     |
| Iprovalicarb       | 321.2/119.0    |
| Kresoxim-methyl    | 314.1/116.0    |
| Mepanipyrim        | 224.4/77.3     |
| 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    |
| Quinoxifen         | 307.8/196.8    |
| Rotenone           | 395.3/213.2    |
| Simazine           | 202.2/131.4    |
| Spinosyn A         | 732.6/142.2    |
| Spinosyn D         | 746.6/142.2    |
| Spiroxamine        | 298.2/144.0    |
| Tebuconazole       | 308.2/70.2     |
| Thiabendazole      | 202.0/175.0    |
| Triadimefon        | 294.0/197.1    |
| Trifloxystrobin    | 409.0/186.0    |
| Triflumizole       | 346.0/278.1    |
| Vamidothion        | 288.1/146.0    |
| Zoxamide           | 336.0/187.0    |

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

# Ordering Information

## HyperSep SPE Formats

Thermo Scientific offers a comprehensive selection of SPE products that have been developed for rapid, effective and economical sample preparation. HyperSep SPE products are available in a range of formats, including columns, 96-well plates and microscale products.

### HyperSep SPE Columns

*Ideal for lower throughput and larger volume samples*

- Column volumes from 1mL to 75mL
- Bed weights ranging from 2.5mg 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60302-601 | 100 Pack |
| 25mg                    | 1mL    | 60302-602 | 100 Pack |
| 50mg                    | 1mL    | 60302-603 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 5mg                     | 1mL    | 60303-201 | 100 Pack |
| 10mg                    | 1mL    | 60303-202 | 100 Pack |
| 30mg                    | 1mL    | 60303-203 | 100 Pack |
| 60mg                    | 1mL    | 60303-204 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 5mg                     | 1mL    | 60303-301 | 100 Pack |
| 10mg                    | 1mL    | 60303-302 | 100 Pack |
| 30mg                    | 1mL    | 60303-303 | 100 Pack |
| 60mg                    | 1mL    | 60303-304 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 5mg                     | 1mL    | 60303-401 | 100 Pack |
| 10mg                    | 1mL    | 60303-402 | 100 Pack |
| 30mg                    | 1mL    | 60303-403 | 100 Pack |
| 60mg                    | 1mL    | 60303-404 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-421 | 100 Pack |
| 25mg                    | 1mL    | 60300-422 | 100 Pack |
| 50mg                    | 1mL    | 60300-423 | 100 Pack |
| 100mg                   | 1mL    | 60300-524 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-441 | 100 Pack |
| 25mg                    | 1mL    | 60300-442 | 100 Pack |
| 50mg                    | 1mL    | 60300-443 | 100 Pack |
| 100mg                   | 1mL    | 60300-444 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-681 | 100 Pack |
| 25mg                    | 1mL    | 60300-682 | 100 Pack |
| 50mg                    | 1mL    | 60300-683 | 100 Pack |
| 100mg                   | 1mL    | 60300-684 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-481 | 100 Pack |
| 25mg                    | 1mL    | 60300-482 | 100 Pack |
| 50mg                    | 1mL    | 60300-483 | 100 Pack |
| 100mg                   | 1mL    | 60300-484 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-561 | 100 Pack |
| 25mg                    | 1mL    | 60300-562 | 100 Pack |
| 50mg                    | 1mL    | 60300-563 | 100 Pack |
| 100mg                   | 1mL    | 60300-564 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-581 | 100 Pack |
| 25mg                    | 1mL    | 60300-582 | 100 Pack |
| 50mg                    | 1mL    | 60300-583 | 100 Pack |
| 100mg                   | 1mL    | 60300-584 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-809 | 100 Pack |
| 25mg                    | 1mL    | 60300-810 | 100 Pack |
| 50mg                    | 1mL    | 60300-811 | 100 Pack |
| 100mg                   | 1mL    | 60300-812 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-801 | 100 Pack |
| 25mg                    | 1mL    | 60300-802 | 100 Pack |
| 50mg                    | 1mL    | 60300-803 | 100 Pack |
| 100mg                   | 1mL    | 60300-804 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-721 | 100 Pack |
| 25mg                    | 1mL    | 60300-722 | 100 Pack |
| 50mg                    | 1mL    | 60300-723 | 100 Pack |
| 100mg                   | 1mL    | 60300-724 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-501 | 100 Pack |
| 25mg                    | 1mL    | 60300-502 | 100 Pack |
| 50mg                    | 1mL    | 60300-503 | 100 Pack |
| 100mg                   | 1mL    | 60300-504 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-817 | 100 Pack |
| 25mg                    | 1mL    | 60300-818 | 100 Pack |
| 50mg                    | 1mL    | 60300-819 | 100 Pack |
| 100mg                   | 1mL    | 60300-820 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|-------------------------|--------|-----------|----------|
| <b>INDIVIDUAL WELLS</b> |        |           |          |
| 10mg                    | 1mL    | 60300-635 | 100 Pack |
| 25mg                    | 1mL    | 60300-636 | 100 Pack |
| 50mg                    | 1mL    | 60300-637 | 100 Pack |
| 100mg                   | 1mL    | 60300-638 | 100 Pack |
| <b>WELL PLATES</b>      |        |           |          |
| 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 |
|--|-----------|----------|
| <b>THERMO SCIENTIFIC, CTC ANALYTICS, HTA AND VARIAN 8400 SYSTEMS</b> |           |          |
| 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   |
| <b>CTC ANALYTICS ONLY</b>  |           |          |
| 250µL removable needle MEPS syringe                                  | 60308-105 | 1 Each   |
| Replacement plunger assembly for 250µL CTC-compatible syringe        | 60308-106 | 1 Each   |

