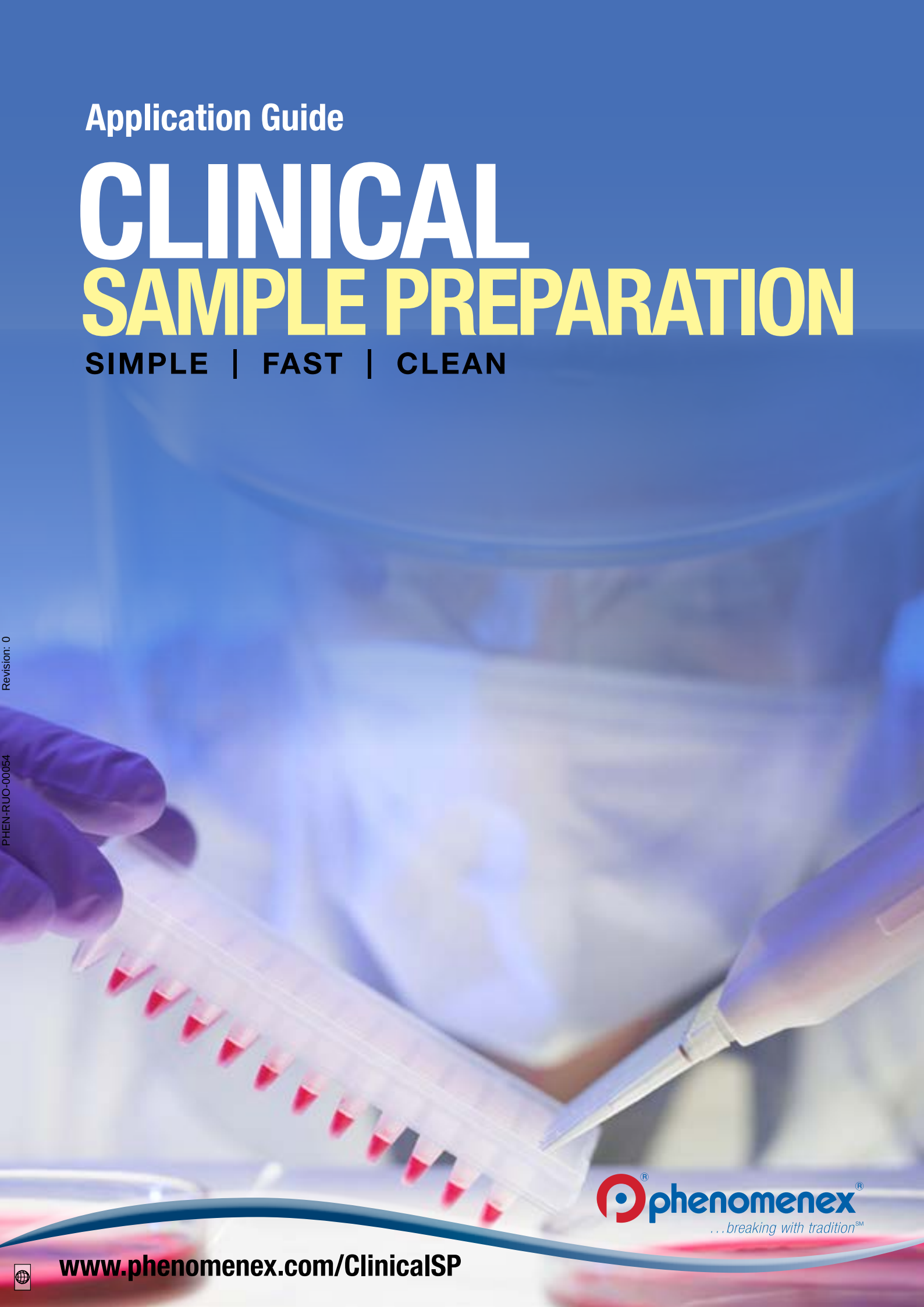
Application Guide

CLINICAL SAMPLE PREPARATION

SIMPLE | FAST | CLEAN



Revision: 0

PHEN-RUO-00054

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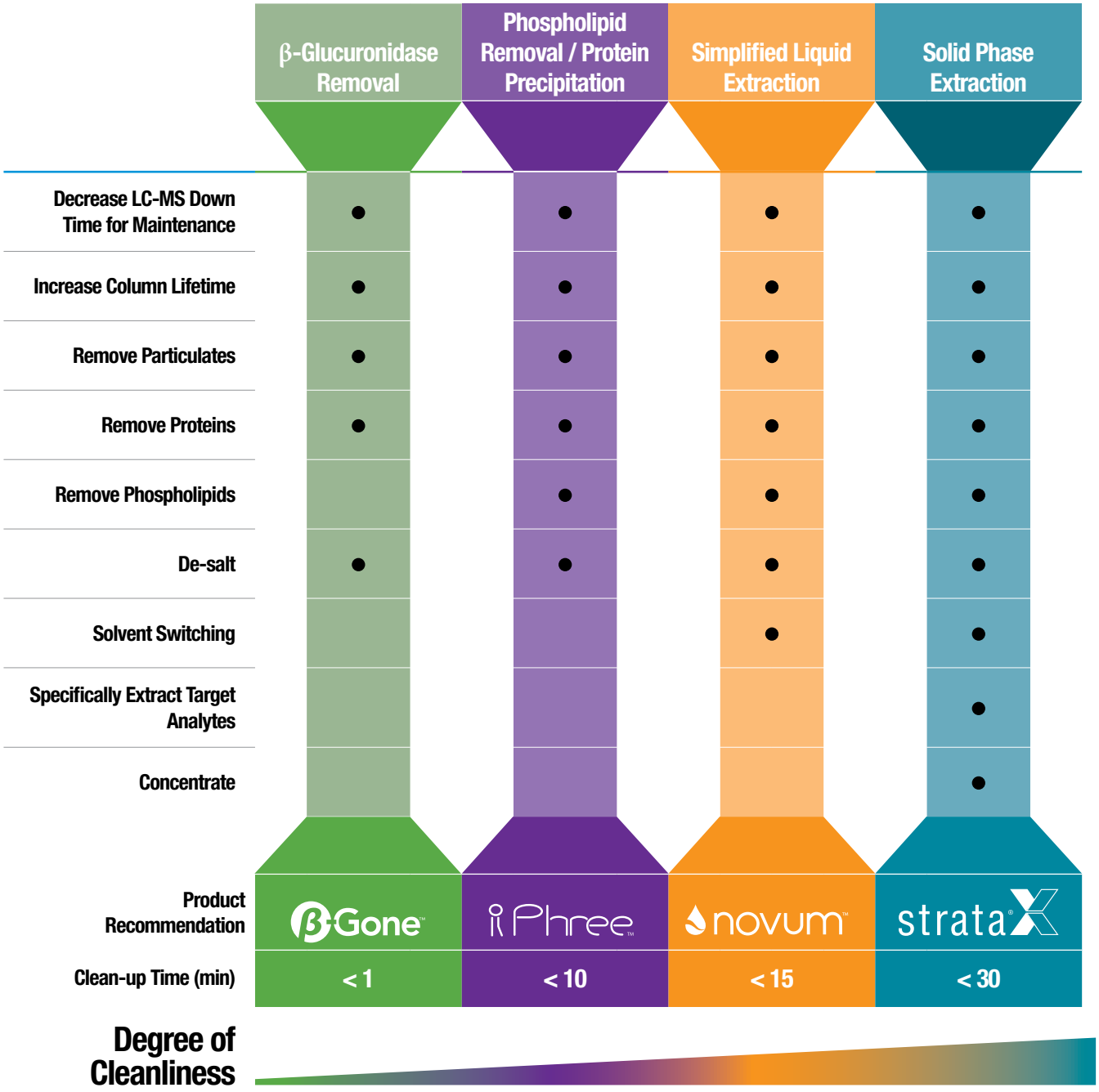
Select the Appropriate Sample Prep Technique for Your Key Requirements



guarantee

If Phenomenex products in this brochure do not provide at least equivalent separation as compared to other products of the same phase and dimensions, return the product with your comparative data within 45 days for a FULL REFUND.

Sample Pre-treatment

Due to their nature, bioanalytical samples often require a pre-treatment step prior to further cleanup.

Plasma/Serum

If the analyte is an acid, 2 % phosphoric acid can be used (20 µL 85 % Phosphoric acid to 1 mL of plasma or serum) to disrupt the drug-protein interaction. If the analyte is basic, 0.1 M sodium hydroxide can be used to disrupt the drug-protein interaction. After addition of acid or base, the sample should be vortexed for 20-30 seconds followed by centrifugation. The supernatant is now ready for further analysis.

