

HUMAN HEALTH

ENVIRONMENTAL HEALTH

FAST, EASY EFFICIENT SAMPLE PREPARATION



Supra-Clean® and Supra-Poly® Solid Phase Extraction (SPE)

A Reference Notebook of SPE Applications



INTRODUCTION AND OVERVIEW

At PerkinElmer, we understand that sample preparation is one of the

most critical steps in the analytical process. Often accounting for 60% of your timetable, it has a fundamental impact on a wide range of operational parameters. Any errors within this process undermine the quality of your data at all subsequent stages of your analysis. Solid Phase Extraction (SPE) helps avoid potential errors in sample preparation, reducing re-runs and dramatically increasing productivity. As one of the most cost-effective and flexible tools within the laboratory environment, SPE also provides efficient sample concentration and purification prior to many of today's most popular analytical techniques, including HPLC, LC/MS, GC and GC/MS. This document is intended to provide you with the tools you need to quickly and efficiently develop extraction methods for your sampling analysis.

PerkinElmer SPE Solutions are ideal for a broad array of analytes and matrices. We offer a variety of formats including Large Reservoir Capacity (LRC) columns, Polypropylene (PP) columns, cartridges 96 well plate format, and glass columns. Each technology is offered with a wide selection of polymer and silica sorbents. Varied column capacities and bed weights allow you to perform scalable analyses depending upon your sample size and required detection limits.

PerkinElmer® Supra-Clean® silica-based and Supra-Poly® polymer-based SPE cartridges and columns.

Answering your needs. Empowering Your Lab.



TABLE OF CONTENTS

ENVIRONMENTAL

• 2,4-Dichlorophenol in Water	12
• 2,4-Dichlorophenoxyacetic Acid in Water	13
• 4'4-Methylenedianiline in Serum	14
• Acephate in Water	15
• Aniline in Solution	16
• Atrazine in Water	17
• Bentazone in Water	18
• Bentazone in Water, Derivitized	19
• Chlorophenol in Water	20
• Chlorophenoxy Acid Herbicides in Water	21
• N,N-Dimethylaniline in Solution	22
• Nitroanalines in Solution	23
• Nitrobenzene in Water	24 - 25

FORENSIC

• 6-Acetylmorphine in Urine	26
• Amphetamines in Urine, Oxidation with Periodate for GC or GC/MS Confirmations	27
• Anabolic Steroids in Urine	28
• Antidepressants/Painkillers in Urine and Blood	29
• Benzoyllecgonine in Urine	30
• Carboxy-delta-9-THC, Delta-9-THC, Delta-9-Hydroxy THC in Whole Blood	31
• Delta-9-THC, Delta-9-Hydroxy THC, Carboxy-Delta-9-THC in Whole Blood	32
• Gamma-Hydroxybutyrate (GHB) in Blood, Urine, and Tissue	33
• Gamma-Hydroxybutyrate (GHB) in Urine without conversion to Gamma-Butyrolactone (GBL)	34
• Opiates in Urine	35
• Sympathomimetic Amines in Urine	36

PHARMACEUTICAL

• Acetaminophen in Serum	37
• Acetaminophen in Solution	38
• Benzodiazepine Screen in Blood, Serum, and Tissue by GC/MS	39
• Benzodiazepine Screen in Urine by GC/MS	40

PHARMACEUTICAL

• Beta Agonists in Urine	41
• Beta Blockers in Blood and Urine	42
• Caffeine, Theophylline and Theobromine in Blood, Plasma/Serum, and Urine	43
• Cefaperazone in Water	44
• Clonazepam and 7-Aminoclonazepam in Urine	45
• DHEA, Testosterone, and Epitestosterone in Urine	46
• Doxepin in Rat Serum	47
• Gabapentin in Blood, Plasma/Serum	48 - 50
• Ketotifen Fumarate in Solution	51
• Lovastatin in Water	52
• Methylmalonic Acid from Serum/Plasma	53
• Olanzapine in Whole Blood.....	54
• Paroxetine in Blood, Plasma/Serum and Urine	55
• Propranolol in Rat Serum	56
• Tacrolimus, Cyclosporin and Rapamycin in Whole Blood.....	57
• Tricyclic Antidepressants in Plasma/Serum	58

Supra-Clean and Supra-Poly Solid Phase Extraction (SPE) Columns with Precise Bed Technology™

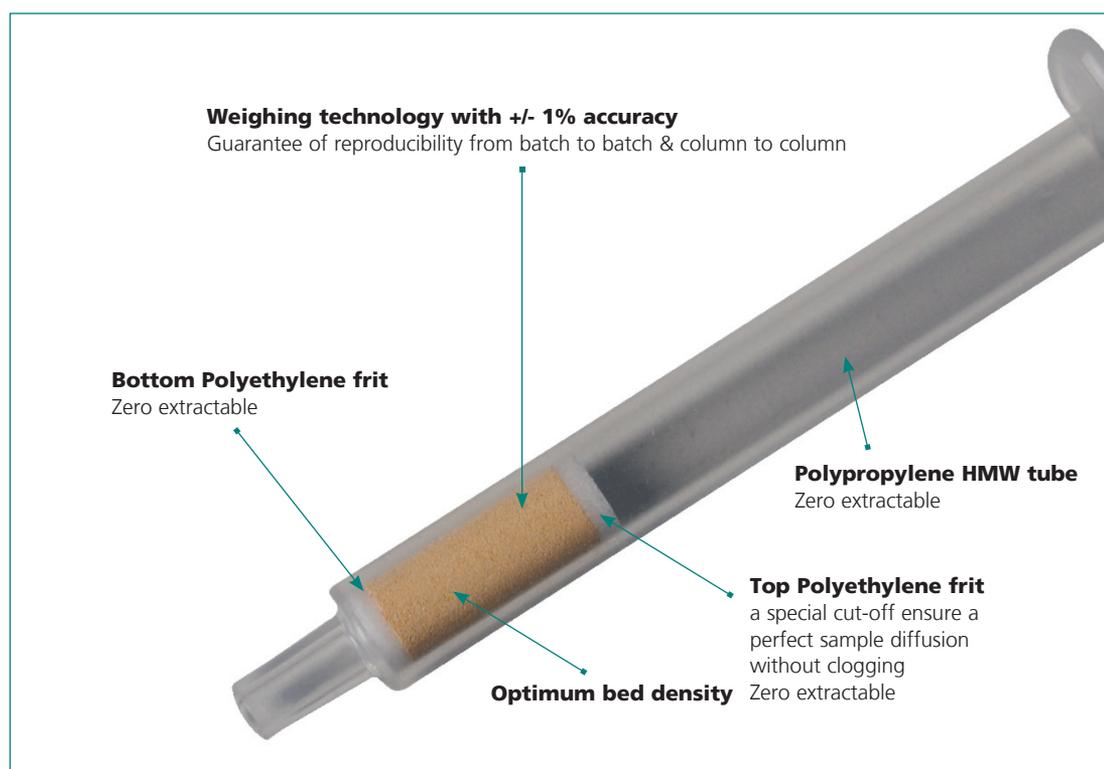
Supra-Clean and Supra-Poly SPE products are designed to offer you a sample preparation solution that decreases your sample preparation time all while maintaining a high level of reproducibility. Supra-Clean uses a silica based media and Supra-Poly uses a polymer based media. Both formats are packed using Precise Bed Technology and spherical particles, which allow columns to be evenly and consistently filled resulting in optimum bed density.

Optimum bed density delivers a level of performance unattainable with the irregularly shaped silica used in competitive solutions. The homogeneous filling and mono-dispersed spherical media not only provide unparalleled reproducibility, it also delivers superior performance.

Homogeneous filling and spherical media yield a +/- 1% variation in bed volume precision, a significant improvement from the industry average. This level of quality control allows customers to experience consistent sample preparation performance column to column, batch to batch, and lot to lot.

Sample diffusion through Supra-Clean/Supra-Poly spherical media is also better than in other systems, improving flow efficiency and avoiding channeling and clogging due to fines. This enables smaller sample sizes to be used without compromising recovery levels, dramatically reducing solvent volumes, analytical costs and processing times. This allows you to work faster and more cost effectively, all while experiencing complete recovery, reproducibility and reliability.

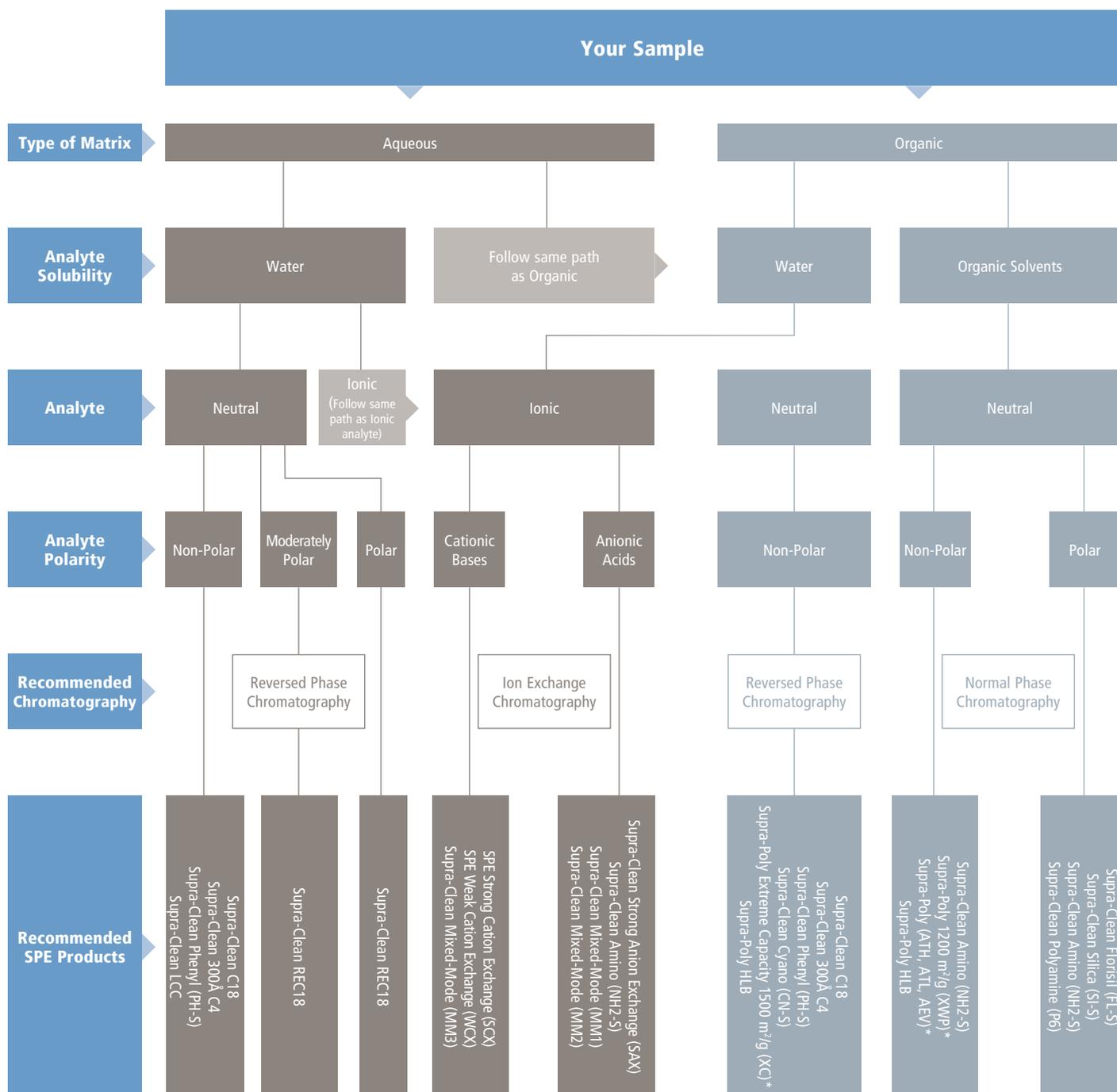
Precise Bed Technology



All columns are thoroughly quality control tested in-house to guarantee tracability. Products are supplied with an individual certificate detailing the specific production number and sorbent batch.

Choose the Best SPE Solution For Your Method

To find the ideal SPE column for your particular application, simply follow the flow diagram below, selecting your path based on the options at each junction.