### MEPS For GC: Thermo Scientific, CTC Analytics, HTA and Varian 8400 Systems\*

| Description                                       | Cat. No.  | Quantity |
|---|-----------|----------|
| HyperSep Retain PEP MEPS                          | 60308-201 | 5 Pack   |
| HyperSep Retain-CX MEPS                           | 60308-202 | 5 Pack   |
| HyperSep Retain-AX MEPS                           | 60308-203 | 5 Pack   |
| HyperSep Hypercarb MEPS                           | 60308-204 | 5 Pack   |
| HyperSep Verify-CX MEPS                           | 60308-205 | 5 Pack   |
| HyperSep Verify-AX MEPS                           | 60308-206 | 5 Pack   |
| HyperSep C18 MEPS                                 | 60308-207 | 5 Pack   |
| HyperSep Silica MEPS                              | 60308-208 | 5 Pack   |
| HyperSep MEPS Development Kit for GC Applications | 60308-209 | 5 Pack   |

*Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18*

### MEPS For GC: CTC Analytics using 250µL Syringes

| Description                                       | Cat. No.  | Quantity |
|---|-----------|----------|
| HyperSep Retain PEP MEPS                          | 60308-301 | 5 Pack   |
| HyperSep Retain-CX MEPS                           | 60308-302 | 5 Pack   |
| HyperSep Retain-AX MEPS                           | 60308-303 | 5 Pack   |
| HyperSep Hypercarb MEPS                           | 60308-304 | 5 Pack   |
| HyperSep Verify-CX MEPS                           | 60308-305 | 5 Pack   |
| HyperSep Verify-AX MEPS                           | 60308-306 | 5 Pack   |
| HyperSep C18 MEPS                                 | 60308-307 | 5 Pack   |
| HyperSep Silica MEPS                              | 60308-308 | 5 Pack   |
| HyperSep MEPS Development Kit for GC Applications | 60308-309 | 5 Pack   |

*Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18*

### MEPS For LC: Thermo Scientific, CTC Analytics, HTA and Varian 8400 Systems\*

| Description                                       | Cat. No.  | Quantity |
|---|-----------|----------|
| HyperSep Retain PEP MEPS                          | 60308-401 | 5 Pack   |
| HyperSep Retain-CX MEPS                           | 60308-402 | 5 Pack   |
| HyperSep Retain-AX MEPS                           | 60308-403 | 5 Pack   |
| HyperSep Hypercarb MEPS                           | 60308-404 | 5 Pack   |
| HyperSep Verify-CX MEPS                           | 60308-405 | 5 Pack   |
| HyperSep Verify-AX MEPS                           | 60308-406 | 5 Pack   |
| HyperSep C18 MEPS                                 | 60308-407 | 5 Pack   |
| HyperSep Silica MEPS                              | 60308-408 | 5 Pack   |
| HyperSep MEPS Development Kit for LC applications | 60308-409 | 5 Pack   |

*Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18*

### MEPS For LC Applications: CTC Analytics using 250µL Syringes

| Description                                       | Cat. No.  | Quantity |
|---|-----------|----------|
| HyperSep Retain PEP MEPS                          | 60308-501 | 5 Pack   |
| HyperSep Retain-CX MEPS                           | 60308-502 | 5 Pack   |
| HyperSep Retain-AX MEPS                           | 60308-503 | 5 Pack   |
| HyperSep Hypercarb MEPS                           | 60308-504 | 5 Pack   |
| HyperSep Verify-CX MEPS                           | 60308-505 | 5 Pack   |
| HyperSep Verify-AX MEPS                           | 60308-506 | 5 Pack   |
| HyperSep C18 MEPS                                 | 60308-507 | 5 Pack   |
| HyperSep Silica MEPS                              | 60308-508 | 5 Pack   |
| HyperSep MEPS Development Kit for LC applications | 60308-509 | 5 Pack   |

*Kit contains 1 each of Retain PEP, Retain-CX, Retain-AX, Hypercarb and C18*

\* For use with 100µL and 250µL MEPS syringes

## HyperSep Online SPE

### HyperSep Javelin Direct-Connect Online SPE Columns

| I.D.  | Length | Retain PEP | Retain-CX | Retain-AX | Hypercarb | Quantity |
|-------|--------|------------|-----------|-----------|-----------|----------|
| 2.1mm | 10mm   | 60310-201  | 60310-301 | 60310-401 | 60310-501 | 4 Pack   |
| 3.0mm | 10mm   | 60310-202  | 60310-302 | 60310-402 | 60310-502 | 4 Pack   |

### HyperSep UNIGUARD Direct-Connect Online SPE Cartridges

| I.D.  | Length | Retain PEP | Retain-CX | Retain-AX | Hypercarb | Quantity |
|-------|--------|------------|-----------|-----------|-----------|----------|
| 2.1mm | 10mm   | 60311-201  | 60311-301 | 60311-401 | 60311-501 | 4 Pack   |
| 3.0mm | 10mm   | 60311-202  | 60311-302 | 60311-402 | 60311-502 | 4 Pack   |

### HyperSep HPLC Columns for Online SPE

| I.D.  | Length | Retain PEP | Retain-CX | Retain-AX | Hypercarb | Quantity |
|-------|--------|------------|-----------|-----------|-----------|----------|
| 2.1mm | 20mm   | 60312-201  | 60312-301 | 60312-401 | 60312-501 | 1 Each   |
| 3.0mm | 20mm   | 60312-202  | 60312-302 | 60312-402 | 60312-502 | 1 Each   |

## HyperSep Vacuum Manifolds



### HyperSep Universal Vacuum Manifold

Accommodates both SPE columns and 96-well plates; system supplied with manifold, base/gauge, flask and stopper, tubing and spigots