Whole Blood

There are several pre-treatment strategies that can be followed for whole blood. If the target analyte is present in red blood cells, a hemolysis step is necessary.

Hemolysis	To 0.2 mL whole blood (spiked with analytes and internal standard (I.S.) in a 1.2 mL centrifuge tube, add 400 µL of 2 % Zinc sulfate/80 % Methanol. Vortex for 10-20 seconds followed by centrifugation at 14,000 rpm for 10 minutes. Collect the supernatant for further analysis. Preparation of zinc sulfate/methanol: Into a 100 mL volumetric flask add 20 mL water and 3.6 g Zinc sulfate · 7 H_2O. After the solution is clear and the salt crystals have dissolved, add 100 % Methanol. Refrigerate the solution at 2-8 °C for 7 days.
Osmotic Breakdown	To 1 mL of whole blood add internal standard and 4 mL of distilled water. Mix/vortex and let stand for 5 minutes. Centrifuge at 670 g for 10 minutes and discard the pellet. Adjust the pH of the supernatant accordingly with the addition of a buffer solution.
Sonication	Sonicate 1 mL whole blood for 15 minutes at room temperature. Add 3-6 mL of an appropriate pH buffer (such as potassium phosphate buffer). Mix/vortex. Let stand for 5 minutes. Centrifuge at 670 g for 15 minutes. Analyze supernatant.

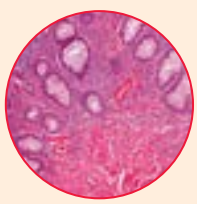
Urine

Enzymatic hydrolysis is necessary in case of conjugated forms (sulfated or glucuronide) of the analyte present and requires specific pH (~ 4-5) and temperature ranges. Depending on the compound's stability, an acid or base hydrolysis can be performed as well.

Enzmatic Hydrolysis	To 500 µL sample (spiked with analyte and I.S.) add 100 µL acidic buffer (see below) and 20 µL ß-glucuronidase. Vortex 5-6 seconds. Incubate in a water bath at 63 °C for 30 minutes. Transfer sample to a 96-well collection plate or autosampler vial. Centrifuge for 10 minutes at 2,000 rpm. Preparation of acetic buffer (1.0 M acetate buffer, pH4.0): Dissolve 3.0 g of glacial acetic acid and 4.1 g of sodium acetate in a 1L volumetric flask.
Base Hydrolysis	To 1 mL urine (spiked with analyte and I.S.) add 100 µL 10 N Potassium hydroxide. Mix, vortex, and hydrolyze for 20 minutes at 60 °C. Cool and adjust pH to 3.5- 4.0 (by adding 200 µL glacial acetic acid).
Acid Hydrolysis	To 1 mL urine add 0.25 mL Hydrogen chloride in a screw capped test tube. Screw the tube top on loosely and heat in a boiling water bath for 60 minutes. Adjust to pH 7 (or as needed) with 1.0 N Sodium hydroxide.

Saliva

No hydrolysis is required for oral fluids and the generic protocol used for plasma/serum pre-treatment may be followed.



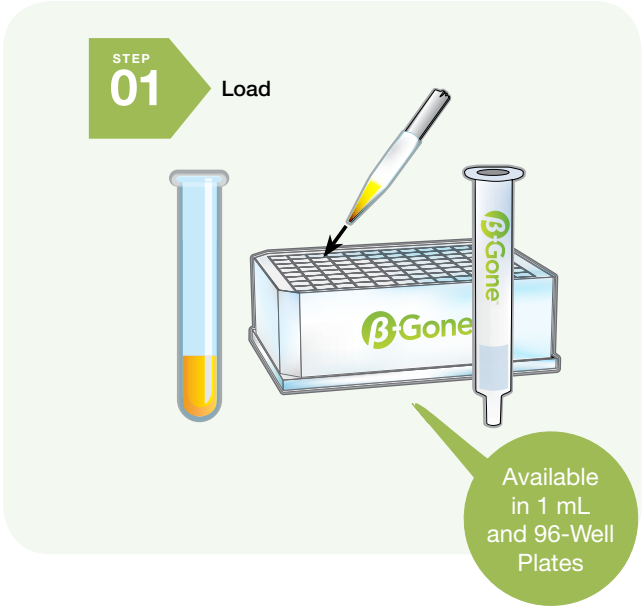
Tissue

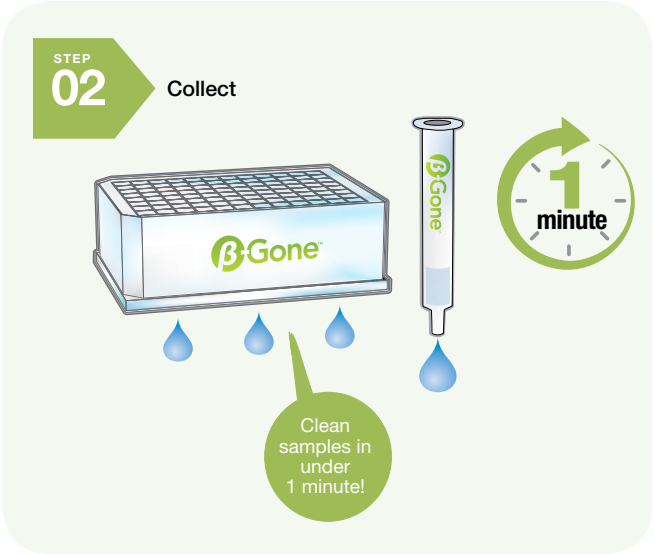
Homogenize with organic or aqueous solvent depending upon analyte solubility. Settle, decant, centrifuge or filter supernatant. Perform direct Matrix Solid Phase Dispersion (MSPD) extraction on tissue.

β-Glucuronidase Removal

β-Glucuronidase is a large enzyme that is present in urine samples after a urine hydrolysis is performed. In order to protect columns, β-Gone β-Glucuronidase Removal Products selectively remove the β-Glucuronidase enzyme and increase the sensitivity of analysis.

- Increase HPLC/UHPLC column lifetime
- Increase sensitivity of analysis
- Save time with a simple two-step method





Increase Analyte Sensitivity

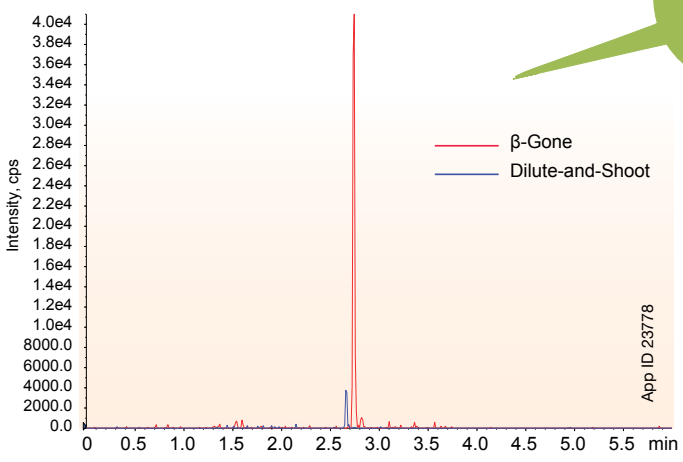
β-Gone Protocol:

1) Dilute 200 µL of Urine Hydrolysate with 133 µL of 0.1 % Formic acid in Methanol
2) Load diluted sample onto β-Gone 96-Well Plate (Part No. 8E-S322-DGA) and apply 2-5 psi using a positive pressure manifold or a vacuum manifold
3) Collect eluent and inject 10 µL for analysis

Recovery

Analyte	% Average Recovery	% CV
Benzoylecognine	109	3
Buprenorphine	93	6
Codeine	109	4
Lorazepam	79	5
Methamphetamine	106	3
Norbuprenorphine	109	5
PCP	102	3

Norbuprenorphine: β-Gone vs. Dilute-and-Shoot



LC-MS/MS Conditions

Column: Kinetex® 2.6 µm Biphenyl
Dimensions: 50 x 3.0mm
Part No.: 00B-4622-Y0
Guard: SecurityGuard™ ULTRA Biphenyl Cartridge: AJ0-9208
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Methanol

Gradient:

Time (min)	% B
0.01	10
1	10
4	100
5	100
5.01	10
6	10

Flow Rate: 0.7 mL/min
Detection: MS/MS (SCIEX, API 4000™)

Phospholipid Removal

Phospholipid Removal Solutions

Traditional protein precipitation does not remove phospholipids that are present in biological samples, such as plasma and whole blood, and phospholipids are a primary source of ion suppression in LC-MS analysis. Easily remove phospholipids and proteins in under 5 minutes using Phree Phospholipid Removal Solutions.