*AEV = Advanced Environmental
 ATH = Hydrophobic/Hydrophilic
 ATL = Lipophilic

HLB = Hydrophilic Lipophilic Balanced
 XC = Extreme Capacity
 XWP = Extra Wide Particle

A Wide Array of Columns for a Broad Range of Applications

SUPRA-CLEAN SPHERICAL SILICA				
Phase	Mechanism	Interaction mode	Compounds	Matrix
C18-S	Hydrophobic	Reversed Phase	Polar to Non-Polar compounds	Biological fluids, aqueous samples
High Recovery REC18	Hydrophobic	Reversed Phase	Non-polar and mid-polar compounds including 100% water solvents	Biological fluids, aqueous samples
Phenyl (PH-S)	Hydrophobic	Reversed Phase	Non-polar to mid-polar aromatic compounds	Biological fluids
Silica (SI-S)	Hydrophilic	Normal Phase	Polar compounds	Non-polar organics, oils, lipids
Amino (NH2-S)	Hydrophilic	Normal Phase	Polar to Mid-Polar aromatic compounds	Biological fluids, aqueous samples, buffered organics
Strong Cation Exchange (SCX)	Ion Exchange	Ion Exchange	Basic compounds	Biological fluids, aqueous samples, buffered organics
Weak cation Exchange (WCX)	Ion Exchange	Ion Exchange	Strong basic compounds	Biological fluids, aqueous samples
Strong Anion Exchange (SAX)	Ion Exchange	Ion Exchange	Acidic compounds	Biological fluids, aqueous samples
Cyano (CN-S)	Hydrophilic	Normal Phase	Polar to Mid-Polar compounds	Non-polar organics, oils, lipids
Florisil (FL-S)	Hydrophilic	Normal Phase	Polar compounds	Ideal for polar compounds in non-polar matrix
Florisil Pesticide (FL-S)	Hydrophilic	Normal Phase	Polar compounds	Ideal for polar compounds in non-polar matrix
Polyamine (P6)	Hydrophilic	Reversed Phase	Carboxylic acids, phenolics and nitroaromatics	Aqueous and mid-polar matrices
300 A (C4)	Hydrophobic	Reversed Phase	Non-polar to mid-polar compounds	Biological Samples
LCC	Hydrophobic	Reversed Phase	Non-polar to mid-polar compounds	Biological fluids, aqueous samples
Mixed mode (MM1)	Ion Exchange/ Hydrophobic	Reversed Phase / SCX	Basic compounds	Biological samples
Mixed mode (MM2)	Ion Exchange/ Hydrophobic	Reversed Phase / WCX	Very basic compounds	Biological samples
Mixed mode (MM3)	Ion Exchange/ Hydrophobic	Reversed Phase / SAX	Acidic compounds	Biological samples
SUPRA-POLY SPHERICAL POLYMER				
Extreme Capacity (XC)	Hydrophobic	Reversed Phase	Polar and non-polar	Aqueous or organic
Extreme Capacity Wide Pore (XWP)	Hydrophobic	Reversed Phase	Polar and non-polar	Biological and viscous samples
Hydrophilic (ATH)	Hydrophilic	Reversed Phase	Mid to non-polar compounds	Aqueous or organic
Lipophilic (ATL)	Lipophilic	Reversed Phase	Mid to non-polar compounds	Crude samples
Environmental (AEV)	Hydrophilic/ Hydrophobic	Reversed Phase	Mid to non-polar compounds	Aqueous or Organic
HLB	Hydrophilic/lipophilic balanced	Reversed Phase	Mid to non-polar compounds	Aqueous or organic

Typical Applications	pH Range	End-capping	Pore Size (Å)	Surface Area (m ² /g)	Particle Size (µm)	Comments
Drugs and drug metabolites in biological matrices, trace organic material in water, toxins in food	2-8	Yes	60	500	50	18% Carbon Load (CL)
Drugs and drug metabolites in biological matrices, trace organic material in water, toxins in food	2-8	Yes	NA	NA	50	High capacity and better recovery especially for high aqueous conditions. 15% CL
Benzodiazepines in biological matrices, extraction of aromatic compounds	2-8	No	60	500	50	9% CL
Aldehydes, amines, pesticides, herbicides, carotenoids, fat soluble vitamins, aflatoxins, fatty acids, and phospholipids	2-8	No	60	500	50	Bare Silica
Basic compounds, polar amine compounds, carbohydrates	2-8	No	60	500	50	5% CL
Cations, antibiotics, drugs, amino acids, catecholamines, herbicides, nucleic acid bases, nucleosides, and surfactants	2-8	No	60	450	60	Strong Acid - Sulfonic acid; Exchange capacity 0.70 meq/g
Cations, amines, antibiotics, drugs, amino acids, catecholamines, nucleic acid bases, nucleosides, and surfactants	2-8	No	60	450	60	Weak Acid - Carboxylic acid; Exchange capacity 0.22 meq/g
Acidic food pigments, organic acids, phenol compounds, nucleic acids, nucleotides, surfactants	2-8	No	60	450	60	Strong Base - quaternary amine; Exchange capacity 0.30 meq/g
Polar compounds in hexane and oil	2-8	Yes	60	500	50	8% CL; Mid-range polarity between silica and C18
Pesticides, Polychlorinated Biphenyls (PCB)	2-8	No	NA	NA	200	Standard grade. Alternative to silica for viscous matrices due to large particle size. Granular shape
Pesticides	2-8	No	NA	NA	200	High purity pesticide grade. Alternative to silica for viscous matrices. Granular shape
Aromatic and natural products; Flavones, Chalcones, Anthraquinones	2-8	No	NA	NA	100	Nylon 6
Hydrophobic peptides and polypeptides	2-8	No	300	-	-	Large pore size for isolation of large biomolecules
Non-polar compounds in aqueous solution	2-8	Yes	60	500	50	10% CL; Lower carbon load than C18-S and REC18
Drugs and drug metabolites	2-8	No	60	450	60	Exchange capacity 0.09 meq/g
Drugs and drug metabolites	2-8	No	60	450	60	Exchange capacity 0.10 meq/g
Drugs and drug metabolites	2-8	No	60	450	60	Exchange capacity 0.14 meq/g
Drugs and drug metabolites biological fluids	0-14	No	NA	1500	70	High capacity polystyrene-divinylbenzene (PSDVB)
Drugs and drug metabolites biological fluids	0-14	No	Wide Pore	1200	90	High capacity PSDVB for large biomolecules and viscous matrices
Mid-polar and non-polar compounds in aqueous and organic solvents	1-13	No	70	800	75	Mixed hydrophilic/hydrophobic interactions
Lipids	0-14	No	70	800	100	PSDVB; Alternative to high flow silica for mid-polar to non-polar compounds (<3000D) in crude samples
Aqueous environmental compounds that are not retained on C18	1-12	No	70	800	75	Advanced environmental; Polystyrene-co-2-hydroxyethyl methacrylate (PSHEMA)
Mid-polar and non-polar compounds in aqueous and organic solvents	0-14	No	80	850	30 & 60	Hydrophilic-lipophilic-balanced reversed-phase sorbent for acids, bases and neutrals

Six Steps for Clean Extract

1. Pretreat Sample

Some samples require initial pretreatment to obtain the optimal extraction. This pretreatment can include homogenization of solid samples, adjustment of the matrix to the proper ionic strength and pH, and removal of particulates via filtration or centrifugation. This step may also include addition of an internal standard.

2. Condition Column

Sorbent activation and functional group activation are achieved by passing a volume of an appropriate solvent or a mixture of solvent, through the column. Column frits are simultaneously conditioned as well.

Methanol or acetonitrile are commonly used for activating hydrophobic sorbents, while hexane or dichloromethane activate hydrophilic sorbents. 2 to 4 bed volumes are typically recommended. See SPE Solvent Selection Guide (*below*).

Note: Once the conditioning is done never let the column dry unless it is specified in the method.

3. Load Sample

Apply your sample onto the upper part of the sorbent bed. Matrix contaminants may pass through the column unretained while other matrix components may be more or less strongly retained on the sorbent surface.

To get a maximum purification efficiency, the sample flow needs to be controlled via vacuum or pressure. To achieve faster flow of viscous sample through a column, 90 µm sorbents or larger particle size can be used. The exchange capacity and selectivity are unaffected.

Note: It is necessary to analyze the unretained fraction to check if all compounds of interest have been retained.

4. Wash Column

Passing solvents through columns washes away interfering compounds while leaving the analyte of interest undisturbed on the sorbent bed.

Different solvents or solvent mixtures may be used to improve the rinsing efficiency. See SPE Solvent Selection Guide (*below*).

5. Dry Column (optional)

A drying step may be required. Solvent traces are evaporated by circulating air through the column over a 2 to 10 minute time period. This improves the extraction yield for certain methods and compounds.

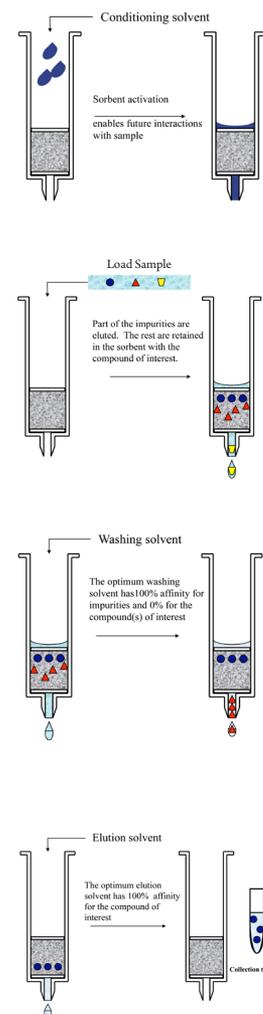
6. Elute Compounds of Interest

An appropriate solvent is passed through the column to disrupt the analyte-sorbent interaction and to elute 100% of compounds of interest.

The appropriate solvent must have maximum interaction with the compound of interest and a minimal interaction with the remaining impurities, leaving them undisturbed on the sorbent bed. See SPE Solvent Selection Guide (*below*).

In addition, the volume of the elution solvent needs to be as small as possible to maximize the concentration factor.

Note: Sorbent with low particle size (e.g 30, 50 µm) gives a lower elution volume than larger sorbent particle size (e.g 90, 140 µm).



SPE Solvent Selection Guide

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

Solvent	Hexane	Isooctane	Petroleum Ether	Cyclohexane	Carbon tetrachloride	Toluene	Chloroform	Methylene Chloride	Tetrahydrofuran	Diethyl Ether	Ethyl Acetate	Acetone	Acetonitrile	Pyridine	Isopropanol	Ethanol	Methanol	Water	Acetic Acid	
Water Miscible	No	No	No	No	No	No	No	No	Yes	No	Poor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
Polarity	NONPOLAR										POLAR									

Method Development for SPE

Imagine how much faster, easier and more efficient your method development would be if your Solid Phase Extraction (SPE) column selection kits were designed to streamline your process. PerkinElmer has created column specific kits for some of the more common analyses performed in today's laboratories. These kits dramatically reduce set-up time for these methods and make it easy to select the appropriate solution based on your specific matrices.

PerkinElmer SPE Column Selection Kits	Media Weight	Volume	Qty.	Part No.
Pre-Concentration of Hydrophobic Compounds from Aqueous Matrix	200 mg	6 mL	50	N9306594
	200 mg	3 mL	50	N9306595
Type of matrix: aqueous Principle: concentration of non-polar, mid-polar compounds Includes 10 each: Supra-Clean C18 with cartridge, REC18; Supra-Poly Lipophilic (ATL), extreme capacity (XC), hydrophobic/hydrophilic (ATH)				
Extraction of Hydrophobic Compounds from Aqueous Matrix	500 mg	6 mL	50	N9306596
	500 mg	3 mL	50	N9306597
Type of matrix: aqueous or (organics and aqueous) mixtures Principle: extraction of non-polar and mid-polar compounds Includes 10 each: Supra-Clean C18 (un-end-capped), C18 with cartridge, REC18, LCC, Phenyl				
Pre-Concentration of Hydrophilic Compounds	500 mg	6 mL	30	N9306598
	500 mg	3 mL	30	N9306599
Type of matrix: aqueous or (organics and aqueous) mixtures Principle: extraction of non-polar and mid-polar compounds Includes 10 each: Supra-Clean Amino, Cyano, Silica with cartridge				
Removal of Polar Compounds from Aqueous and Organic Matrix	500 mg	6 mL	30	N9306600
	500 mg	3 mL	30	N9306601
Type of matrix: aqueous or organics Principle: Extraction and concentration of acidic, basic and neutral compounds Includes 10 each: Supra-Clean Amino, Florisil, Silica with cartridge				
Extraction of Acidic Basic and Neutral Compounds from Aqueous or Organic Matrix	100 mg	3 mL	50	N9306602
Type of matrix: aqueous or organics Principle: Extraction and concentration of acidic, basic and neutral compounds Includes 10 each: Supra-Poly Lipophilic (ATL), extra wide particle (XWP), extreme capacity (XC), advanced environmental (AEV), hydrophobic/hydrophilic (ATH)				
Extraction of Carboxylic Acids and Strong Bases from Aqueous Matrix	500 mg	6 mL	40	N9306603
Type of matrix: aqueous Principle: selective extraction of weak acids and strong bases with the same protocol Includes 10 each: Supra-Clean Strong Anion Exchange, Weak Cation Exchange, Mixed-Mode 2, 3				
Extraction of Weak Bases from Aqueous Matrix	500 mg	6 mL	30	N9306604
Type of matrix: aqueous Principle: extraction of weak bases Includes 15 each: Supra-Clean Strong Cation Exchange, Mixed-Mode 1				