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



### HyperSep-96 Well Plate Manifold

Accommodates 96-well plates; system includes base, lid and waste collection tray

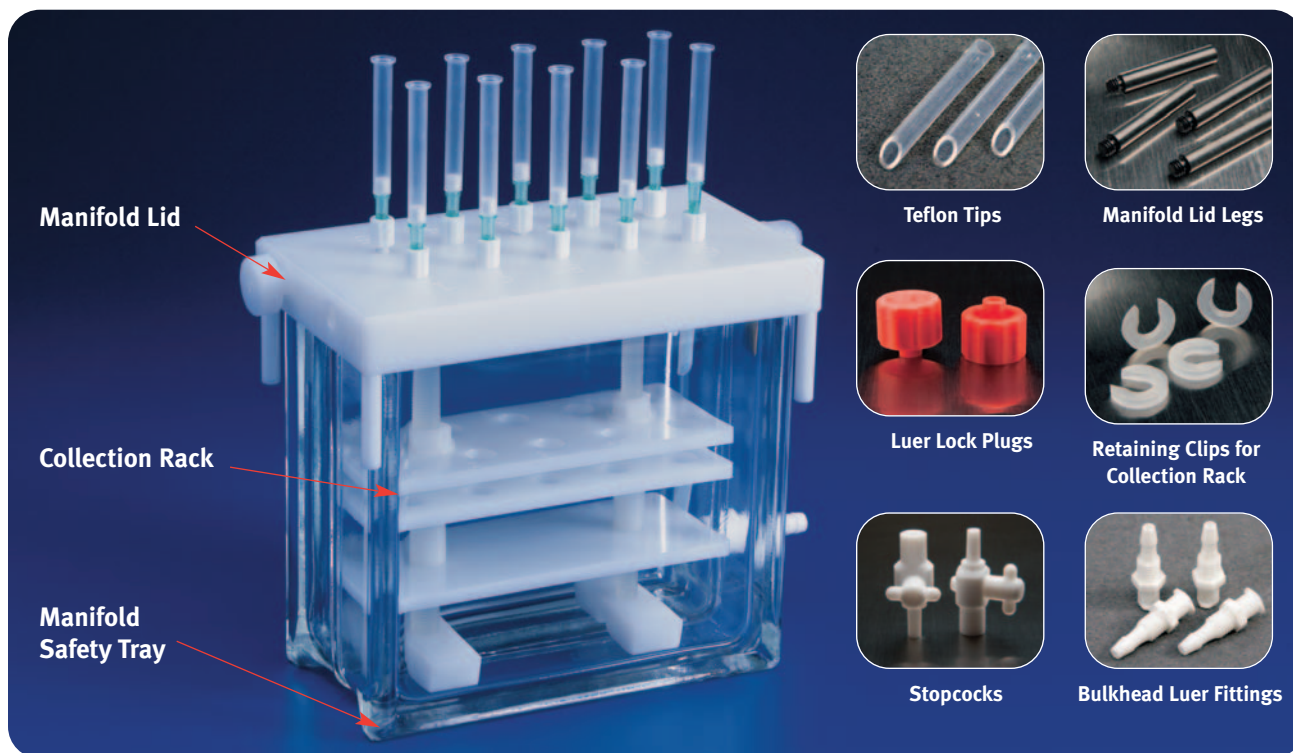
| Description                      | Cat. No.  | Quantity |
|----------------------------------|-----------|----------|
| HyperSep-96 vacuum manifold      | 60103-351 | 1 Each   |
| Vacuum pump, European plug       | 60104-241 | 1 Each   |
| Vacuum pump, North American plug | 60104-243 | 1 Each   |

#### ACCESSORIES

|   |           |          |
|---|-----------|----------|
| Base plate for HyperSep-96 well plate     | 60300-301 | 1 Each   |
| Base plate for HyperSep-96 well plate     | 60300-303 | 5 Pack   |
| Sample collection plate, 1mL              | 60300-402 | 50 Pack  |
| Sample collection plate, 2mL              | 60300-403 | 50 Pack  |
| Adaptors for 1mL, 3mL and 6mL SPE columns | 60104-259 | 15 Pack  |
| Empty 1mL wells                           | 60300-318 | 100 Pack |
| Empty 1mL wells, fritted                  | 60300-311 | 100 Pack |



## HyperSep Glass Block Vacuum Manifolds



### 16-Port Vacuum Manifold

- Glass block, Corian® manifold lid, cover gasket, vacuum gauge and valve assembly, 16 Teflon® tips, adjustable collection rack, bulkhead Luer fittings, 16 plugs and manifold safety tray

### 24-Port Vacuum Manifold

- Glass Block, Corian manifold lid, cover gasket, vacuum gauge and valve assembly, 24 Teflon tips, adjustable collection rack, bulkhead Luer fittings, 24 plugs and manifold safety tray