- Remove phospholipids
- Remove proteins
- No method development required

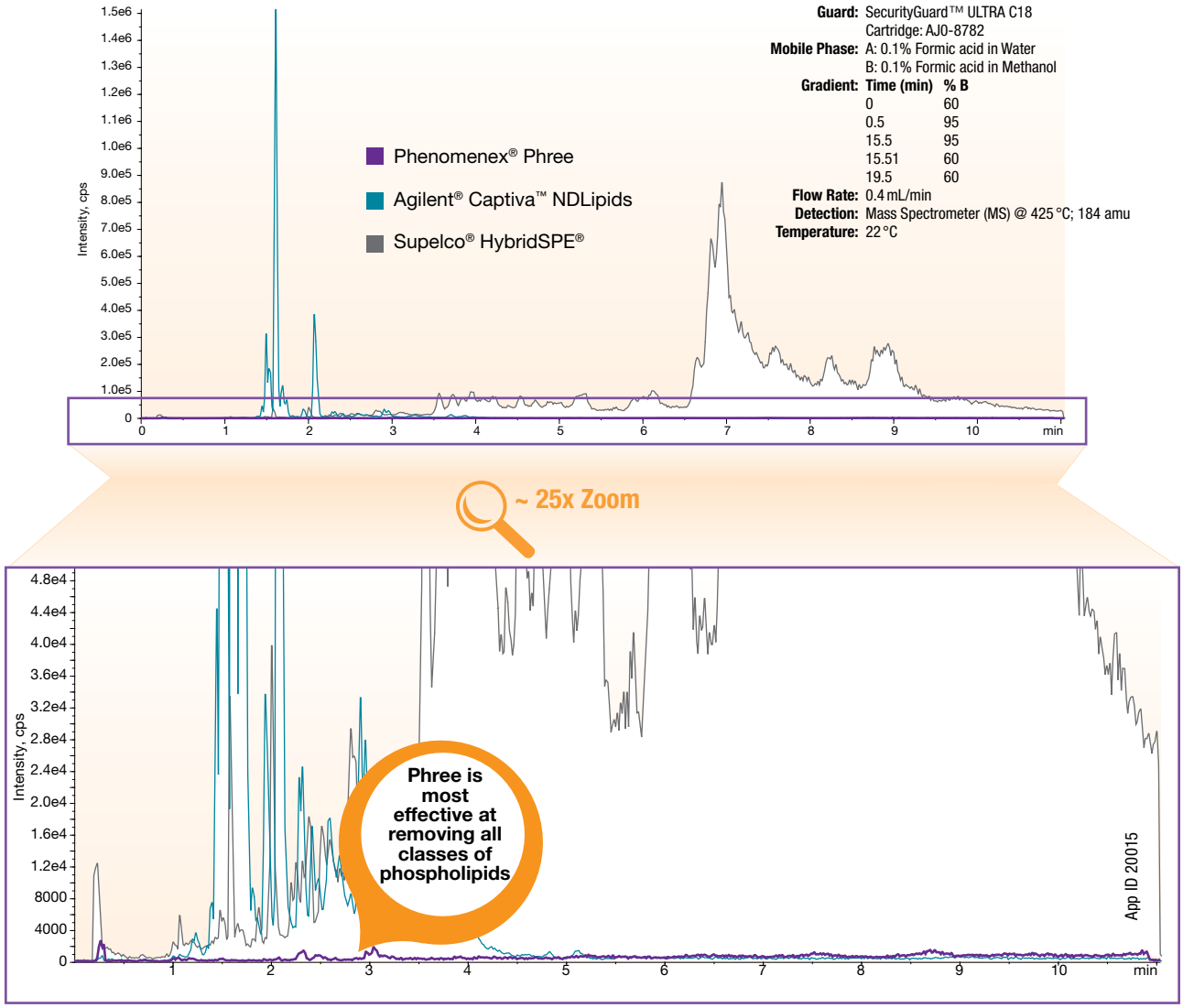
Phospholipid Profiles

HPLC Conditions

Plasma Cleanup: 100 µL plasma plus 300 µL Acetonitrile with 1 % Formic acid
Column: Kinetex® 2.6 µm C18 100 Å
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Guard: SecurityGuard™ ULTRA C18 Cartridge: AJ0-8782
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Methanol
Gradient:

Time (min)	% B
0	60
0.5	95
15.5	95
15.51	60
19.5	60

Flow Rate: 0.4 mL/min
Detection: Mass Spectrometer (MS) @ 425 °C; 184 amu
Temperature: 22 °C



Phospholipid profile monitored using *m/z 184-184*

Removal of Phospholipids from 100 µL Plasma

	Lyso 1	Lyso 2	PC 1	PC 2	PC 4
Phenomenex Phree	>99.9 %	>99.9 %	>99.9 %	>99.9 %	>99.9 %
Agilent Captiva NDLipids	28.9 %	36.4 %	>99.9 %	99.9 %	>99.9 %
Supelco HybridSPE	97.8 %	98.4 %	>96.3 %	>99.7 %	>80.9 %

Lyso 1: 1-Palmitoyl-2-OH-sn-glycero-phosphocholine, (16:0) *m/z 496-184*
Lyso 2: 1-Oleoyl-2-OH-sn-glycero-phosphocholine, (18:1) *m/z 522-184*
PC 1: 1-Palmitoyl-2-Oleoyl-sn-glycero-phosphocholine, (16:0, 18:1) *m/z 760-184*
PC 2: 1-Stearoyl-2-Lindoleoyl-sn-glycero-phosphocholine, (18:0, 18:2) *m/z 786-184*
PC 4: 1-Oleoyl-2-Linoleoyl-sn-glycero-phosphocholine, (18:1, 18:2) *m/z 784-184*

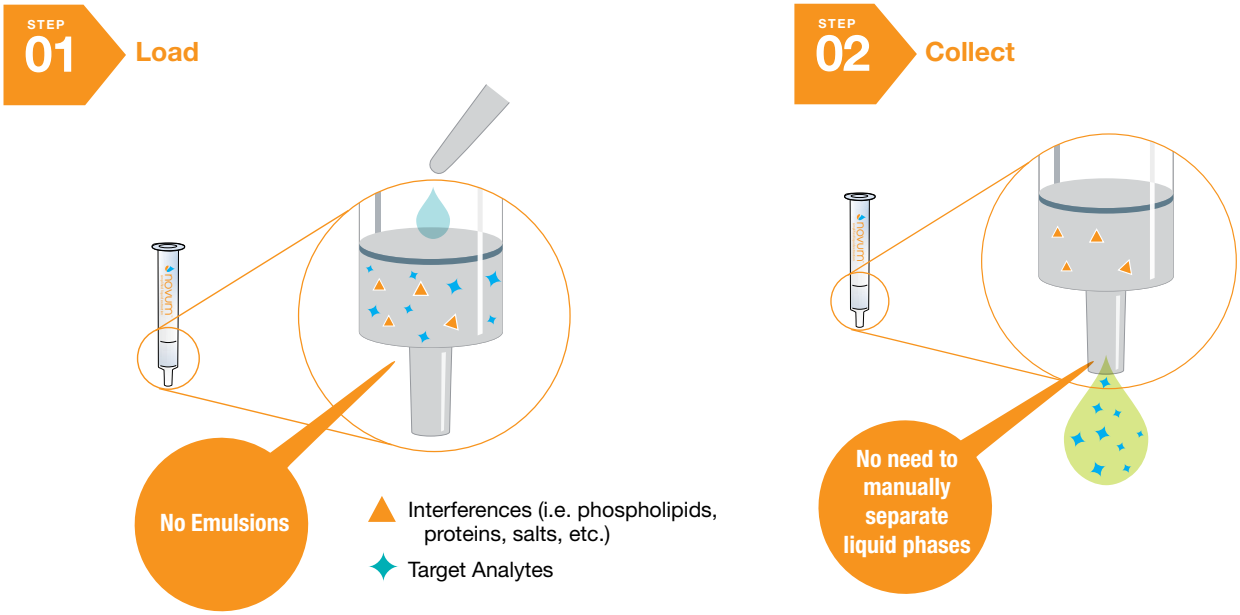
Phenomenex is not affiliated with Agilent Technologies or Sigma-Aldrich Co. Comparitive chromatograms may not be representative of all separations.

Simplified Liquid Extraction

Simplified Liquid Extraction (SLE) is a faster, easier, and more reliable way to perform liquid-liquid extractions (LLE).