ENVIRONMENTAL

2,4-Dichlorophenol in Water

PerkinElmer SPE Supra-Poly XC 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306405

SAMPLE PRETREATMENT

Adjust pH to 2 with H₂SO₄

COLUMN CONDITIONING

5 mL methanol
5 mL water

SAMPLE LOADING

Load 500 mL water sample
Elute at 5 mL/minute

COLUMN WASHING

5 mL water
1 mL methanol

DRYING

Dry column (20 minutes under nitrogen)

ELUTION

3 mL tetrahydrofuran

CONCENTRATION

Concentrate sample to 3 mL under nitrogen

ANALYSIS RECOMMENDATIONS

HPLC

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated PFP 250 mm x 4.6 mm x 5 µm
Part No. N9303600

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

2,4-Dichlorophenoxyacetic Acid in Water

PerkinElmer SPE Supra-Poly XC 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306405

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

3 mL of tetrahydrofuran

5 mL of methanol

5 mL of DI water

SAMPLE LOADING

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

COLUMN WASHING

5 mL of DI water

DRYING

Dry column (20 minutes at >10" Hg/full flow for positive pressure manifold)

ELUTION

Use 0.8 mL of methanol to replace the residual water in the XC packing

Discard the water which passes through the SPE column

Let methanol infiltrate the packing material for 2 minutes

Elute the column with 3 mL of THF

Collect the eluate and reconstitute to 3 mL using mobile phase

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

4'4-Methylenedianaline in Serum

PerkinElmer SPE Supra-Clean C18-S 100 mg/1 mL, Plastic Tube (Straight) Part No. N9306478

COLUMN CONDITIONING

3 mL of methanol
3 mL of DI water

SAMPLE LOADING

Load sample at 1 mL/minute

COLUMN WASHING

1 mL of DI water

ELUTION

0.25 mL of methanol containing 1 M ammonium hydroxide

ANALYSIS RECOMMENDATIONS

Inject 10 µL onto HPLC system
Extracted 100 µg/mL sample
Mobile phase: methanol/water (50:50)
Flow rate: 1.2 mL/min
Injection volume: 10 µL
Wavelength: UV 254 nm

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated C18 250 mm x 4.6 mm x 5 µm
Part No. N9303561

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Acephate in Water

PerkinElmer SPE Supra-Clean C18-S 100 mg/1 mL, Plastic Tube (Straight) Part No. N9306478

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

2 mL of methanol
2 mL of DI water

SAMPLE LOADING

Load 4 mL samples at 1 to 2 mL/minute

COLUMN WASHING

1 mL of DI water

ELUTION

5 mL of methanol

CONCENTRATION

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

ANALYSIS RECOMMENDATIONS

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

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated C18 250 mm x 4.6 mm x 5 µm
Part No. N9303561

RELATED ITEMS

Vacuum Manifold -12 position; Part No. N9306619
Vacuum Manifold -24 position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Aniline in Solution

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

Prepare solutions of aniline in DI water at concentrations of 200, 20 and 2 ppm

COLUMN CONDITIONING

3 mL of methanol
3 mL of DI water

SAMPLE LOADING

Load 3 mL sample at 1 to 2 mL/minute

COLUMN WASHING

1 mL of DI water

ELUTION

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

CONCENTRATION

Evaporate to full dryness at room temperature under a stream of nitrogen
Reconstitute the sample to 1 mL with methanol

ANALYSIS RECOMMENDATIONS

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

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated Aqueous C18
250 mm x 4.6 mm x 5 um Part No. N9303549

RELATED ITEMS

Vacuum Manifold -12 position; Part No. N9306619
Vacuum Manifold -24 position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Atrazine in Water

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

2 mL of methanol

2 mL of DI water

SAMPLE LOADING

Load 4 mL samples at 1 to 2 mL/minute

COLUMN WASHING

1 mL of DI water

ELUTION

5 mL of methanol

CONCENTRATION

Evaporate to full dryness under a stream of nitrogen

Reconstitute the sample to 0.5 mL with water

ANALYSIS RECOMMENDATIONS

Mobile phase: ACN/0.2% acetic acid (10:90, v/v)

Flow: 1mL/minute

Temperature: 30°C

Detection: UV 214nm

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated C18 250 mm x 4.6 mm x 5 µm

Part No. N9303561

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Bentazone in Water

PerkinElmer SPE Supra-Poly XC 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306405

SAMPLE PRETREATMENT

Adjust the pH of 500 mL of water to ≤ 3 using 0.5 M H_2SO_4

COLUMN CONDITIONING

3 mL of tetrahydrofuran

5 mL of methanol

5 mL of DI water

SAMPLE LOADING

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

COLUMN WASHING

5 mL of DI water

DRYING

Dry column (20 minutes at >10 " Hg/full flow for positive pressure manifold)

ELUTION

Use 0.8 mL of methanol to replace the residual water in the XC 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 3 mL of tetrahydrofuran

Evaporate the tetrahydrofuran and reconstitute to 3 mL using mobile phase

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Bentazone in Water, Derivatized

PerkinElmer SPE Supra-Poly XC 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306405

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

3 mL of tetrahydrofuran
5 mL of methanol
5 mL of DI water

SAMPLE LOADING

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

COLUMN WASHING

5 mL of DI water

DRYING

Dry column (20 minutes under nitrogen)

ELUTION

3 mL tetrahydrofuran
Concentrate sample to 3 mL under N₂

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Chlorophenol in Water

PerkinElmer SPE Supra-Poly XC 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306405

SAMPLE PRETREATMENT

Collect 500 mL of water
Adjust pH to 2 with H₂SO₄

COLUMN CONDITIONING

5 mL methanol
5 mL water

SAMPLE LOADING

Load 500 mL water sample
Elute at 5 mL/minute

COLUMN WASHING

5 mL water
1 mL methanol

ELUTION

3 mL tetrahydrofuran

CONCENTRATION

Concentrate sample to 3 mL under nitrogen

ANALYSIS RECOMMENDATIONS

HPLC

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated PFP 250 mm x 4.6 mm x 5 um
Part No. N9303600

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Chlorophenoxy Acid Herbicides in Water

PerkinElmer SPE Supra-Clean C18 1 g /6 ml, Plastic Tube (Straight) Part No. N9306422

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

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

SAMPLE LOADING

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

COLUMN WASHING

10 mL of DI water adjusted to pH 1.0 with HCl

DRYING

Dry column using maximum vacuum pressure for 15 to 30 minutes

ELUTION

10 mL of hexane/acetone (50:50)

CONCENTRATION

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

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 µL onto GC

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

N,N-Dimethylaniline in Solution

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

3 mL of methanol
3 mL of DI water

SAMPLE LOADING

Load 3 mL sample at 1 to 2 mL/minute

COLUMN WASHING

1 mL of DI water

ELUTION

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

CONCENTRATION

Evaporate to full dryness at room temperature under a stream of nitrogen
Reconstitute the sample to 1 mL with methanol

ANALYSIS RECOMMENDATIONS

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

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated Aqueous C18
250 mm x 4.6 mm x 5 um Part No. N9303549

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Nitroanalines in Solution

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

3 mL of methanol

3 mL of DI water

SAMPLE LOADING

Load 3 mL samples at 1 to 2 mL/minute

COLUMN WASHING

1 mL of 0.1% ammonia aqueous solution

ELUTION

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

CONCENTRATION

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

Reconstitute the sample to 1 mL with methanol

ANALYSIS RECOMMENDATIONS

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

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated Aqueous C18
250 mm x 4.6 mm x 5 um Part No. N9303549

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Nitrobenzene in Water

PerkinElmer SPE Supra-Poly XC 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306405

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

3 mL of hexane
5 mL of methanol
5 mL of DI water

SAMPLE LOADING

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

COLUMN WASHING

5 mL of DI water

DRYING

Dry column (20 minutes at >10" Hg/full flow for positive pressure manifold)

ELUTION

Use 0.8 mL of acetone to replace the residual water in the XC 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 Supra-Poly XC column to a column packed with 5 g of anhydrous sodium sulphate which has been washed with 3 mL of acetone, 3 mL of hexane and 3 mL acetone

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

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

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

ENVIRONMENTAL

Nitrobenzene in Water

PerkinElmer SPE Supra-Poly XC 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306405

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

3 mL n-hexane
5 mL methanol

SAMPLE LOADING

Load 10 mL water sample
Elute at 5 mL/minute

COLUMN WASHING

10 mL of DI water

DRYING

Dry column (20 minutes at >10" Hg)

ELUTION

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

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated C18 250 mm x 4.6 mm x 5 um
Part No. N9303561

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

FORENSIC

6-Acetylmorphine in Urine

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/15 ml, Plastic Tube (Straight) Part No. N9306713

SAMPLE PRETREATMENT

To 2 mL of 100 mM phosphate buffer (pH= 6.0) add internal standard*

Mix/vortex

Add 4 mL of urine

Centrifuge for 10 minutes at 2,000 rpm

Discard pellet

Adjust pH to 6 with 100 mM monobasic or dibasic sodium phosphate

* *Suggested internal standard D6-6-AM-TMS*

COLUMN CONDITIONING

3 mL methanol

3 mL DI water

1 mL 100 mM phosphate buffer (pH 6)

SAMPLE LOADING

Load the previously prepared sample onto the SPE column at a flow rate of 1 mL/min

COLUMN WASHING

3 mL DI water

2 mL 100 mM acetate buffer (pH 4.5)

3 mL methanol

DRYING

Dry the column for 10 minutes under vacuum

ELUTION

3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2) at a flow rate of 1-2 mL/min

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

Add 50 µL ethyl acetate

Mix/vortex

Add 50 µL BSTFA (with 1% TMCS)

Overlay with N₂ and cap

Mix/vortex

React 45 minutes at 70 °C; cool.

ANALYSIS RECOMMENDATIONS

Inject 2 µl to a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

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

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 30 m x 0.25 mm x 0.25 µm
Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

Amphetamines in Urine, Oxidation with Periodate for GC or GC/MS Confirmations

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200mg/15ml, Plastic Tube (Straight) Part No. N9306713

SAMPLE PRETREATMENT

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

Mix/vortex

Incubate at room temperature for 20 minutes

Adjust pH to 6.0±0.5 with 100 mM monobasic or dibasic sodium phosphate

* Suggested internal standards D5-amphetamine and D5-methamphetamine

COLUMN CONDITIONING

3 mL methanol

3 mL DI water

1 mL 100 mM phosphate buffer (pH 6)

SAMPLE LOADING

Load the previously prepared sample onto the SPE column at a flow rate of 1-2 mL/min

COLUMN WASHING

3 mL DI water

1 mL 100 mM acetic acid

3 mL methanol

DRYING

Dry the column for 5 minutes

ELUTION

3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2) at a flow rate of 1-2 mL/min

CONCENTRATION

Add 30 µL silylation grade DMF to eluate
Evaporate to 30 µL at < 40°C

POST TREATMENT

Add 50 µL PFPA (PFAA)

Overlay with N₂ and cap

Improve derivatization by addition of 50 µL PFPOH

React for 20 minutes at 70 °C

Evaporate to dryness at < 40 °C

ANALYSIS RECOMMENDATIONS

Inject 2 µL to a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

Compound	Primary Ion	Secondary	Tertiary
D5-amphetamine	194	92	123
Amphetamine	190	91	118
D5-methamphetamine	208	92	163
Methamphetamine	204	91	160

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 30 m x 0.25 mm x 0.25 µm
Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

FORENSIC

Anabolic Steroids in Urine

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/15 ml, Plastic Tube (Straight) Part No. N9306713

SAMPLE PRETREATMENT

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

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

Mix/vortex

Hydrolyze for 3 hours at 65 °C; cool.