Vacuum Gauge and Valve Assembly

| Description                                 | Cat. No.  | Quantity |
|---|-----------|----------|
| 16-port vacuum manifold                     | 60104-232 | 1 Each   |
| 24-port vacuum manifold                     | 60104-233 | 1 Each   |
| Vacuum pump, European plug                  | 60104-241 | 1 Each   |
| Vacuum pump, North American plug            | 60104-243 | 1 Each   |
| <b>REPLACEMENT PARTS</b>                    |           |          |
| 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 <sub>4</sub> , 1.5g Sodium Acetate   | 50mL     | 60105-210 | 250      |
| 4g MgSO <sub>4</sub> , 1g sodium Chloride  | 50mL     | 60105-211 | 250      |
| 1.5g Sodium Chloride, 1.5g Sodium Citrate Tribasic Dihydrate, 750mg Sodium citrate Dibasic                             | 50mL     | 60105-212 | 250      |
| 4g Anhydrous Magnesium Sulfate, 1g Sodium Chloride, 1g Sodium Citrate Tribasic Dihydrate, 500mg Sodium citrate Dibasic | 50mL     | 60105-216 | 250      |

### QuEChERS Clean-Up Products

| Description  | Capacity | Cat. No.  | Quantity |
|--|----------|-----------|----------|
| 6g Anhydrous Magnesium Sulfate, 1.5g Anhydrous Sodium Acetate  | 50mL     | 60105-310 | 25       |
| 4g Anhydrous Magnesium Sulfate, 1g NaCl  | 50mL     | 60105-311 | 25       |
| 6g Anhydrous Magnesium Sulfate, 1.5g Sodium Chloride, 1.5g Sodium Citrate Tribasic Dihydrate, 750mg Sodium citrate Dibasic | 50mL     | 60105-312 | 25       |
| 4g Anhydrous Magnesium Sulfate, 1g Sodium Chloride, 1g Sodium Citrate Tribasic Dihydrate, 500mg Sodium citrate Dibasic     | 50mL     | 60105-316 | 25       |
| Centrifuge Tubes with 900mg MgSO <sub>4</sub> , 300mg PSA and 150mg Carbon   | 15mL     | 60105-205 | 50       |
| 900mg MgSO <sub>4</sub> , 300mg PSA and 150mg C18  | 15mL     | 60105-206 | 50       |
| 750mg MgSO <sub>4</sub> , 250mg PSA, 250mg endcapped C18 and 250 Carbon  | 15mL     | 60105-213 | 50       |
| 900mg Anhydrous MgSO <sub>4</sub> , 300mg PSA  | 15mL     | 60105-214 | 50       |
| 900mg Anhydrous MgSO <sub>4</sub> , 150mg PSA  | 15mL     | 60105-215 | 50       |
| 900mg MgSO <sub>4</sub> , 150mg PSA and 45mg Carbon  | 15mL     | 60105-217 | 50       |
| 900mg MgSO <sub>4</sub> , 150mg PSA and 15mg Carbon  | 15mL     | 60105-218 | 50       |
| 1200mg MgSO <sub>4</sub> , 400mg PSA   | 15mL     | 60105-224 | 50       |
| 1200mg MgSO <sub>4</sub> , 400mg PSA and 400mg C18   | 15mL     | 60105-225 | 50       |