- Eliminate interferences from your analysis without extensive method development
- Achieve consistent, reliable results from lot-to-lot
- Available as a high quality synthetic sorbent (Novum) or as a traditional diatomaceous earth sorbent (Strata® DE)

An Easy, Automatable Procedure



Determine Which SLE Sorbent is Right for Your Extraction

novum™ simplified liquid extraction		strata® DE Supported Liquid Extraction
Synthetic	Sorbent Type	Diatomaceous Earth
Lot-to-lot consistency and reproducibility	Advantages	Cost effective and large volume capabilities
Ethyl Acetate, MTBE	Extraction Solvents	Hexane, DCM, MTBE Ethyl Acetate
MINI 96-Well Plates, MAX 96-Well Plates	Plate Fomats	200 µL 96-Well Plates, 400 µL 96-Well Plates
1 cc, 3 cc, 6 cc, 12 cc	Tube Fomats	12 cc and 60 cc

SLE sorbent selections are dependent on extraction solvents and sample volume.

Extraction of Corticosteroids

from Plasma Using Strata DE SLE

We developed a method using Strata DE SLE for a wide range of corticosteroid compounds from plasma, which are then analyzed by LC-MS/MS. All compounds in the suite provided recovery greater than 90% with the exception of Triamcinolone. Triamcinolone is the most polar compound in the suite and is simply too hydrophilic to be extracted by DCM. Acceptable recoveries can be obtained by changing to ethyl acetate as an elution solvent. All compounds show a % CV of less than 12%.

Pre-treatment

Dilute 100 µL of spiked plasma (125 ng/mL) with 200 µL of Water

SLE Protocol

96-Well Plate:	Strata DE SLE 400 µL 96-Well Plate
Part No.:	8E-S325-5GB
Load:	Pre-treated sample onto plate (apply vacuum or positive pressure to pull/push sample into sorbent)
Wait:	5 minutes
Elution:	3x 600 µL Dichloromethane (DCM) or 3x 600 µL Ethyl Acetate
Apply:	Vacuum or apply positive pressure at 5-10" Hg for 10 seconds
Dry Down:	Sample under slow stream of Nitrogen at 30 °C
Reconstitute:	200 µL Acetonitrile/Water (20:80)

LC-MS/MS Conditions

Column: Kinetex® 2.6 µm Biphenyl
Dimensions: 50 x 2.1 mm
Part No.: 00B-4622-AN
Guard Cartridge: SecurityGuard™ ULTRA Biphenyl
Guard Part No.: AJ0-9209
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Acetonitrile

Gradient:

Time (min)	% B
0	20
3	95
3.5	95
3.51	20
6	20

Injection Volume: 5 µL
Flow Rate: 0.5 mL/min
Detection: MS/MS (SCIEX API 4000™), ESI+

Recovery Values and % CVs

Elution Solvent:	Dichloromethane		Ethyl Acetate	
	% Recovery	% RSD (n=4)	% Recovery	% RSD (n=10)
ß-Methasone	92	4	98	6
Cortisone	96	10	96	8
Coritcosterone	92	3	74	10
Cortisone Acetate	90	12	112	12
Triamcinolone	13	8	92	9
Prednisone	94	7	93	10
Testosterone	95	5		

**Testosterone was not extracted using Ethyl Acetate*

High recoveries and low RSD values using Strata DE!

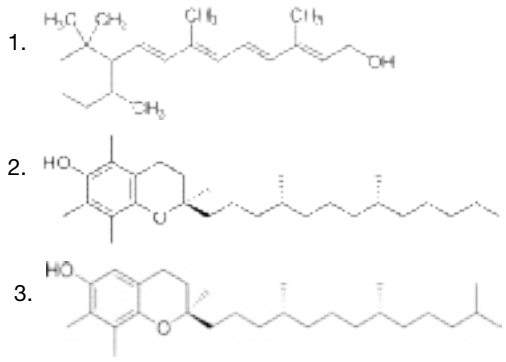
Learn more about Strata DE at www.phenomenex.com/StrataDE

A Fast Method for Vitamin A and E Extraction

from Human Serum Using Novum SLE

We have developed a simple and reliable method to extract vitamin A and E from human serum, using Novum Simplified Liquid Extraction (SLE). A Kinetex® 5 µm, EVO C18, 100 x 2.1 mm HPLC column was utilized to obtain the best selectivity of the two vitamers of vitamin E, alpha and gamma tocopherol, along with vitamin A, while a polarity switching technique in mass spectrometric ionization was employed.

Structures of Vitamin A and E

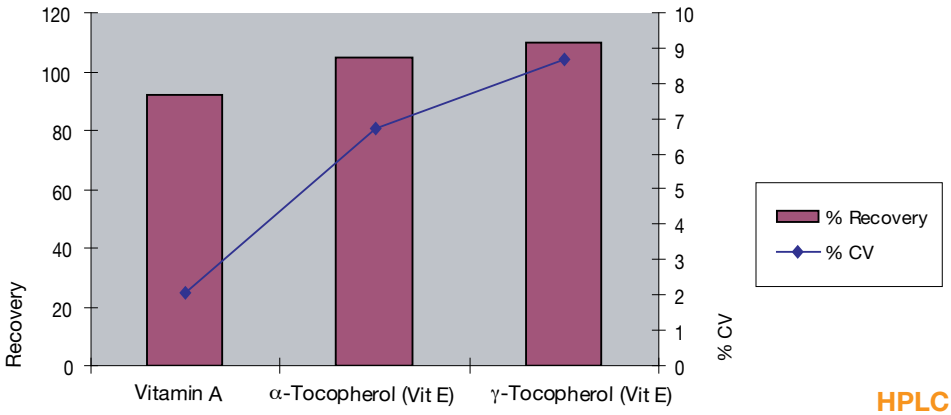


1. Vitamin A (retinol) 2. γ-Tocopherol (vitamin E) 3. α-Tocopherol (vitamin E)

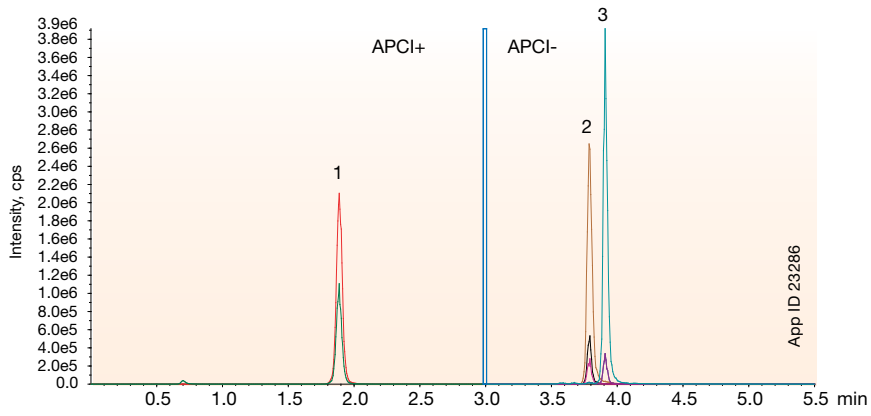
SLE Protocol

96-Well Plate:	Novum SLE MAX 96-Well Plate
Part No.:	8E-S138-5GA
Pre-treatment:	Dilute 200 µL human serum with 100 µL Isopropanol (IPA) and 150 µL of Water. Vortex 30 seconds.
Load:	Sample on the Novum plate and apply a short pulse of vacuum for 10-15 seconds. Wait for 5 minutes.
Elution:	2x 900 µL of Ethyl acetate/Acetone (90:10) and elute by gravity (~ 5 minutes) and collect the eluent. Apply vacuum at 5" of Hg for 20-30 seconds to complete the extraction.
Dry Down:	Evaporate to complete dryness under steady stream of nitrogen at 45 °C.
Reconstitute:	In 200 µL of initial mobile phase.

Recovery and Cleanliness on Novum MAX SLE



LC-MS/MS Analysis of Vitamin A and E Using Dual Polarity



HPLC Conditions

Column:	Kinetex 5 µm EVO C18
Dimensions:	100 x 2.1 mm
Part No.:	00D-4633-AN
Guard:	SecurityGuard™ ULTRA EVO C18 Cartridge: AJ0-9298
Mobile Phase:	A: Water B: Isopropanol/Acetonitrile (1:1)

Gradient:	**Time (min)**	**B (%)**
	0	65
	3	95
	4	95
	4.1	65
	5.55	65

Injection Volume:	5 µL
Flow Rate:	0.6 mL/min
Temperature:	Ambient
Detection:	MS/MS (SCIEX API 5000™)
Instrument:	Agilent® 1260
Sample:	1. Vitamin A 2. γ-Tocopherol (Vit E) 3. α-Tocopherol (Vit E)

Extraction of NSAIDs

from Plasma Using Novum SLE

In this application, we use the Novum SLE MAX 96-Well Plates to extract an eight compound NSAID suite from plasma.

Sample Pre-treatment

Dilute 200 µL of human plasma with 200 µL of 1 % Formic acid in water. Vortex 3-5 seconds.