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

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

COLUMN CONDITIONING

3 mL methanol then aspirate

3 mL DI water then aspirate

1 mL 100 mM phosphate buffer (pH 6) then aspirate

SAMPLE LOADING

Load the previously prepared sample onto the SPE column at 1-2 mL/min flow rate

COLUMN WASHING

3 mL of 10% (v/v) methanol in DI water

Dry the column for 5 minutes

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

DRYING

Dry the column for 5 minutes

ELUTION

Option 1: Elute with 3 mL of CH₂Cl₂/IPA/NH₄OH (78:20:2) at 1-2 mL/min

Option 2: 3 mL CH₂Cl₂/IPA (80:20)

Option 3: 3 mL ethyl acetate

Option 4: 3 mL methanol

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

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

Overlay with N₂ and cap

Mix/vortex

React for 20 minutes at 70 °C

Remove from heat source to cool

ANALYSIS RECOMMENDATIONS

Inject 2 μ L to a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

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- β -hydroxyandosterone	522	417	158	
Methandienone	409	313	281	
19-norandosterone-2TMS	405	315	225	
Alpha-hydroxyetiocholanone	504	417		
17- α -epitestosterone-TMS	432	341	327	209
Stanozolol	472	381	342	149

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 15 m x 0.25 mm x 0.25 μ m
Part No. N9316279

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

FORENSIC

Antidepressants/Painkillers in Urine and Blood

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/15 ml, Plastic Tube (Straight) Part No. N9306713

SAMPLE PRETREATMENT

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

Add 1 mL of urine or blood

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

Mix/vortex

Adjust pH 6.0±0.5 with 100 mM of monobasic or dibasic sodium phosphate

Mix/vortex

Centrifuge

* Suggested internal standards Amitriptyline-D3, Diphenhydramine-D3, Methadone-D9, Norpropoxyphene-D5, Propoxyphene-D11, Tramadol-D3

COLUMN CONDITIONING

3 mL methanol

3 mL DI water

1 mL 100 mM phosphate buffer (pH 6)

SAMPLE LOADING

Load the previously prepared sample onto the SPE column at 1-2 mL/min

COLUMN WASHING

3 mL DI water

3 mL of 1% acetic acid

3 mL methanol

DRYING

Dry the column for 5 minutes

ELUTION

Elute with 3 mL of ethyl acetate: acetonitrile: ammonia (78:20:2, v/v/v) at 1-2 mL/min

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

Reconstitute sample in 100 µL of methanol

ANALYSIS RECOMMENDATIONS

Inject 2 µL onto a LC

RESULTS

Ions to monitor (Mass Spectrometry)

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

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated C 18 150 mm x 2.1 mm x 5 µm Part No. N9303556

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

FORENSIC

Benzoyllecgonine in Urine

PerkinElmer SPE Supra-Clean C18-S 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306462

SAMPLE PRETREATMENT

Add deuterated internal standard (ISTD) to 1-2 mL of urine
2 mL 100 mM phosphate buffer (pH 6)

COLUMN CONDITIONING

3 mL methanol
3 mL DI water
1 mL 100 mM phosphate buffer (pH 6)

SAMPLE LOADING

Load the previously prepared sample at 1-2 mL/min

COLUMN WASHING

3 mL DI water
100 mM HCL
1 mL of methanol

ELUTION

3 mL Methylene chloride:
Isopropanol:Ammonium Hydroxide (78:20:2) into conical tube

CONCENTRATION

Evaporate to dryness < 50 °C

POST TREATMENT

Reconstitute in 50 µL PFPA
Add 25 µL PFPOH
Cover with N₂, cap, mix, heat to 70 °C and hold for 20 min.
Evaporate to dryness < 50 °C.
Reconstitute in 100 µL ethyl acetate
Transfer final treated sample to low volume autosampler vial insert

ANALYSIS RECOMMENDATIONS

Inject 1 µL into GC/MS

RESULTS

Three ion ratio chromatograms must all apex within ±2 scans of standard retention time. Ion ratios must fall within ±20% of standard ratios. Deuterated internal standards may use only 2 ions, a primary ion and only 1 confirmation ion.

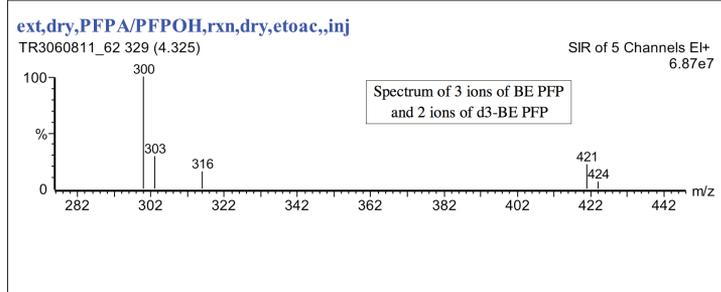
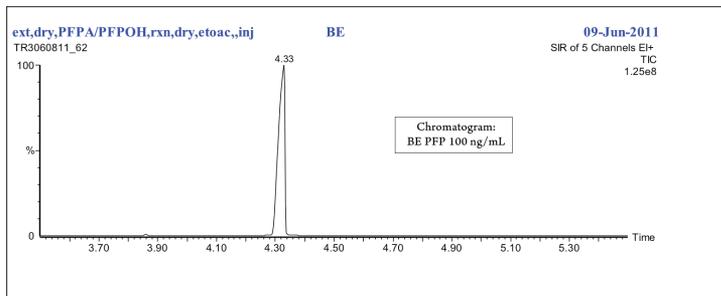
PFPA SIM Ions:

Compound	Primary Ion	Secondary	Tertiary
Benzoyllecgonine	300	421	316
d3-benzoyllecgonine	303	424	

BSTFA Ions:

Compound	Primary Ion	Secondary	Tertiary
Benzoyllecgonine	240	361	256
d3-benzoyllecgonine	243	364	

CHROMATOGRAM / SPECTRUM



RECOMMENDED COLUMN

PerkinElmer Elite-5 12 m x 0.20 mm x 0.33 µm Part No. N9316110
Helium carrier – 2 mL/min

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

NOTES

Acetic Acid, 100 mM = 2.86 mL glacial acetic acid diluted to 500 mL DI water. Phosphate buffer, 100 mM pH6 = 1.7 g Na₂HPO₄ + 12.14 g Na₂HPO₄ dilute to 1000 mL with DI water, adjust to pH6 with 100 mM Na₂HPO₄ (raises pH) or 100 mM Na₂HPO₄ (lowers pH). Methylene Chloride/Isopropanol/Ammonium Hydroxide(78:20:2) extraction solvent = 40 mL IP-OH + 4 mL concentrated NH₄OH + 156 mL MeCl₂. Make fresh daily.

REFERENCES

Benzoyllecgonine in Urine by SAMHSA GC/MS, Timothy D. Ruppel, PerkinElmer Application Note, Project I.D. 008424A_01
Timothy D. Ruppel, PerkinElmer Application Note, Order No. 009784_01

FORENSIC

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

PerkinElmer SPE Supra-Clean Mixed-Mode MM3 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306649

SAMPLE PRETREATMENT

Add internal standards* to 1 to 2 mL of whole blood

Mix/vortex

Vortex and add dropwise 1 mL of cold acetonitrile

Centrifuge and transfer acetonitrile to a clean tube

Adjust sample pH to 3.0 ± 0.5 with approximately 2.0 mL of 100 mM sodium acetate buffer

* Suggested internal standards D₃-Carboxy-delta-9-THC; D₃-delta-9-THC

COLUMN CONDITIONING

5 mL methanol

5 mL DI water

2 mL acetate buffer (pH 3.0) then aspirate

SAMPLE LOADING

Load sample at 1 to 2 mL/min

COLUMN WASHING

5 mL DI water

4 mL 100 mM HCl/acetonitrile (95:5)

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

400 μ L hexane then aspirate

DRYING

Optional: Dry the column for 5 minutes

ELUTION

4 mL hexane

5 mL hexane/ethyl acetate (50:50)

CONCENTRATION

Evaporate to dryness < 40 °C

POST TREATMENT

Add 50 μ L of ethyl acetate and 50 μ L of BSTFA (with 1% TMCS)

Mix/vortex

React for 20 minutes at 70 °C

Cool

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 μ L to a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

Compound	Primary Ion	Secondary	Tertiary
D ₃ -Carboxy-delta-9-THC	374	476	491
D ₉ -Carboxy-delta-9-THC	380	479	497
Carboxy-delta-9-THC	371	473	488
D ₃ -Hydroxy-delta-9-THC	374	462	477
Hydroxy-delta-9-THC	371	459	474
D ₃ -delta-9-THC	374	389	
Delta-9-THC	371	386	

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 15 m x 0.25 mm x 0.25 μ m
Part No. N9316279

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

FORENSIC

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

PerkinElmer SPE Supra-Clean Mixed-Mode MM3 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306649

SAMPLE PRETREATMENT

Add internal standards* to 1 to 2 mL of whole blood

Vortex and add dropwise 2 mL of cold acetonitrile

Mix and centrifuge; transfer acetonitrile to a clean tube

Evaporate acetonitrile under air or nitrogen to 200 µL

Add 2 mL DI water (pH=6.0 to 7.0)

* *Suggested internal standards D3-Carboxy-delta-9-THC; D3-delta-9-THC; D3-Hydroxy-delta-9-THC*

SAMPLE LOADING

Load sample directly onto column (no preconditioning)

COLUMN WASHING

Wash the column with 2.5 mL DI water/acetonitrile/ammonium hydroxide (84:15:1)

DRYING

Dry the column for 5 minutes

ELUTION

5 mL hexane/ethyl acetate/glacial Acetic acid (49:29:2) at a flow rate of 1 to 2 mL/min

CONCENTRATION

Evaporate to dryness with air or nitrogen at < 40 °C

POST TREATMENT

Add 50 µL of ethyl acetate

Mix/vortex

Add 50 µL BSTFA (with 1% TMCS)

React for 20 minutes at 70 °C

Cool

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 µL to a GC/MS

RECOMMENDED COLUMN

PerkinElmer Elite-35 MS 30 m x 0.25 mm x 0.25 µm
Part No. N9316440

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

FORENSIC

Gamma-Hydroxybutyrate (GHB) in Blood, Urine, and Tissue

PerkinElmer SPE Supra-Clean Mixed-Mode MM3 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306649

SAMPLE PRETREATMENT

Pipet 200 µL sample into 1.5 mL plastic tubes

Add 25 µL internal standard*

Add 1 mL of acetone

Centrifuge

Transfer acetone layer to culture tube

Evaporate extract with nitrogen at 70 °C

Reconstitute extracts with 200 µL of 100 mM phosphate buffer (pH 6.0)

Mix/vortex

* Suggested internal standard D6-GHB

COLUMN CONDITIONING

5 mL methanol

5 mL DI water

2 mL 100 mM phosphate buffer (pH 6.0)

SAMPLE LOADING

Load the previously prepared sample onto the SPE column

ELUTION

2 mL methanol/ammonium hydroxide (99:1) in the original sample test tube

Mix/vortex

Pour into column and collect extract

Aspirate at ~1 "Hg

CONCENTRATION

Evaporate to dryness at 70 °C under nitrogen

POST TREATMENT

Add 100 µL ethyl acetate and 100µL BSTFA with 1% TCMS

Mix/vortex

Heat at at 70 °C for 30 minutes

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 µL to a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

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

RECOMMENDED COLUMN

PerkinElmer Elite-35 MS 30 m x 0.25 mm x 0.25 µm
Part No. N9316440

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

FORENSIC

Gamma-Hydroxybutyrate (GHB) in Urine without conversion to Gamma-Butyrolactone (GBL)

PerkinElmer SPE Supra-Clean Mixed-Mode MM3 500 mg/6 mL, Plastic Tube (Straight) Part No. N9306649

SAMPLE PRETREATMENT

Pipet 200 µL sample into a test tubes

Add internal standard* and 100 µL 100mM phosphate buffer (pH 6.0)

Mix/vortex

* *Suggested internal standard D6-GHB*

COLUMN CONDITIONING

5 mL methanol

5 mL DI water

2 mL 100 mM phosphate buffer (pH 6.0)

SAMPLE LOADING

Load the previously prepared sample onto the SPE column and aspirate at ~1 "Hg

Sample load and wash are both collected

COLUMN WASHING

Add 2 mL methanol/sodium hydroxide (99:1) to the original sample test

Mix/vortex thoroughly

Decant wash onto column and combine eluate with sample load collected in previous step

DRYING

Evaporate to dryness at 60 °C under air or nitrogen

CLEANUP

Add 200 µL of dimethylformamide

Add 1 mL of hexane saturated with dimethylformamide

Mix by inversion for 5 minutes

Centrifuge at 3000 rpm for 5 minutes.