**QuEChERS Clean-Up Products** *Continued*

| Description  | Capacity | Cat. No.  | Quantity |
|--|----------|-----------|----------|
| 1200mg MgSO <sub>4</sub> , 400mg PSA, 400mg C18 and 400mg Carbon             | 15mL     | 60105-226 | 50       |
| 900mg MgSO <sub>4</sub> , 150mg PSA and 150mg C18                            | 15mL     | 60105-227 | 50       |
| 150mg MgSO <sub>4</sub> , 50mg PSA and 50mg Carbon                           | 15mL     | 60105-230 | 50       |
| 150mg MgSO <sub>4</sub> , 300mg PSA and 150mg Chlorofiltr                    | 15mL     | 60105-231 | 50       |
| 900mg Anhydrous MgSO <sub>4</sub> , 300mg PSA and 150mg Carbon               | 15mL     | 60105-305 | 10       |
| 900mg Anhydrous MgSO <sub>4</sub> , 300mg PSA and 150mg C18                  | 15mL     | 60105-306 | 10       |
| 150mg MgSO <sub>4</sub> , 50mg PSA, 50mg Carbon                              | 15mL     | 60105-313 | 10       |
| 900mg Anhydrous MgSO <sub>4</sub> , 300mg PSA                                | 15mL     | 60105-314 | 10       |
| 900mg Anhydrous MgSO <sub>4</sub> , 150mg PSA                                | 15mL     | 60105-315 | 10       |
| 900mg MgSO <sub>4</sub> , 150mg PSA and 45mg Carbon                          | 15mL     | 60105-317 | 10       |
| 900mg MgSO <sub>4</sub> , 150mg PSA and 15mg Carbon                          | 15mL     | 60105-318 | 10       |
| 1200mg MgSO <sub>4</sub> , 400mg PSA   | 15mL     | 60105-324 | 10       |
| 1200mg MgSO <sub>4</sub> , 400mg PSA and 400mg C18                           | 15mL     | 60105-325 | 10       |
| 1200mg MgSO <sub>4</sub> , 400mg PSA, 400mg C18 and 400mg Carbon             | 15mL     | 60105-326 | 10       |
| 900mg MgSO <sub>4</sub> , 150mg PSA and 150mg C18                            | 15mL     | 60105-327 | 10       |
| 150mg MgSO <sub>4</sub> , 50mg PSA and 50mg Carbon                           | 15mL     | 60105-330 | 10       |
| 150mg MgSO <sub>4</sub> , 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 <sub>4</sub> , 50mg PSA and 50mg Carbon                           | 2mL      | 60105-202 | 100      |
| 150mg MgSO <sub>4</sub> , 50mg PSA   | 2mL      | 60105-203 | 100      |
| 150mg MgSO <sub>4</sub> , 50mg PSA and 50mg C18                              | 2mL      | 60105-204 | 100      |
| 150mg MgSO <sub>4</sub> , 25mg PSA   | 2mL      | 60105-219 | 100      |
| 150mg MgSO <sub>4</sub> , 25mg PSA and 25mg C18                              | 2mL      | 60105-220 | 100      |
| 150mg MgSO <sub>4</sub> , 25mg PSA and 2.5mg Carbon                          | 2mL      | 60105-221 | 100      |
| 150mg MgSO <sub>4</sub> , 25mg PSA and 7.5mg Carbon                          | 2mL      | 60105-222 | 100      |
| 150mg MgSO <sub>4</sub> , 50mg PSA, 50mg C18 and 50mg Carbon                 | 2mL      | 60105-223 | 100      |
| 150mg Anhydrous MgSO <sub>4</sub> , 50mg PSA and 50mg Carbon                 | 2mL      | 60105-302 | 10       |
| 150mg Anhydrous MgSO <sub>4</sub> , 50mg PSA                                 | 2mL      | 60105-303 | 10       |
| 150mg Anhydrous MgSO <sub>4</sub> , 50mg PSA and 50mg C18                    | 2mL      | 60105-304 | 10       |
| 150mg MgSO <sub>4</sub> , 25mg PSA   | 2mL      | 60105-319 | 10       |
| 50mg MgSO <sub>4</sub> , 25mg PSA and 25mg C18                               | 2mL      | 60105-320 | 10       |
| 2mL Centrifuge Tube with 150mg MgSO <sub>4</sub> , 25mg PSA and 2.5mg Carbon | 2mL      | 60105-321 | 10       |
| 150mg MgSO <sub>4</sub> , 25mg PSA and 7.5mg Carbon                          | 2mL      | 60105-322 | 10       |
| 150mg MgSO <sub>4</sub> , 50mg PSA, 50mg C18 and 50mg Carbon                 | 2mL      | 60105-323 | 10       |



# Resources

## for Chromatographers

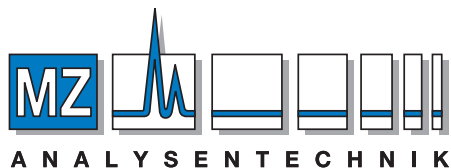
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