SLE Protocol

96-Well Plate:	Novum SLE MAX 96-Well Plate
Part No.:	8E-S138-5GA
Load:	400 µL of pre-treated sample onto plate and apply a short pulse of vacuum (~5" Hg) for 5-10 seconds or until sample has completely entered the sorbent.
Elute:	2x 900 µL of 10 % Ethyl acetate in dichloromethane (DCM) and allow the solvent to elute by gravity (~5 minutes) and collect eluant. At the completion of the second aliquot, apply vacuum at 5" Hg for 30 seconds to complete the extraction.
Dry Down:	Evaporate the final extract to complete dryness under a slow stream of nitrogen at 40 °C for about 60 minutes.
Reconstitute:	200 µL of Methanol/Water (10:90) by vortexing the plate at 1400 rpm for 2 minutes.

Recovery

Analyte	% Average Recovery	% CV
Ibuprofen	82	6.70
Diclofenac	79	3.20
Naproxen	96	2.80
Ketoprofen	96	3.10
Mefenamic Acid	77	12.6
Flurbiprofen	82	12.0
Sulindac	87	10.1
Salicylic Acid	93	7.30

High recoveries across all NSAIDs!

Acids, Neutrals, and Bases

from Urine Using Novum SLE

In this application, we will show how a specific pH manipulation can lead to extraction conditions of a relatively hydrophobic acid (THC-COOH) along with more polar bases (buprenorphine and norbuprenorphine) and neutrals (barbiturates). We developed a SLE application for acids, neutrals, and bases from a urine matrix containing β-glucuronidase followed by two LC-MS/MS methods.

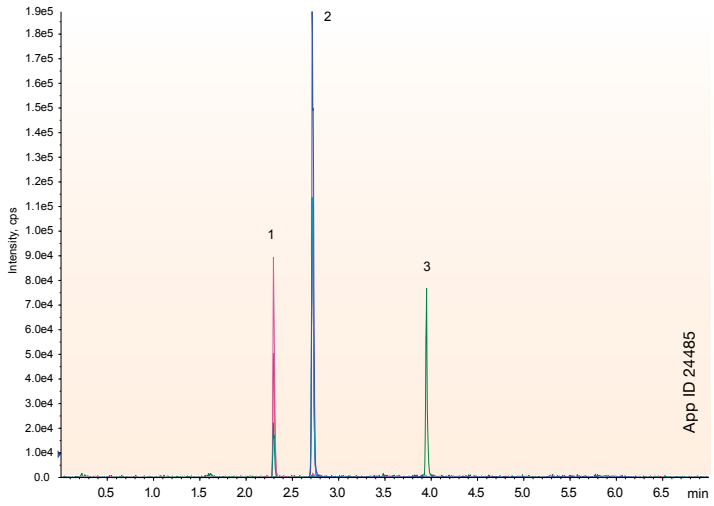
Pre-treatment

To 200 µL of urine, add 25 µL of β-Glucuronidase Enzyme, 25 µL Ammonium Acetate Buffer (100 mM, pH 4), 180 µL Ammonium Bicarbonate Buffer (100 mM, pH 9) and 20 µL Internal Standard (1 µg/mL). Final total volume is 450 µL.

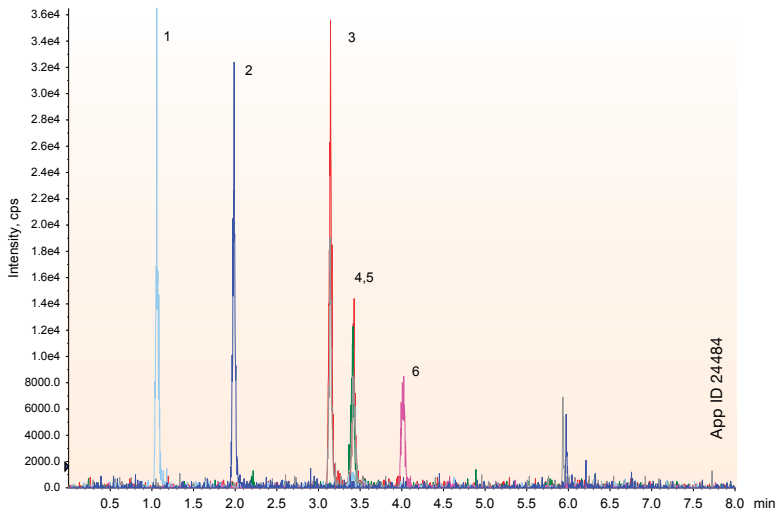
SLE Protocol

96-Well Plate:	Novum Max SLE 96-Well Plate
Part No.:	8E-S138-5GA
Load:	Pre-treated sample and pulse vacuum at 5" Hg for 2-3 seconds, or until the sample completely enters the sorbent bed. Wait for 6 minutes.
Elute:	2x 900 µL Ethyl acetate and elute by gravity. Apply 5" vacuum at end of elution to collect residual solvent from tips in collection plate.
Dry Down:	Under a gentle stream of nitrogen at 30°C.
Reconstitute	For ESI+ samples (THC-COOH, Buprenorphine and Norbuprenorphrine) reconstitute in Methanol/0.1% Formic acid in water (1:4). For ESI- samples (Barbiturates) reconstitute in Methanol/1% NH_4OH in water (1:4).

ESI+ Chromatogram (Buprenophrine/Norbuprenorphine/THC)



ESI- Chromatogram (Barbiturates Mix)



Positive Mode LC-MS/MS Conditions

Column:	Kinetex® 2.6 µm Biphenyl
Dimensions:	50 x 2.1 mm
Part No.:	00B-4622-AN
Guard:	SecurityGuard™ ULTRA Biphenyl Cartridge: AJ0-9209
Mobile Phase:	A: 0.1 % Formic acid in Water B: 0.1 % Formic acid in Acetonitrile
Gradient:	**Time (min) B (%)** 0 5 5 100 5.1 5 7 5
Injection:	4 µL
Flow Rate:	0.5 mL/min
Temperature:	Ambient
Detection:	MS/MS (SCIEX API 4000™)
Sample:	1. Norbuprenorphine 2. Buprenorphine 3. THC-COOH

Negative Mode LC-MS/MS Conditions

Column:	Kinetex 2.6 µm EVO C18 100Å
Dimensions:	50 x 2.1 mm
Part No.:	00B-4725-AN
Guard:	SecurityGuard ULTRA EVO-C18 Cartridge: AJ0-9298
Mobile Phase:	A: 10 mM Ammonium bicarbonate, pH 9 B: Acetonitrile
Gradient:	**Time (min) B (%)** 0 5 2 15 5 20 5.01 60 6 60 6.1 5 7.5 5
Injection:	4 µL
Flow Rate:	0.5 mL/min
Temperature:	Ambient
Detection:	MS/MS (SCIEX API 4000)
Sample:	1. Phenobarbital 2. Butalbital 3. Pentobarbital 4. Amobarbital 5. Amobarbital-DS 6. Secobarbital

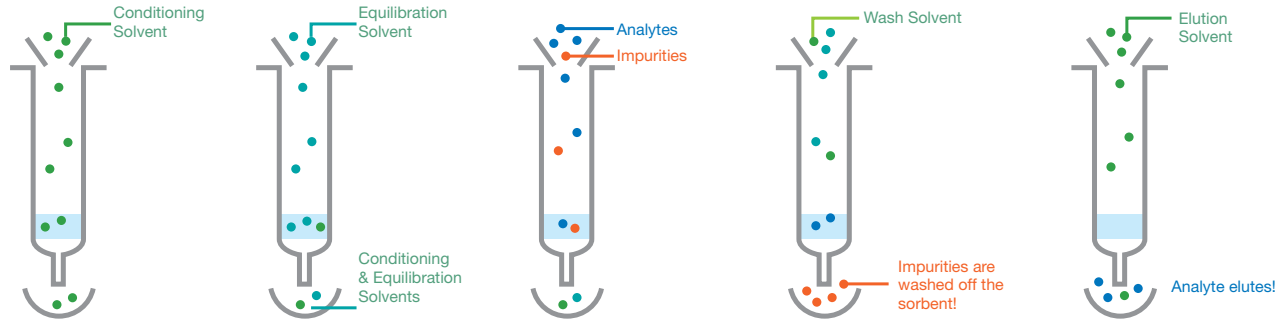
Solid Phase Extraction (SPE)

strata® X Polymeric SPE

Solid Phase Extraction (SPE) is a very targeted form of sample preparation that allows you to isolate your analyte of interest, while removing any interfering compounds that may be in your sample.