Transfer lower dimethylformamide layer to a clean test tube

Evaporate to dryness at < 50 °C under air or nitrogen

POST TREATMENT

Add 100 µL ethyl acetate and 100 µL BSTFA with 1% TCMS

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 µL onto a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

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

RECOMMENDED COLUMN

PerkinElmer Elite-35 MS 30 m x 0.25 mm x 0.25 µm
Part No. N9316440

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

Opiates in Urine

PerkinElmer SPE Supra-Clean C18-S 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306462

SAMPLE PRETREATMENT

Acid Hydrolysis: Combine 3 mL urine sample with ISTD and 500 mL conc. HCl, vortex, heat 30 min at 120 °C

Cool, centrifuge, decant and keep top layer. Add 1 mL of 7.4 N ammonium hydroxide, vortex. Adjust pH to 6.0 with 1-3 mL of 500 mM phosphoric acid

COLUMN CONDITIONING

- 3 mL methanol
- 3 mL DI water
- 1 mL 100 mM phosphate buffer (pH6)

SAMPLE LOADING

Load the prepared sample onto the SPE column at 1-2 mL/min

COLUMN WASHING

- 3 mL DI water
- 1 mL 100 mM acetic acid
- 1 mL of methanol

ELUTION

3 mL Methylene chloride:Isopropanol:Ammonium Hydroxide (78:20:2) into conical tube

CONCENTRATION

Evaporate to dryness < 50 °C

POST TREATMENT

Reconstitute and derivatize dried extract with 50 mL ethyl acetate, 50 mL BSTFA with 1% TWCS
Cover with nitrogen, cap, mix, heat 70 °C (20 min), cool, do not evaporate

ANALYSIS RECOMMENDATIONS

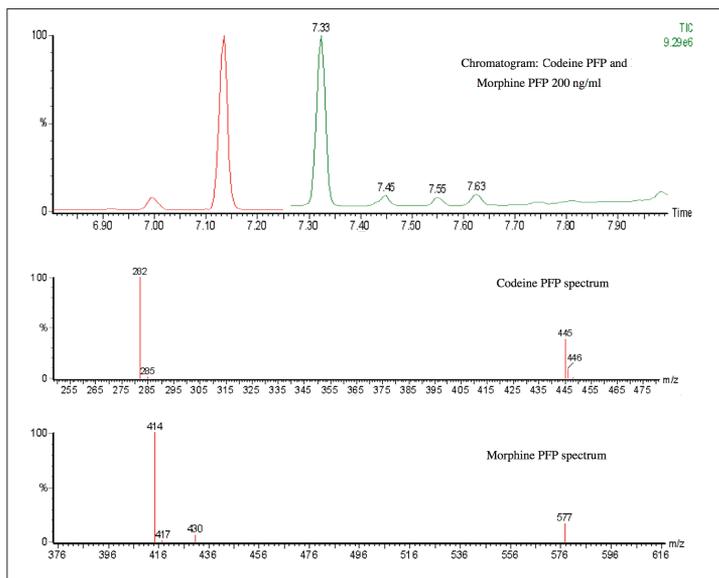
Transfer final treated sample to low volume autosampler vial insert, inject 1 µL into GC/MS

RESULTS

BSTFA SIM Ions:

Drug	Primary Ion**	Secondary	Tertiary
Codeine	371	234	343
d3-Codeine	374	237	
Morphine	429	287	324
d3-Morphine	432	290	

CHROMATOGRAM / SPECTRUM



RECOMMENDED COLUMN

PerkinElmer Elite-5 12 m x 0.20 mm x 0.33 µm Part No. N9316010
Helium carrier – 2 mL/min.

RELATED ITEMS

- Vacuum Manifold -12 Position; Part No. N9306619
- Vacuum Manifold -24 Position; Part No. N9306626
- Vacuum Pump 20 L/min 115V, Part No. N9308035
- Vacuum Pump 17 L/min 230V, Part No. N9308331

NOTES

Acetic Acid, 100 mM = 2.86 mL glacial acetic acid diluted to 500 mL DI water. Phosphate buffer, 100 mM pH6 = 1.7 g Na₂HPO₄ + 12.14 g Na₂HPO₄ dilute to 1000 mL with DI water, adjust to pH6 with 100 mM Na₂HPO₄ (raises pH) or 100 mM Na₂HPO₄ (lowers pH). Methylene chloride/Isopropanol/Ammonium Hydroxide (78:20:2) extraction solvent = 40 mL IP-OH + 4 mL concentrated NH₄OH + 156 mL MeCl₂. Make fresh daily.

REFERENCES

Opiates in Urine by SAMHSA GC/MS, Timothy D. Ruppel, PerkinElmer Application Note, Project I.D. 009816_01

Sympathomimetic Amines in Urine

PerkinElmer SPE Supra-Clean C18-S 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306462

SAMPLE PRETREATMENT

Add deuterated internal standard (ISTD) to 2 mL of urine

Add 2 mL 100 mM phosphate buffer (pH 6)

COLUMN CONDITIONING

3 mL methanol

3 mL DI water

1 mL 100 mM phosphate buffer (pH 6)

SAMPLE LOADING

Load previously prepared sample at 1 to 2 mL/min

COLUMN WASHING

3 mL DI water

1ml of 100 mM acetic acid

1 mL of methanol

ELUTION

3 mL Methylene chloride:

Isopropanol:Ammonium Hydroxide (78:20:2) into conical tube

CONCENTRATION

Evaporate to dryness < 50 °C

POST TREATMENT

Reconstitute in 50 µL derivatization reagent (PFPA, HFBA or TFAA)

Cover with N₂, cap, mix, heat 70 °C (20 min) Evaporate todryness < 50 °C.

Reconstitute in 100 µL ethyl acetate

ANALYSIS RECOMMENDATIONS

Transfer final treated sample to low volume autosampler vial insert, inject 1 µL into GC/MS

RESULTS

PFPA SIMS Ions:

Drug	Primary Ion	Secondary	Tertiary
Amphetamine	190	118	91
d5-amphetamine	194	123	
d8-amphetamine	193	126	
Methamphetamine	204	160	118
d5-methamphetamine	208	163	
d8-methamphetamine	211	163	

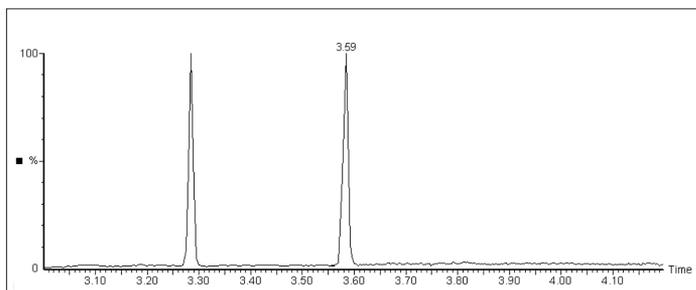
HFBA SIMS Ions:

Drug	Primary Ion	Secondary	Tertiary
Amphetamine	118	240	91
d5-amphetamine	244	123	
d8-amphetamine	243	126	
Methamphetamine	118	210	254
d5-methamphetamine	258	213	
d8-methamphetamine	261	213	

TFAA SIMS Ions:

Drug	Primary Ion	Secondary	Tertiary
Amphetamine	118	140	91
d5-amphetamine	144	123	
d8-amphetamine	143	126	
Methamphetamine	154	118	110
d5-methamphetamine	158	113	
d8-methamphetamine	161	113	

CHROMATOGRAM



RECOMMENDED COLUMN

PerkinElmer Elite-5 12 m x 0.20 mm x 0.33 µm Part No. N9316010

Helium carrier – 2 mL/min.

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

NOTES

Acetic Acid, 100 mM = 2.86 mL glacial acetic acid diluted to 500 mL DI water. Phosphate buffer, 100 mM pH6 = 1.7 g Na₂HPO₄ + 12.14 g Na₂HPO₄ dilute to 1000 mL with DI water, adjust to pH6 with 100 mM Na₂HPO₄ (raises pH) or 100 mM Na₂HPO₄ (lowers pH). Methylene chloride/Isopropanol/Ammonium Hydroxide(78:20:2) extraction solvent = 40 mL IP-OH + 4 mL concentrated NH₄OH + 156 mL MeCl₂. Make fresh daily.

REFERENCES

Sympathomimetic Amines in Urine by SAMHSA GC/MS, Timothy D. Ruppel, PerkinElmer Application Note, Project I.D. 009784_01

PHARMACEUTICAL

Acetaminophen in Serum

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

Dilute 10 mg of acetaminophen in 100 mL of water to give a 100 ppm solution

Mix 25 mL of acetaminophen (100 ppm) solution in a 50 mL flask and dilute to volume with water (standard solution 50 ppm)

Mix 25 mL of acetaminophen (100 ppm) solution in a 50 mL flask and dilute to volume with serum and 1% H_3PO_4 (sample 50% serum and 1% H_3PO_4)

COLUMN CONDITIONING

2 mL of methanol
2 mL of DI water

SAMPLE LOADING

Load 2 mL of prepared sample

COLUMN WASHING

2 mL of methanol/DI water (5/95, v/v)

DRYING

Dry column (5 to 10 minutes)

ELUTION

2 mL of methanol

CONCENTRATION

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

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Acetaminophen in Solution

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

Prepare a 5 ppm sample in 0.2% ammonium acetate

Adjust to pH 5

COLUMN CONDITIONING

2 mL of methanol

2 mL of DI water

SAMPLE LOADING

Load 4 mL samples at 1-2 mL/minute

COLUMN WASHING

1 mL of DI water

ELUTION

5 mL of methanol

CONCENTRATION

Evaporate to full dryness under a stream of nitrogen

Reconstitute the sample to 1 mL with water

ANALYSIS RECOMMENDATIONS

Mobile phase: acetonitrile:1 mM KH_2PO_4 (30:70, v/v)

Flow: 1 mL/minute

Temperature: 30 °C

Detection: UV 254 nm

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated C18, 5 μm , 250 x 4.6 mm

Part No. N9303561

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Benzodiazepine Screen in Blood, Serum, and Tissue by GC/MS

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

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

Add 1 mL blood or 1 g of (1:4) tissue homogenate

Mix/vortex

Add 3 mL of 100 mM phosphate buffer (pH= 6)

Sample pH should be 6.0 ±0.5 (Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate)

Mix/vortex and centrifuge as appropriate

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

COLUMN CONDITIONING

3 mL methanol

3 mL 100 mM phosphate buffer 9pH 6)

SAMPLE LOADING

Load previously prepared sample at 1 to 2 mL/min

COLUMN WASHING

3 mL of 5% (v/v) acetonitrile in 100 mM phosphate buffer (pH=6.0)

Dry the column for 5 minutes under Vacuum

3 mL hexane

DRYING

Dry the column for 5 minutes under vacuum

ELUTION

3 mL ethyl acetate/ammonia (98:2 v/v)

CONCENTRATION

Evaporate to dryness at < 40 °C under nitrogen

POST TREATMENT

Add 50 µl acetonitrile and 50 µl BSTFA with 1% TCMS

Heat at 70 °C for 30 minutes

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 µl to a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

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*

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 30 m x 0.25 mm x 0.25 µm
Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Benzodiazepine Screen in Urine by GC/MS

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

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

Add 1 mL Urine

Mix/vortex

Hydrolyze for 3 hours at 65 °C then cool

Centrifuge for 10 minutes at 2000 rpm and discard pellet

Add 3 mL of 100 mM phosphate buffer (pH 6.0) and mix

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

COLUMN CONDITIONING

3 mL methanol

3 mL 100 mM phosphate buffer 9pH 6)

SAMPLE LOADING

Load previously prepared sample at 1 to 2 mL/min

COLUMN WASHING

3 mL of 5% (v/v) acetonitrile in 100 mM phosphate buffer (pH=6.0)

Dry the column for 5 minutes under Vacuum

3 mL hexane

DRYING

Dry the column for 5 minutes under vacuum

ELUTION

3 mL ethyl acetate/ammonia (98:2 v/v)

CONCENTRATION

Evaporate to dryness at < 40 °C under nitrogen

POST TREATMENT

Add 50 μ l acetonitrile and 50 μ l BSTFA with 1% TCMS

Heat at 70 °C for 30 minutes

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 μ l to a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

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

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 30 m x 0.25 mm x 0.25 μ m
Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Beta Agonists in Urine

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

To 1 mL of 100 mM acetate buffer (pH 4.5) add 1 mL of urine

Add 2 mL of 100 mM Acetate buffer (pH 4.5)

Mix/vortex

Centrifuge

COLUMN CONDITIONING

3 mL of methanol

3 mL of DI water

3 mL 100 mM acetate buffer (pH 4.7)

SAMPLE LOADING

Load the previously prepared sample onto the column at 1-2 mL/min

COLUMN WASHING

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

DRYING

Dry the column for 5 minutes under vacuum

ELUTION

Elute with 1 mL dichloromethane/isopropanol/ammonium hydroxide (78:20:2) at 1-2 mL/min

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

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

Add 100 µL of the methaneboronic acid solution to eluate

Mix/vortex

React 15 minutes at 70 °C; cool

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 µL to a GC/MS

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 30 m x 0.25 mm x 0.25 µm

Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Beta Blockers in Blood and Urine

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

To 1 mL of 100 mM acetate buffer (pH 4.5) add 1 mL of blood or urine

Add 2 mL of 100 mM Acetate buffer (pH 4.5)

Mix/vortex

Centrifuge

COLUMN CONDITIONING

3 mL of methanol

3 mL of DI water

3 mL of 100 mM acetate buffer (pH 4.5)