- Targeted analyte extraction for cleaner analysis
- Concentration of samples for better chromatographic results
- Solvent switching for GC or LC compatibility

Solid Phase Extraction General Protocol



A Choice for Every Analyte

OPTION 01

Acidic Compounds

Strong Acids (pK_a < 2)	Weak Acids (pK_a 2-4)
Strata-X-AW	Strata-X-A

OPTION 02

Neutral Compounds

Neutral Compounds
Strata-X

OPTION 03

Basic Compounds

Weak Bases (pK_a 8-10)	Strong Bases (pK_a > 10)
Strata-X-C	Strata-X-CW

Learn more, visit www.phenomenex.com/SPE

Urinary Catecholamines

Using Strata-X Microelution SPE

Metanephrine and normetanephrine are both metabolites of epinephrine and norepinephrine. In this application, Strata-X-CW Microelution SPE 96-Well Plates were used in conjunction with a Kinetex® Biphenyl HPLC column in order to resolve an interference that coelutes with 3-Methoxytyramine on a standard C18 HPLC column, while reaching low limits of quantification for specific urinary catecholamines, metanephrine, and normetanephrine.

Urine Pre-treatment

500 µL of urine was diluted with 500 µL of 50 mM Ammonium acetate buffer, (pH 7). Urine was pre-spiked from 10 ng/mL to 63 pg/mL with metanephrine, normetanephrine, and 3-methoxytyramine (standards provided by Cerilliant®).

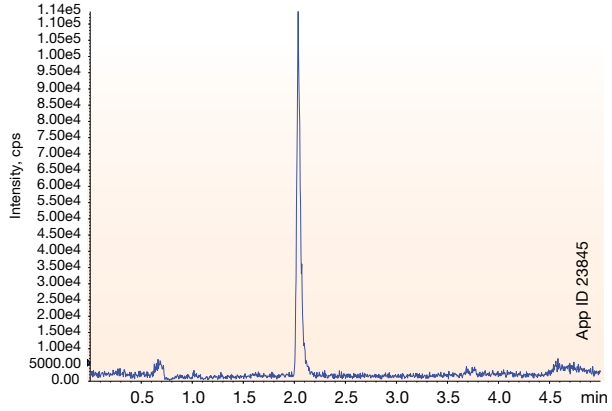
SPE Method

Microelution 96-Well Plate:	Strata-X-CW Microelution 96-Well Plate, 2 mg/well
Part No.:	8M-S035-4GA
Condition:	200 µL Methanol
Equilibrate:	200 µL 50 mM Ammonium acetate buffer, pH 7
Load:	1 mL of pre-treated sample
Wash 1:	200 µL of 50 mM Ammonium acetate buffer, pH 7
Wash 2:	200 µL Acetonitrile/IPA (1:1)
Elute:	2x 25 µL of Water/Acetonitrile/Formic acid (85:10:5)
Injection:	Dilute eluent with 100 µL of 0.1 % Formic acid in water (Metanephrine-D3 internal standard was included at 1ng/mL)

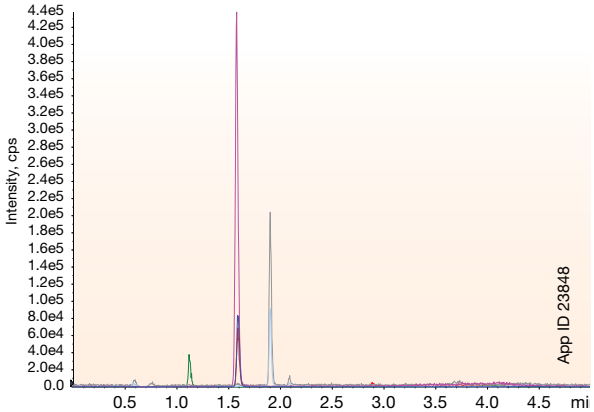
Recovery Values from 10 ng/mL to 63 pg/mL

Analyte Concentration (ng/mL)	Average % Recovery	% CV (n=6)
Metanephrine		
10	102	5
1	102	3
0.5	99	2
0.25	99	3
0.125	97	3
0.063	94	6
Normetanephrine		
10	100	10
1	87	12
0.5	110	10
0.25	89	9
0.125	110	13
0.063	108	15
Amobarbital		
10	91	3
1	89	6
0.5	95	4
0.25	86	5
0.125	87	6
0.063	92	7

Chromatogram of unresolved interference for 3-methoxytyramine using a HPLC C18 column



Representative Chromatogram of Urinary Catecholamines



LC-MS/MS Conditions

Column:	Kinetex 5 µm Biphenyl
Dimensions:	50 x 4.6 mm
Part No.:	00B-4627-E0
Guard:	SecurityGuard™ ULTRA Biphenyl Cartridges: AJ0-9207
Mobile Phase:	A: 0.1 % Formic acid in Water B: 0.1 % Formic acid in Methanol
Gradient:	**Time (min) B (%)** 0 5 3 90 3.1 5 5 5
Injection:	30 µL
Flow Rate:	0.7 mL/min
Temperature:	Ambient
Detection:	MS/MS (SCIEX API 4000™)

Underivatized Methylmalonic Acid (MMA)

from Plasma Using Strata-X SPE

Methylmalonic acid (MMA) is a small dicarboxylic acid. This hydrophilic molecule can present chromatographic challenges both in achieving adequate retention under reversed phase conditions as well as resolution from the isomeric/isobaric species such as succinic acid, especially at low analyte concentrations. To combat these challenges, many published LC-MS/MS methods require a sample derivatization step, however, this step can add time to the overall analysis. Presented is a fast, reproducible LC-MS/MS method to analyze underivatized MMA by utilizing a unique Luna® Omega 1.6 µm PS C18 UHPLC column. The method runtime is 5 minutes including column re-equilibration. For the sample preparation procedure we used Strata-X-AW Solid Phase Extraction (SPE) to produce a clean sample from plasma. Analyte detection was performed using negative mode electrospray ionization of a triple quadrupole MS.

Sample Pre-treatment

Combine 0.5 mL of 1 % aqueous acetic acid and 50 µL of internal standard with 100 µL blank, standard, or sample.

SPE Protocol

Cartridge:	Strata-X-AW 30 mg/1 mL
Part No.:	8B-S038-TAK
Condition:	1 mL of Methanol
Equilibrate:	1 mL of 1 % Acetic acid in Water
Load:	Pre-treated sample (see above)
Wash:	0.5 mL of Methanol/Water (50:50)
Dry:	5 to 10 minutes at max vacuum (or apply positive pressure using Presston™ 100 Positive Pressure Manifold)
Elute:	2x 0.6 mL 2 % Ammonium hydroxide in Methanol
Dry Down:	Evaporate solvent to dryness @ 45-50 °C under a gentle stream of nitrogen
Reconstitute:	200 µL of mobile phase A (0.1 % Formic acid in Water)

Analyte Recovery

Sample Name	Spike (nmol/L)	Average Concentration N=3 (nmol/L)	% CV	% Recovery
Prespiked 250 nmol/L in plasma	250	696	9.44	114
Prespiked 750 nmol/L in plasma	750	1157	2.00	102
Extracted unspiked plasma	0	385	3.01	N/A

LC Conditions

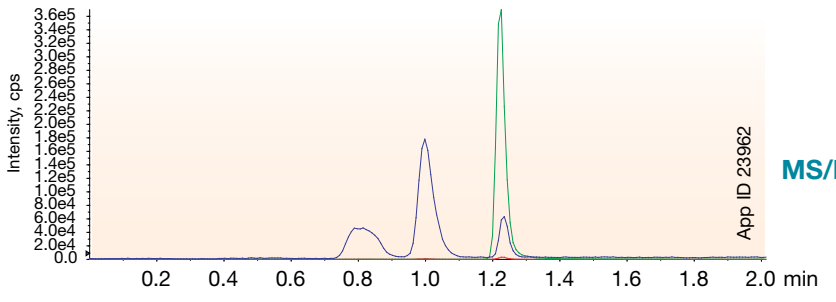
Analytical Column: Luna Omega 1.6 µm PS C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4752-AN
Guard: SecurityGuard™ ULTRA PS C18 Cartridges: AJ0-9508
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Acetonitrile

Gradient:

Time (min)	B (%)
0.01	2
2	90
3	90
3.01	2
5	2

Injection: 5 µL
Flow Rate: 0.4 mL/min
Temperature: 40°C

MMA Representative Chromatogram



Peaks in order of elution: plasma interference (0.81 min), succinic acid (1.00 min), methyl-D3-malonic acid (1.20 min), and methylmalonic acid (1.23 min).

MS/MS Conditions

Detector: SCIEX 4000 QTRAP®
Mode: Negative Ionization Mode
Scan Type: MRM
Curtain Gas (CUR): 10.0 psi
Collision Gas (CAD): Medium
IonSpray Voltage (IS): -4500 V
Temperature (TEM): 600 °C
Ion Source Gas 1 (Gas1): 50 psi
Ion Source Gas 2 (Gas2): 50 psi
Interface Heater (ihe): On

Comprehensive Drug Research Panel

from Oral Fluid Collection Devices Using Strata-X SPE

Drug testing in oral fluid has steadily gained popularity due to the easy and non-intrusive sample collection procedure. The oral fluid collection device provides a buffer solution that contains a number of antibacterial agents and surfactants that act to prevent bacterial growth and increase the analytes stability during the sample transit to testing laboratories. The buffer solution poses many chromatography challenges, such as ion suppression. Here, we present a fast sample preparation procedure to reduce the effects of the device's buffer solution while maintaining good recovery of analytes using a Strata-X-C SPE method for different classes of analytes from a comprehensive drug research panel. For a more selective SPE solution, our dual cartridge SPE method is recommended (page 16).

Sample Pre-treatment

Transfer 1 mL of oral fluid collected on an applicator tip in its preservative buffer. Leave it for 2 hours followed by centrifugation for 15 minutes at 600 g. Remove 0.5 mL of supernatant and combine with 1 mL of 1% Formic acid. Vortex for 5-10 seconds.

SPE Protocol

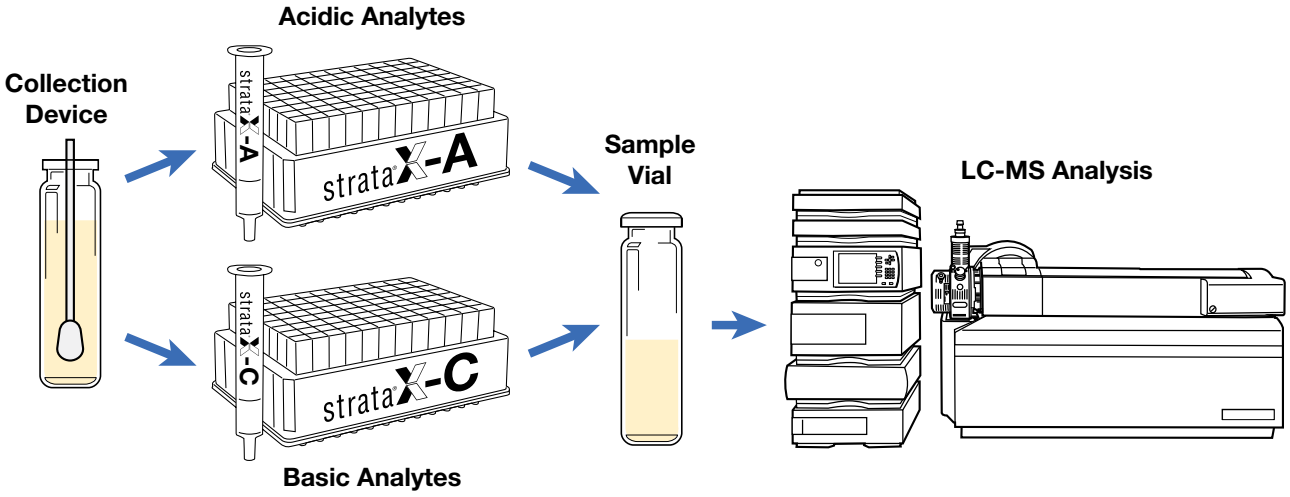
96-Well Plate:	Strata-X-CW 30 mg/well
Part No.:	8E-S035-TGB
Condition:	1mL Methanol
Equilibrate:	1 mL DI Water
Load:	Pre-treated Sample
Wash 1:	1 mL 1% Formic acid in DI Water
Wash 2:	1 mL DI Water
Dry:	5-6 minutes at maximum vacuum (20"Hg or higher)
Elution:	2x 500 µL (2 aliquots of 500uL) Methylene chloride/ Isopropanol/30% Ammonium hydroxide in Water (80:18:2)
Dry Down:	Evaporate to dryness under nitrogen at 45-50°C
Reconstitute:	200 µL of 0.1% Formic acid in Water/0.1% Formic acid in Methanol (90:10)

Analyzing additional analytes?
Use our Strata-X double cartridge solution for increased clean-up and increased sensitivity across all classes of drug compounds.
pg. 16

Analyte Concentration (ng/mL)	Average % Recovery
6-MAM	79
α-Hydroxyalprazolam	88
Alprazolam	79
Amphetamine	94
Benzoylecgonine	89
Carisoprodol	95
Citalopram	81
Cocaine	84
Codeine	89
Diazepam	74
EDDP	72
Fentanyl	74
Hydrocodone	102
Hydromorphone	98
Meperidine	84
Mephedrone	84
Meprobamate	92
Methamphetamines	81
Naloxone	83
Norbuprenorphine	87
Nordiazepam	75
Norfentanyl	85
Norhydrocodone	89
Noroxycodone	85
Oxycodone	84
PCP	84
Phenobarbital	83
Secobarbital	82
Tapentadol	83
Temazepam	86
Tramadol	87

Expanded Comprehensive Drug Research Panel

from Oral Fluid Collection Devices Using Double Cartridge Strata-X SPE



SPE Method

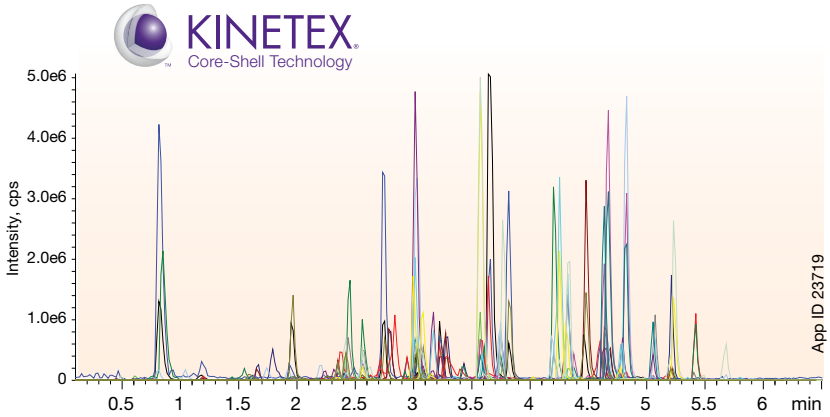
Step	Basic analyte extraction	Acidic analyte extraction
Cartridge:	Strata-X-C, 30 mg/3 mL	Strata-X-A, 30 mg/3 mL
Part No.:	8B-S029-TBJ	8B-S123-TBJ
Condition:	1 mL 100 % Methanol	1 mL 100 % Methanol
Equilibrate:	1 mL DI Water	1 mL DI Water
Load:	Combine 0.5 mL of pre-treated sample with 1 mL 1 % Formic acid, mix/vortex 5-10 sec and load on Strata-X-C	Combine 0.5 mL of pre-treated sample with 1 mL 1 % Ammonium hydroxide, mix/vortex 5-10 sec and load on Strata-X-A
Weak Wash:	1 mL DI Water	1 mL DI Water
Strong Wash:	1 mL Acetone / Water (50:50)	1 mL Acetone / Water (50:50)
Dry down:	3-4 minutes at maximum vacuum (15" Hg or higher)	3-4 minutes at maximum vacuum (15" Hg or higher)
Elute:	2x 500 µL Methanol / Acetonitrile / Ammonium hydroxide (5:5:2)	2x 500 µL Methanol / Acetonitrile / Formic acid (50:50:5)
Dry down:	Evaporate to dryness under a gentle steam of Nitrogen at 45-50 °C	Evaporate to dryness under a gentle steam of Nitrogen at 45-50 °C
Reconstitute:	With 125 µL initial Mobile Phase	With 125 µL initial Mobile Phase
Combine into a single sample vial		

Note: If not testing for THC-COOH, lorazepam and other select barbiturates, use Strata-X-C only.