SAMPLE LOADING

Load the previously prepared sample onto the SPE column at 1-2 mL/min

COLUMN WASHING

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

DRYING

Dry the column for 5 minutes under vacuum

ELUTION

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

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

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

Add 100 µL of the methaneboronic acid solution to eluate

Mix/vortex

React 15 minutes at 70 °C; cool

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 µL to a GC/MS

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 30 m x 0.25 mm x 0.25 µm

Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

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

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

To 1 mL of 100 mM Acetic Acid add internal standard*

Add 1 ml blood, plasma/serum, or urine

Add 2 mL of 100 mM Acetic Acid

Mix/vortex

Centrifuge

* Suggested internal standard 8-Chlorotheophylline

COLUMN CONDITIONING

3 mL of methanol

3 mL of DI water

1 mL of 100 mM acetic acid

SAMPLE LOADING

Load the previously prepared sample onto the SPE column at 1-2 mL/min

COLUMN WASHING

3 mL DI water

3 mL 100 mM acetic acid

DRYING

Dry the column for 5 minutes under vacuum

ELUTION

3 mL ethyl acetate/methanol (90:10)

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

Reconstitute sample in 1000 µl of 0.1% formic acid (aq)

ANALYSIS RECOMMENDATIONS

Inject 20 µl on to an LC

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Cefaperazone in Water

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

5 ppm of cefoperazone in water

COLUMN CONDITIONING

2 mL of methanol
2 mL of DI water

SAMPLE LOADING

Load 10 mL samples at 1 to 2 mL/minute

COLUMN WASHING

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

ELUTION

6 mL of methanol

ANALYSIS RECOMMENDATIONS

Mobile phase: 0.005 M tetrabutyl ammonium phosphate (pH 3.63)/ACN (70:30, v/v)
Flow: 1 mL/minute
Temperature: 30 °C
Detection: UV 254 nm

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated Aqueous C18 250 mm x 4.6 mm x 5 um Part No. N9303549

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Clonazepam and 7-Aminoclonazepam in Urine

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

β -Glucuronidase hydrolysis: To 2 mL of urine add internal standard and 1 mL β -Glucuronidase solution*

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

Mix/vortex

Hydrolyze for 3 hours at 65 °C; cool

* *Suggested internal standard Clonazepam-D4, 7-aminoclonazepam-D4*

COLUMN CONDITIONING

3 mL methanol

3 mL water

Continue to condition with 1 ml 100 mM phosphate buffer (pH 6.0)

SAMPLE LOADING

Load the previously prepared sample onto the SPE column

COLUMN WASHING

2 mL DI water

Wash the column with 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6.0)

Dry the column for 5 minutes

2 mL hexane

ELUTION

Elute with 3 mL ethyl acetate with 2% ammonium hydroxide at 1-2 mL/min flow

CONCENTRATION

Evaporate to dryness at <40 °C under nitrogen

POST TREATMENT

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

Mix/vortex

React 20 minutes 90°C; cool

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 μ L to a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

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

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 30 m x 0.25 mm x 0.25 μ m
Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

DHEA, Testosterone, and Epitestosterone in Urine

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

To 5 mL of urine, add internal standard* and adjust sample pH to 5.5-6.5 using concentrated sodium phosphate monobasic or dibasic

Mix

Centrifuge samples at 3,000 rpm for 5 minutes

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

COLUMN CONDITIONING

3 mL methanol

3 mL DI water

1 mL 100 mM phosphate buffer (pH 6.0)

SAMPLE LOADING

Load the previously prepared sample onto the SPE column and allow to flow via gravity

COLUMN WASHING

3 mL DI water

DRYING

Dry the column for 10 minutes under vacuum

ELUTION

3 mL methanol at 1-2 mL/min flow rate

CLEANUP

Dry eluate under a stream of nitrogen.

Add 2 mL of 200 mM phosphate buffer (pH 7.0) and 250 units of β -glucuronidase

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

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

CONCENTRATION

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

POST TREATMENT

Add 50 μ L of MSTFA/NH₄/dithioerythritol (1,000:2:5, V/W/W) and incubate at 70 °C for 20 minutes

Centrifuge at 3,000 rpm for 1 minutes and transfer to GC vial

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 μ L to a GC/MS

RESULTS

Ions to monitor (Mass Spectrometry)

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

RECOMMENDED COLUMN

PerkinElmer Elite-5 MS 30m x 0.25mm x 0.25 μ m Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Doxepin in Rat Serum

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

Mix 10 mL of doxepin aqueous solution (20mg/L) and 30 mL of rat serum into a 100 mL vessel

Dilute to 100 mL with 0.5% ammonia solution to give a 2 ppm solution

COLUMN CONDITIONING

2 mL of methanol
2 mL of DI water

SAMPLE LOADING

Load 2mL samples

COLUMN WASHING

2 mL of 0.5% ammonia solution containing 5% methanol

ELUTION

2 mL of 1% acetic acid in methanol

CONCENTRATION

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

Reconstitute the sample to 1 mL with ACN:20 mM sodium acetate (pH 4) (40:60, v/v)

ANALYSIS RECOMMENDATIONS

Mobile phase: ACN:20 mM sodium acetate (pH 4) (40:60, v/v)

Flow: 1.0 mL/minute

Injection: 10 μ L

Temperature: 30 °C

Detection: 290 nm

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated C18 250 mm x 4.6 mm x 5 μ m
Part No. N9303561

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Gabapentin in Blood, Plasma/Serum

PerkinElmer SPE Supra-Clean C18-S 100 mg/1 mL, Plastic Tube (Straight) Part No. N9306478

SAMPLE PRETREATMENT

To 500 μ L of 20% acetic acid add internal standard*

Mix thoroughly

Add 500 μ L of blood, plasma/serum

Mix thoroughly

Centrifuge as appropriate

COLUMN CONDITIONING

3 mL of methanol

3 mL of DI water

1 mL 100 mM HCl

SAMPLE LOADING

Load at 1 to 2 mL/minute

COLUMN WASHING

3 mL DI H₂O

3 mL ethyl acetate

3 mL hexane

DRYING

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

ELUTION

1 mL 2% ammonium hydroxide in methanol

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

Derivatize by adding 50 μ L of ethyl acetate and 50 μ L of BSTFA (1%

TCMS) and 50 μ L ethyl acetate

Cap and heat at 70 °C for 30 minutes

Remove and allow to cool

ANALYSIS RECOMMENDATIONS

Inject 1 to 2 μ L onto GC/MS

RECOMMENDED COLUMN

PerkinElmer Elite-5MS 30 m x 0.25 mm x 0.25 μ m

Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

NOTES

* Internal standard: 1-aminomethyl-1-cycloheptyl acetic acid (FID):Gabapentin-D10 (GC/MS). BSTFA + TCMA = N,O-bis[Trimethylsilyl]trifluoroacetamide plus Trimethylchlorosilane

PHARMACEUTICAL

Gabapentin in Blood, Plasma/Serum

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

To 0.2 to 0.5 mL of sample add 1 mL of acetone (dropwise) while vortexing

Add internal standards*

Mix/Vortex and centrifuge

Transfer organic phase to clean test tube and evaporate to dryness

Add 3 mL of 100 mM HCL

Mix/Vortex and centrifuge

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

COLUMN CONDITIONING

3 mL methanol

3 mL water

1 mL 100mM HCl

SAMPLE LOADING

Load the previously prepared sample onto the SPE column at 1-2 mL/min

COLUMN WASHING

3 mL DI water

3 mL ethyl acetate

3 mL hexane

DRYING

Dry the column for 10 minutes under vacuum

ELUTION

3 mL methanol containing 2% ammonium hydroxide at 1-2 mL/min flow rate

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

Dissolve residue in 100 µL methanol

ANALYSIS RECOMMENDATIONS

Inject 5 µL on to an LC/MS

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated C18 100 mm x 2.1 mm x 3 µm Part No. N9303551

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Gabapentin in Blood, Plasma/Serum

PerkinElmer SPE Supra-Clean C18-S 100 mg/1 mL, Plastic Tube (Straight) Part No. N9306478

SAMPLE PRETREATMENT

Add internal standard to 500 mL of 20% Acetic acid and mix thoroughly

Add 500 μ L of blood, plasma/serum and mix thoroughly and centrifuge if necessary

COLUMN CONDITIONING

3 mL methanol
3 mL DI water
1 mL 100 mM HCl

SAMPLE LOADING

Load at 1 to 2 mL/minute

COLUMN WASHING

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

ELUTION

1 mL 2% ammonium hydroxide in methanol

CONCENTRATION

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

POST TREATMENT

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

ANALYSIS RECOMMENDATIONS

Transfer final treated sample to low volume autosampler vial insert, inject 1 -2 μ L into GC/MS

RECOMMENDED COLUMN

PerkinElmer Elite-5MS 30 m x 0.25 mm x 0.25 μ m
Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619
Vacuum Manifold -24 Position; Part No. N9306626
Vacuum Pump 20 L/min 115V, Part No. N9308035
Vacuum Pump 17 L/min 230V, Part No. N9308331

NOTES

Internal standard: 1-aminomethyl-1-cycloheptyl acetic acid (FID): Gabapentin-D10 (GC/MS). BSTFA + TCMA = N,O-bis[Trimethylsilyl] trifluoroacetamide plus Trimethylchlorosilane.

PHARMACEUTICAL

Ketotifen Fumarate in Solution

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

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

COLUMN CONDITIONING

2 mL of methanol
2 mL of DI water

SAMPLE LOADING

Load 2mL sample

COLUMN WASHING

2mL of DI water

ELUTION

2mL of methanol

POST TREATMENT

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

Reconstitute sample in methanol for analysis

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Lovastatin in Water

PerkinElmer SPE Supra-Clean C18-S 100 mg/1 mL, Plastic Tube (Straight) Part No. N9306478

SAMPLE PRETREATMENT

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

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

COLUMN CONDITIONING

2 mL of methanol

2 mL of DI water

SAMPLE LOADING

Load 10 mL samples at 1 to 2 mL/minute

COLUMN WASHING

1 mL of DI water

ELUTION

4 mL of acetonitrile

CONCENTRATION

Evaporate to full dryness under a stream of nitrogen

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

ANALYSIS RECOMMENDATIONS

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

Flow: 1 mL/minute

Temperature: 30 °C

Detection: UV 230 nm

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated Aqueous C18

150 mm x 4.6 mm x 5 µm Part No. N9303546

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Methylmalonic Acid from Serum/Plasma

PerkinElmer SPE Supra-Clean C18-S 100 mg/1 mL, Plastic Tube (Straight) Part No. N9306478

SAMPLE PRETREATMENT

Add 100 μ L of internal standard D3-MMA and 1 mL of acetonitrile to 1 mL of sample

Mix thoroughly

Centrifuge for 5 minutes at 2,000 rpm

COLUMN CONDITIONING

3 mL methanol

3 mL DI water

SAMPLE LOADING

Decant supernatant onto SAX column

COLUMN WASHING

10 mL of DI water

5 mL of methanol

2 mL of MTBE

DRYING

Dry with Vacuum for 3 minutes after each wash step

ELUTION

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

CONCENTRATION

Dry under a stream of nitrogen at < 35 $^{\circ}$ C

POST TREATMENT

Derivatize by Reconstituting with 25 μ L of MSTFA + 1% TMCS and 25 μ L ethyl acetate

Heat for 20 minutes at 60 $^{\circ}$ C

ANALYSIS RECOMMENDATIONS

Transfer final treated sample to low volume autosampler vial insert, inject 1 -2 μ L into GC/MS

RECOMMENDED COLUMN

PerkinElmer Elite-5MS 30 m x 0.25 mm x 0.2 5 μ m

Part No. N9316282

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

NOTES

MTBE - Methyl tertiary-butyl ether

PHARMACEUTICAL

Olanzapine in Whole Blood

PerkinElmer SPE Supra-Clean Cyano 500 mg/3 mL, Plastic Tube (Straight) Part No. N9306645

SAMPLE PRETREATMENT

Add internal standard* to 1 ml of DI water

Add 1 ml blood

Add 8 ml of DI water

Mix/vortex and centrifuge

**Suggested internal standard Prazepam*

COLUMN CONDITIONING

5 mL of methanol

5 mL of DI water

SAMPLE LOADING

Load at 1 to 2 mL/minute

COLUMN WASHING

5 mL of 1% acetic acid in water

DRYING

Dry the column for 5 minutes

ELUTION

5 mL 1% acetic acid in methanol at 1 to 2 mL/min

Repeat the previous elution step

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

Reconstitute sample in 100 µL 0.1% aqueous trifluoroacetic acid

ANALYSIS RECOMMENDATIONS

Inject 50 µL into HPLC

RECOMMENDED COLUMN

PerkinElmer Brownlee Choice C18 150 mm x 4.6 mm x 3 µm
Part No. N9303624

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Paroxetine in Blood, Plasma/Serum and Urine

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

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

Add 1 mL whole blood, serum/plasma or urine

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

Mix/vortex and centrifuge as appropriate

* *Suggested internal standard: Paroxetine-D6*

COLUMN CONDITIONING

3 mL methanol

3 mL water

3 mL 100 mM phosphate buffer (pH 6)

SAMPLE LOADING

Load the previously prepared sample onto the SPE column

COLUMN WASHING

3 mL DI water

3 mL 100 mM acetic acid

3 mL methanol

ELUTION

3 mL ethyl acetate:acetonitrile:ammonium hydroxide (78:20:2) at a flow rate of 1-2 mL/min

CONCENTRATION

Evaporate to dryness at <40 °C under nitrogen

POST TREATMENT

Dissolve residue in 100 µL methanol

ANALYSIS RECOMMENDATIONS

Inject 5 µL onto LC/MS

RESULTS

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

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated C 8 100 mm x 4.6 mm x 5 µm
Part No. N9303565

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Propranolol in Rat Serum

PerkinElmer SPE Supra-Poly XC 60 mg/3 mL, Plastic Tube (Straight) Part No. N9306502

SAMPLE PRETREATMENT

Mix 10 mL of Propranolol aqueous solution (100 mg/L) and 30mL of rat serum in to a 100 mL vessel

Dilute to 100 mL with 0.5% ammonia solution to give a 10 ppm solution

COLUMN CONDITIONING

2 mL of methanol

2 mL of DI water

SAMPLE LOADING

Load 2mL samples

COLUMN WASHING

2 mL of 0.5% ammonia solution containing 5% methanol

ELUTION

2 mL of 1% acetic acid in methanol

CONCENTRATION

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

Reconstitute the sample to 1 mL with ACN:20 mM sodium acetate (pH 4) (30:70, v/v)

ANALYSIS RECOMMENDATIONS

Mobile phase: ACN:20 mM sodium acetate (pH 4) (40:60, v/v)

Flow: 1.0 mL/minute

Injection: 10 µL

Temperature: 30 °C

Detection: UV 290 nm

RECOMMENDED COLUMN

PerkinElmer Brownlee Validated Aqueous C18

250 mm x 4.6 mm x 5 µm Part No. N9303549

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

PHARMACEUTICAL

Tacrolimus, Cyclosporin and Rapamycin in Whole Blood

PerkinElmer SPE Supra-Poly XC 200 mg/6 mL, Plastic Tube (Straight) Part No. N9306635

SAMPLE PRETREATMENT

Add 50 mL whole blood and 50 mL of 0.1 M ZnSO₄ to a centrifuge tube

Thoroughly mix

Add 500 mL methanol and internal standards*

Thoroughly mix

Centrifuge for 2 min at 2000 rpm

Transfer supernate to a clean tube, add 500 mL DI H₂O

Thoroughly mix

COLUMN CONDITIONING

2 mL of methanol

2 mL of DI water

SAMPLE LOADING

Decant the sample onto the column

Load at 1 to 2 mL/minute

COLUMN WASHING

2 mL DI water

DRYING

Dry column (20 minutes at >10" Hg)

ELUTION

Add 750 mL of ethyl acetate

Collect eluate at 1 to 2 mL/minute

RECOMMENDED COLUMN

PerkinElmer Brownlee Analytical C18 150 mm x 4.6 mm x 3 um
Part No. N9303508

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

NOTES

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

PHARMACEUTICAL

Tricyclic Antidepressants in Plasma/Serum

PerkinElmer SPE Supra-Clean Mixed-Mode MM1 200 mg/3 mL, Plastic Tube (Straight) Part No. N9306543

SAMPLE PRETREATMENT

To 2 mL of 100 mM phosphate buffer (pH= 6.0) add internal standard*

Add 1 mL of plasma/serum

Mix/vortex

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

Adjust pH to 6.0±0.5 with 100 mM monobasic or dibasic sodium phosphate

* Suggested internal standards: Trimipramine, Protriptyline

COLUMN CONDITIONING

3 mL methanol

3 mL water

3 mL 100 mM phosphate buffer (pH=6)

SAMPLE LOADING

Load at 1 mL/min flow rate

COLUMN WASHING

3 mL DI water

1 mL of 100 mM acetic acid

3 mL methanol

DRYING

Dry the column for 5 minutes under vacuum

ELUTION

3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2) at 1 mL/min or gravity flow

CONCENTRATION

Evaporate to dryness at < 40 °C

POST TREATMENT

Reconstitute with 200 µL ethyl acetate/DI H₂O (1:3)

Mix/vortex

ANALYSIS RECOMMENDATIONS

Inject 100 µL onto HPLC

RELATED ITEMS

Vacuum Manifold -12 Position; Part No. N9306619

Vacuum Manifold -24 Position; Part No. N9306626

Vacuum Pump 20 L/min 115V, Part No. N9308035

Vacuum Pump 17 L/min 230V, Part No. N9308331

ORDERING INFORMATION

Supra-Clean Columns and 96 Well Plates

PerkinElmer offers a comprehensive selection of SPE products that have been precisely manufactured for guaranteed reproducibility. Not just column to column. Not just batch to batch. But lot to lot — no matter how many days, weeks or years between production times. Supra-Clean and Supra-Poly SPE products are available in a range of media weights, volumes and bundles, including easy to order selection kits and application packs.

SUPRA-CLEAN COLUMNS AND 96 WELL PLATES

- Pure spherical silica
- Pore size 60 - 120 Å
- 20 chemistries with pH range 2-8

Supra-Clean C18

Supra-Clean C18 Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306476	50
100 mg	1 ml	N9306478	100
100 mg	3 ml	N9306523	50
200 mg	3 ml	N9306462	50
500 mg	3 ml	N9306438	50
500 mg	3 ml*	N9306642	50
200 mg	6 ml	N9306634	30
500 mg	6 ml	N9306448	30
500 mg	6 ml*	N9306640	30
1 g	6 ml	N9306422	30
2 g	6 ml	N9306430	30
2 g	15 ml	N9306479	20
2 g	25 ml	N9306475	20

* Not end-capped

Supra-Clean C18 96 Well Plates

Media Weight	Volume	Part No.	Quantity
25 mg	2 ml	N9306566	1
50 mg	2 ml	N9306567	1
100 mg	2 ml	N9306568	1

Supra-Clean C18 Cartridges

Media Weight	Volume	Part No.	Quantity
390 mg	--	N9306587	50
910 mg	--	N9306588	50
1690 mg	--	N9306589	50

Supra-Clean REC18

Supra-Clean REC18 Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306519	50
100 mg	1 ml	N9306520	100
100 mg	3 ml	N9306455	50
200 mg	3 ml	N9306521	50
500 mg	3 ml	N9306522	50
200 mg	6 ml	N9306633	30
500 mg	6 ml	N9306457	30
1 g	6 ml	N9306491	30

Supra-Clean REC18 96 Well Plates

Media Weight	Volume	Part No.	Quantity
25 mg	2 ml	N9306563	1
50 mg	2 ml	N9306564	1
100 mg	2 ml	N9306565	1

Supra-Clean 300Å C4

Supra-Clean 300Å C4 Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306590	50
100 mg	1 ml	N9306591	100
100 mg	3 ml	N9306592	50
200 mg	3 ml	N9306593	50

Supra-Clean Strong Anion Exchange (SAX)

Supra-Clean Strong Anion Exchange (SAX) Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306553	50
100 mg	1 ml	N9306471	100
100 mg	3 ml	N9306554	50
200 mg	3 ml	N9306482	50
500 mg	3 ml	N9306555	50
500 mg	6 ml	N9306556	30

ORDERING INFORMATION

Supra-Clean Columns and 96 Well Plates

Supra-Clean Strong Anion Exchange (SAX) 96 Well Plates

Media Weight	Volume	Part No.	Quantity
25 mg	2 ml	N9306581	1
50 mg	2 ml	N9306582	1
100 mg	2 ml	N9306583	1

Supra-Clean Strong Cation Exchange (SCX)

Supra-Clean Strong Cation Exchange (SCX) Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306536	50
100 mg	1 ml	N9306432	100
100 mg	3 ml	N9306537	50
200 mg	3 ml	N9306538	50
500 mg	3 ml	N9306539	50
500 mg	6 ml	N9306540	30

Supra-Clean Strong Cation Exchange (SCX) 96 Well Plates

Media Weight	Volume	Part No.	Quantity
25 mg	2 ml	N9306575	1
50 mg	2 ml	N9306576	1
100 mg	2 ml	N9306577	1

Supra-Clean Weak Cation Exchange (WCX)

Supra-Clean Weak Cation Exchange (WCX) Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306544	50
100 mg	1 ml	N9306545	100
100 mg	3 ml	N9306546	50
200 mg	3 ml	N9306547	50
500 mg	3 ml	N9306420	50
500 mg	6 ml	N9306407	30

Supra-Clean Mixed-Mode (MM1)

Supra-Clean Mixed-Mode (MM1) Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306541	50
100 mg	1 ml	N9306542	100
100 mg	3 ml	N9306419	50
200 mg	3 ml	N9306543	50
500 mg	3 ml	N9306481	50
500 mg	6 ml	N9306416	30
200 mg	15 ml	N9306713	20

Supra-Clean Mixed-Mode (MM1) 96 Well Plates

Media Weight	Volume	Part No.	Quantity
25 mg	2 ml	N9306578	1
50 mg	2 ml	N9306579	1
100 mg	2 ml	N9306580	1

Supra-Clean Mixed-Mode (MM2)

Supra-Clean Mixed-Mode (MM2) Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306548	50
100 mg	1 ml	N9306549	100
100 mg	3 ml	N9306550	50
200 mg	3 ml	N9306551	50
500 mg	3 ml	N9306411	50
500 mg	6 ml	N9306552	30

Supra-Clean Mixed-Mode (MM3)

Supra-Clean Mixed-Mode (MM3) Columns

Media Weight	Volume	Part No.	Quantity
500 mg	6 ml	N9306649	30

Supra-Clean Florisil (FL-S)

Supra-Clean Florisil (FL-S) Columns

Media Weight	Volume	Part No.	Quantity
200 mg	3 ml	N9306511	50
500 mg	3 ml	N9306512	50
500 mg	6 ml	N9306494	30
1 g	6 ml	N9306413	30
2 g	6 ml	N9306513	20
2 g	15 ml	N9306514	20
2 g	25 ml	N9306515	20

* Granular shape

Supra-Clean Florisil (FL-S) Pesticide Grade

Supra-Clean Florisil (FL-S) Pesticide Grade Columns

Media Weight	Volume	Part No.	Quantity
200 mg	3 ml	N9306516	50
500 mg	3 ml	N9306400	50
500 mg	6 ml	N9306517	30
1 g	6 ml	N9306436	30
2 g	6 ml	N9306470	30
2 g	15 ml	N9306443	20
2 g	25 ml	N9306447	20

* Granular shape

ORDERING INFORMATION

Supra-Clean Columns and 96 Well Plates

Supra-Clean Silica (SI-S)

Supra-Clean Silica (SI-S) Columns

Media Weight	Volume	Part No.	Quantity
100 mg	3 ml	N9306532	50
200 mg	3 ml	N9306444	50
500 mg	3 ml	N9306402	50
500 mg	6 ml	N9306466	30
1 g	6 ml	N9306404	30
2 g	6 ml	N9306533	20
2 g	15 ml	N9306534	20
2 g	25 ml	N9306535	20

Supra-Clean Silica (SI-S) Cartridges

Media Weight	Volume	Part No.	Quantity
300 mg	--	N9306584	50
700 mg	--	N9306585	50
1300 mg	--	N9306586	50

Supra-Clean Cyano (CN-S)

Supra-Clean Cyano (CN-S) Columns

Media Weight	Volume	Part No.	Quantity
500 mg	3 ml	N9306645	50
500 mg	6 ml	N9306644	30

Supra-Clean Amino (NH2-S)

Supra-Clean Amino (NH2-S) Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306528	50
100 mg	1 ml	N9306410	100
100 mg	3 ml	N9306529	50
500 mg	3 ml	N9306414	50
200 mg	6 ml	N9306530	50
500 mg	6 ml	N9306531	30

Supra-Clean Amino (NH2-S) 96 Well Plates

Media Weight	Volume	Part No.	Quantity
25 mg	2 ml	N9306572	1
50 mg	2 ml	N9306573	1
100 mg	2 ml	N9306574	1

Supra-Clean Polyamine (P6)

Supra-Clean Polyamine (P6) Columns

Media Weight	Volume	Part No.	Quantity
500 mg	3 ml	N9306518	50
500 mg	6 ml	N9306434	30

Supra-Clean Phenyl (PH-S)

Supra-Clean Phenyl (PH-S) Columns

Media Weight	Volume	Part No.	Quantity
50 mg	1 ml	N9306401	50
100 mg	1 ml	N9306524	100
100 mg	3 ml	N9306525	50
200 mg	3 ml	N9306490	50
500 mg	3 ml	N9306421	50
500 mg	6 ml	N9306526	30
1 g	6 ml	N9306527	30

Supra-Clean Phenyl (PH-S) 96 Well Plates

Media Weight	Volume	Part No.	Quantity
25 mg	2 ml	N9306569	1
50 mg	2 ml	N9306570	1
100 mg	2 ml	N9306571	1

Supra-Clean LCC

Supra-Clean LCC Columns

Media Weight	Volume	Part No.	Quantity
500 mg	3 ml	N9306643	50
500 mg	6 ml	N9306641	30

ORDERING INFORMATION

Supra-Poly HLB Columns and 96 Well Plates

SUPRA-POLY HLB COLUMNS AND 96 WELL PLATES

- Contains macro-porous polymers with ultra-pure, pharmaceutical grade spherical particles
- Shorter analysis times, greater load capacity and reduced solvent usage
- Ideal for high throughput assays

Supra-Poly HLB

Supra-Poly HLB 30 µm Columns

Media Weight	Volume	Part No.	Quantity
30 mg	1 ml	N9306650	50
50 mg	1 ml	N9306655	50
60 mg	1 ml	N9306656	50
100 mg	1 ml	N9306657	50
30 mg	3 ml	N9306651	50
60 mg	3 ml	N9306658	50
100 mg	3 ml	N9306659	50
200 mg	3 ml	N9306660	50
500 mg	3 ml	N9306661	30
100 mg	6 ml*	N9306672	30
150 mg	6 ml	N9306662	30
200 mg	6 ml	N9306663	30
200 mg	6 ml*	N9306673	30
500 mg	6 ml*	N9306674	30
500 mg	6 ml	N9306664	30
500 mg	15 ml	N9306665	20
1 g	15 ml	N9306666	20
30 mg	15 ml**	N9306668	50
60 mg	15 ml**	N9306669	50
100 mg	15 ml**	N9306670	50
200 mg	15 ml**	N9306671	50
1 g	25 ml	N9306667	20

Supra-Poly HLB 60 µm Columns

Media Weight	Volume	Part No.	Quantity
30 mg	1 ml	N9306652	50
50 mg	1 ml	N9306675	50
60 mg	1 ml	N9306676	50
100 mg	1 ml	N9306677	50
30 mg	3 ml	N9306653	50
60 mg	3 ml	N9306678	50
100 mg	3 ml	N9306679	50
200 mg	3 ml	N9306680	50
500 mg	3 ml	N9306681	30
100 mg	6 ml*	N9306692	30
150 mg	6 ml	N9306682	30
200 mg	6 ml	N9306683	30

Media Weight	Volume	Part No.	Quantity
200 mg	6 ml*	N9306693	30
500 mg	6 ml	N9306684	30
500 mg	15 ml	N9306685	20
500 mg	6 ml*	N9306694	30
1 g	15 ml	N9306686	20
30 mg	15 ml**	N9306688	50
60 mg	15 ml**	N9306689	50
100 mg	15 ml**	N9306690	50
200 mg	15 ml**	N9306691	50
1 g	25 ml	N9306687	20

* Glass columns ** LRC columns

Supra-Poly HLB 30 µm 96 Well Plates

Media Weight	Volume	Part No.	Quantity
30 mg	2 ml	N9306698	1
50 mg	2 ml	N9306699	1
60 mg	2 ml	N9306700	1

Supra-Poly HLB 60 µm 96 Well Plates

Media Weight	Volume	Part No.	Quantity
30 mg	2 ml	N9306695	1
50 mg	2 ml	N9306696	1
60 mg	2 ml	N9306697	1

ORDERING INFORMATION

Supra-Poly Columns and 96 Well Plates

SUPRA-POLY COLUMNS AND 96 WELL PLATES

- Contains macro-porous polymers with ultra-pure, pharmaceutical grade spherical particles
- Shorter analysis times, greater load capacity and reduced solvent usage
- Ideal for high throughput assays

Supra-Poly Extreme Capacity 1500 m2/g (XC)

Supra-Poly Extreme Capacity 1500 m2/g (XC) Columns

Media Weight	Volume	Part No.	Quantity
30 mg	1 ml	N9306441	50
50 mg	1 ml	N9306500	50
60 mg	1 ml	N9306501	50
100 mg	1 ml	N9306403	50
60 mg	3 ml	N9306502	50
100 mg	3 ml	N9306440	50
200 mg	3 ml	N9306428	50
200 mg	6 ml	N9306635	30
500 mg	6 ml	N9306405	30
1 g	15 ml	N9306503	20

Supra-Poly Extreme Capacity 1500 m2/g (XC) 96 Well Plates

Media Weight	Volume	Part No.	Quantity
30 mg	2 ml	N9306557	1
50 mg	2 ml	N9306558	1
60 mg	2 ml	N9306559	1

Supra-Poly Extra Wide Particle 1200 m2/g (XWP)

Supra-Poly Extra Wide Particle 1200 m2/g (XWP) Columns

Media Weight	Volume	Part No.	Quantity
30 mg	1 ml	N9306504	50
50 mg	1 ml	N9306427	50
60 mg	1 ml	N9306505	50
100 mg	1 ml	N9306506	50
60 mg	3 ml	N9306507	50
100 mg	3 ml	N9306508	50
200 mg	3 ml	N9306509	50
500 mg	6 ml	N9306418	30
1 g	15 ml	N9306510	20

Supra-Poly Extra Wide Particle 1200 m2/g (XWP)

96 Well Plates

Media Weight	Volume	Part No.	Quantity
30 mg	2 ml	N9306560	1
50 mg	2 ml	N9306561	1
60 mg	2 ml	N9306562	1

Supra-Poly Environmental (AEV)

Supra-Poly AEV Columns

Media Weight	Volume	Part No.	Quantity
100 mg	3 ml	N9306648	50

Supra-Poly Hydrophilic (ATH)

Supra-Poly ATH Columns

Media Weight	Volume	Part No.	Quantity
100 mg	3 ml	N9306646	50
200 mg	3 ml	N9306638	50
200 mg	6 ml	N9306636	30

Supra-Poly Lipophilic (ATL)

Supra-Poly ATL Columns

Media Weight	Volume	Part No.	Quantity
100 mg	3 ml	N9306647	50
200 mg	3 ml	N9306639	50
200 mg	6 ml	N9306637	30

ORDERING INFORMATION

SPE Kits and Packs

SPE SELECTION KITS

Selection kits enable quick column selection for development of reproducible and repeatable SPE methods.

SPE Selection Kits

Description	Media Weight	Volume	Part No.	Quantity
Pre-Concentration of Hydrophobic Compounds from Aqueous Matrix	200 mg	6 ml	N9306594	50
	200 mg	3 ml	N9306595	50
Extraction of Hydrophobic Compounds from Aqueous Matrix	500 mg	6 ml	N9306596	50
	500 mg	3 ml	N9306597	50
Pre-Concentration of Hydrophilic Compounds	500 mg	6 ml	N9306598	30
	500 mg	3 ml	N9306599	30
Removal of Polar Compounds from Aqueous and Organic Matrix	500 mg	6 ml	N9306600	30
	500 mg	3 ml	N9306601	30
Extraction of Acidic Basic and Neutral Compounds from Aqueous or Organic Matrix	100 mg	3 ml	N9306602	50
Extraction of Carboxylic Acids and Strong Bases from Aqueous Matrix	500 mg	6 ml	N9306603	40
Extraction of Weak Bases from Aqueous Matrix	500 mg	6 ml	N9306604	30

SPE APPLICATION PACKS

Ideal for extraction of known entities from a variety of matrices, our packs are expertly tailored to meet your application needs and are designed to support major EPA methods and applications.

SPE Application Packs

Description	Media Weight	Volume	Part No.	Quantity
Extraction of Basic Drugs from Biological Fluids	200 mg	3 ml	N9306605	50
Extraction of Bisphenol A from Aqueous Matrix	1 g	6 ml	N9306613	30
Extraction of Oil and Grease from Aqueous Matrix-EPA 1664	500 mg	3 ml	N9306612	50
	1 g	6 ml	N9306611	30
Extraction of PAH from Soil and Oil	1.5 g	6 ml	N9306609	30
Extraction of PAH from Soil and Oil (Glass Straight Column)	1.5 g	6 ml	N9306610	30
Extraction of PAH from Water Containing Humic Acids	1.5 g	6 ml	N9306608	30
Extraction of PAH from Water or Soil	4 g	6 ml	N9306606	30
Extraction of PAH from Water or Soil (Glass Straight Column)	4 g	6 ml	N9306607	30
Extraction of PCB from Oil	1 g	6 ml	N9306617	30
	1 g	3 ml	N9306616	50
Extraction of Pesticides and Herbicides from Aqueous Matrix	500 mg	3 ml	N9306614	50
Extraction of Steroids from Biological Fluids	500 mg	6 ml	N9306615	30
Extraction of SVOC from Water-EPA 525	1 g	6 ml	N9306618	30

ORDERING INFORMATION

SPE Vacuum Pumps, Manifolds and Accessories

SPE VACUUM PUMPS, MANIFOLDS AND ACCESSORIES

PerkinElmer SPE Vacuum Manifolds allow multiple samples to be processed simultaneously by conveniently holding SPE media cartridges in place and allowing samples to pass through them. SPE Vacuum Manifolds are available to accommodate either 12 or 24 cartridges; 1, 3, 6, 15, and 25 mL cartridges can be used with the manifolds. Manifolds are equipped with a vacuum port where a standard laboratory vacuum pump can be connected. Vacuum pulls the sample through the stationary phase, metered by the stopcocks, to control the speed of the extraction process and the sample flow over the media bed in the SPE cartridge. Waste and wash solvents are discarded and analytes are collected in sample tubes below the manifold completing the extraction.

SPE Manifolds and Accessories

Description	Part No.	Quantity
Vacuum Manifold - 12 Position	N9306619	1 each
Vacuum Manifold - 24 Position	N9306626	1 each
Replacement Chamber (Glass) - 12 Position	N9306620	1 each
Replacement Chamber (Glass) - 24 Position	N9306627	1 each
Cover Gasket - 12 Position	N9306621	1 each
Cover Gasket - 24 Position	N9306628	1 each
Stopcocks - 12 Position	N9306624	12 pack
Stopcocks - 24 Position	N9306631	24 pack
Needles - Polypropylene - 12 Position	N9306622	12 pack
Needles - Polypropylene - 24 Position	N9306629	24 pack
Needles - Stainless Steel - 12 Position	N9306623	12 pack
Needles - Stainless Steel - 24 Position	N9306630	24 pack
Drying Attachment - 12 Position	N9306625	1 each
Drying Attachment - 24 Position	N9306632	1 each

Vacuum Pumps

Description	Part No.	Quantity
Vacuum Pump 20 L/min 115V	N9308065	1 each
Vacuum Pump 60 L/min 115V	N9308063	1 each
Vacuum Pump 17 L/min 230V	N9308331	1 each
Vacuum Pump 58 L/min 230V	N9308332	1 each

THE PERFECT PARTNERSHIP FROM START TO FINISH



At PerkinElmer, we understand that sample preparation is one of the most critical steps in the analytical process. Often accounting for 60% of your timetable, it has a fundamental impact on a wide range of operational parameters. Any errors within this process undermine the quality of your data at all subsequent stages of your analysis.

Solid Phase Extraction helps avoid potential errors in sample preparation, reducing re-runs and dramatically increasing productivity. As one of the most cost-effective and flexible tools within the laboratory environment, SPE also provides efficient sample concentration and purification prior to many of today's most popular analytical techniques, including HPLC, LC/MS, GC and GC/MS.

SPE columns are part of PerkinElmer's complete offering of analytical solutions—an industry-leading portfolio that encompasses instruments, accessories, consumables, supplies, training and service. It's a breadth of capabilities that enables us to offer the ease and convenience of having a single supplier for all your needs at every stage of your workflow. So you can benefit from greater responsiveness, superior reliability, and dramatic cost savings.

Couple SPE With Leading Instrumentation For Ultimate Laboratory Efficiency

Clarus® 680/580/480 GC

Clarus SQ 8 GC/MS

Flexar™ SQ 300 MS

Flexar LC and UHPLC

The right accessories, consumables, methods and application support are as integral to the success of your laboratory as your instrumentation. That's why we invest heavily in testing and validating our complete portfolio of solutions to ensure you receive accurate, repeatable results on time, every time.

Our OneSource Laboratory Services division even takes it a step further. With more than 1,500 trained and certified field service engineers and service personnel around the world, OneSource offers the most comprehensive portfolio of professional laboratory services in the industry, including complete care programs for virtually every technology and manufacturer.

So turn to PerkinElmer. For the experience. For the confidence. For the better.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com



For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

Copyright ©2012, PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.

010097_01

Printed in USA