Expanded Comprehensive Drug Research Panel (cont'd)

from Oral Fluid Collection Devices Using Double Cartridge Strata-X SPE

Comprehensive Drug Research Panel Chromatogram



Positive ESI Panel

Column: Kinetex 2.6 µm Biphenyl
Dimensions: 50 x 3.0 mm
Part No.: 00B-4622-Y0
Guard: SecurityGuard™ ULTRA Biphenyl Cartridge: AJ0-9208
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol

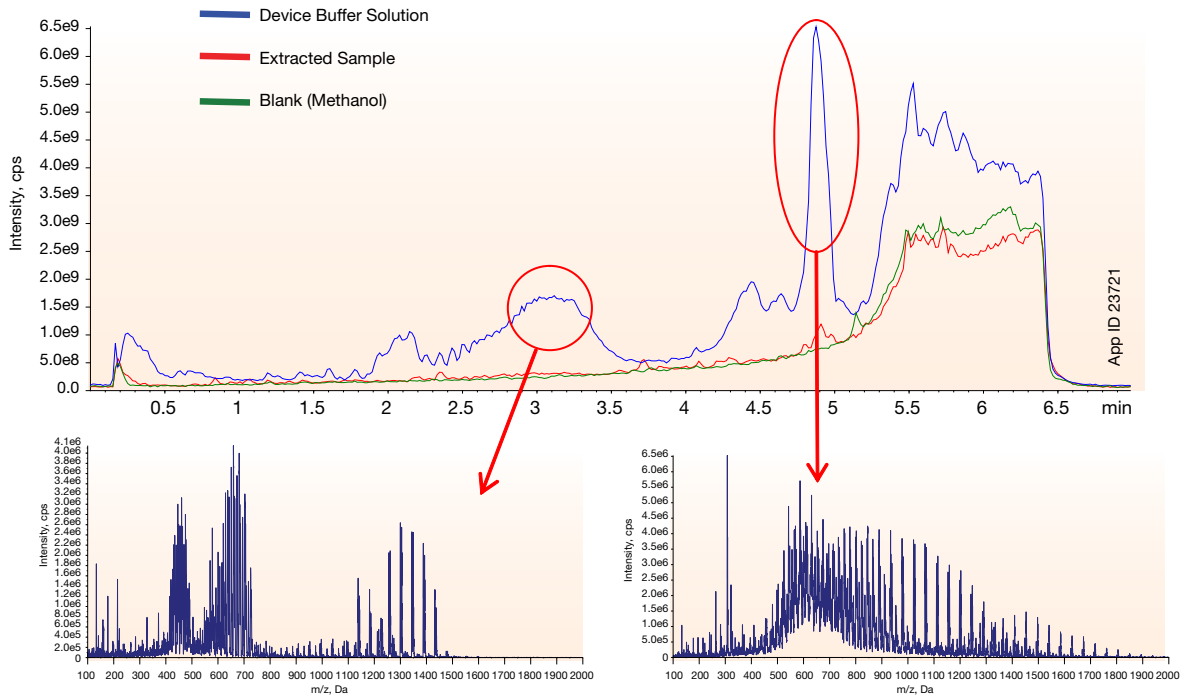
Gradient:

Time (min)	%B
0	10
4	95
5.5	95
5.51	10
7.5	10

Injection: 10 µL
Flow Rate: 0.5 mL/min
Temperature: Ambient
Detector: MS/MS (SCIEX API 5000™) ESI+

Representative LC-MS Chromatogram of Buffer Solution

See the reduction in buffer interferences!



Ordering Information

β-Gone β-Glucuronidase Removal

Part No.	Description	Unit
8B-S139-TAK	1 mL Tubes, Recombinant Enzyme	100 / Box
8B-S322-DAK	1 mL Tubes, Non-Recombinant Enzyme	100 / Box
8E-S139-TGA	96-Well Plate, Recombinant Enzyme	1 / Box
8E-S322-DGA	96-Well Plate, Non-Recombinant Enzyme	1 / Box
8N-S323-TUK	2 mL Centrifuge Tubes, Recombinant and Non-Recombinant Enzyme	100 / Box

Strata DE SLE

Strata DE Diatomaceous Earth (SLE)		
Part No.	Description	Unit
8E-S325-FGB	Strata DE SLE 200 µL 96-Well Plate	2/pk
8E-S325-5GB	Strata DE SLE 400 µL 96-Well Plate	2/pk
8B-S325-KDG	Strata DE SLE 12 cc Tubes	20/pk
8B-S325-VFF	Strata DE SLE 60 cc Tubes	16/pk

Phree Phospholipid Removal

Part No.	Description	Unit
8B-S133-TAK	Phree Phospholipid Removal 1 mL Tube	100/box
8E-S133-TGB	Phree Phospholipid Removal 96-Well Plates	2/box

guarantee

If Phenomenex products in this brochure do not provide at least equivalent separation as compared to other products of the same phase and dimensions, return the product with your comparative data within 45 days for a FULL REFUND.

Novum SLE

Part No.	Description	Unit
8E-S138-FGA	Novum SLE MINI 96-Well Plate	1 / Box
8E-S138-5GA	Novum SLE MAX 96-Well Plate	1 / Box
8B-S138-FAK	Novum SLE 1 cc tubes	100 / Box
8B-S138-5BJ	Novum SLE 3 cc tubes	50 / Box
8B-S138-JCH	Novum SLE 6 cc tubes	30 / Box
8B-S138-KDG	Novum SLE 12 cc tubes	20 / Box

Strata-X SPE Tubes

Tubes	1 mL (100/box)		3 mL (50/box)			6 mL (30/box)		
Phase	30 mg	60 mg	60 mg	200 mg	500 mg	100 mg	200 mg	500 mg
Strata-X	8B-S100-TAK	8B-S100-UAK	8B-S100-UBJ	8B-S100-FBJ	8B-S100-HBJ	8B-S100-ECH	8B-S100-FCH	8B-S100-HCH
Strata-X-C	8B-S029-TAK	—	8B-S029-UBJ	8B-S029-FBJ	8B-S029-HBJ	8B-S029-ECH	8B-S029-FCH	8B-S029-HCH
Strata-X-CW	8B-S035-TAK	—	8B-S035-UBJ	8B-S035-FBJ	8B-S035-HBJ	8B-S035-ECH	8B-S035-FCH	8B-S035-HCH
Strata-X-A	8B-S123-TAK	—	8B-S123-UBJ	8B-S123-FBJ	8B-S123-HBJ	8B-S123-ECH	8B-S123-FCH	8B-S123-HCH
Strata-X-AW	8B-S038-TAK	—	8B-S038-UBJ	8B-S038-FBJ	8B-S038-HBJ	8B-S038-ECH	8B-S038-FCH	8B-S038-HCH
Strata-XL	8B-S043-TAK	—	8B-S043-UBJ	8B-S043-FBJ	8B-S043-HBJ	8B-S043-ECH	8B-S043-FCH	8B-S043-HCH
Strata-XL-C	8B-S044-TAK	—	8B-S044-UBJ	8B-S044-FBJ	8B-S044-HBJ	8B-S044-ECH	8B-S044-FCH	8B-S044-HCH
Strata-XL-CW	8B-S052-TAK	—	8B-S052-UBJ	8B-S052-FBJ	8B-S052-HBJ	8B-S052-ECH	8B-S052-FCH	8B-S052-HCH
Strata-XL-A	8B-S053-TAK	—	8B-S053-UBJ	8B-S053-FBJ	8B-S053-HBJ	8B-S053-ECH	8B-S053-FCH	8B-S053-HCH
Strata-XL-AW	8B-S051-TAK	—	8B-S051-UBJ	8B-S051-FBJ	8B-S051-HBJ	8B-S051-ECH	8B-S051-FCH	8B-S051-HCH

Strata-X SPE 96-Well Plates

96-Well Plates (2/Box)			
Phase	10 mg	30 mg	60 mg
Strata-X-AW	8E-S038-AGB	8E-S038-TGB	8E-S038-UGB
Strata-X-A	8E-S123-AGB	8E-S123-TGB	8E-S123-UGB
Strata-X	8E-S100-AGB	8E-S100-TGB	8E-S100-UGB
Strata-X-C	8E-S029-AGB	8E-S029-TGB	8E-S029-UGB
Strata-X-CW	8E-S035-AGB	8E-S035-TGB	8E-S035-UGB
Strata-XL-AW	–	8E-S051-TGB	–
Strata-XL-A	–	8E-S053-TGB	–
Strata-XL	–	8E-S043-TGB	–
Strata-XL-C	–	8E-S044-TGB	–
Strata-XL-CW	–	8E-S052-TGB	–

Strata-X Microelution SPE Plates

96-Well Plates (ea)	
Phase	2 mg / well
Strata-AW	8M-S038-4GA
Strata-A	8M-S123-4GA
Strata-X	8M-S100-4GA
Strata-X-C	8M-S029-4GA
Strata-X-CW	8M-S035-4GA

Ordering Information

Presston 100 Positive Pressure Manifold

Part No.	Description	Unit
AH0-9334	Presston 100 Positive Pressure Manifold, 96-Well Plate	1 / Box
AH0-9342	Presston 100 Positive Pressure Manifold, 1 mL Tube Complete Assembly	1 / Box
AH0-9347	Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly	1 / Box
AH0-9343	Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly	1 / Box

Phenomenex warrants that for a period of 12 months following delivery, the Presston 100 Positive Pressure Manifold you have purchased will perform in accordance with the published specifications and will be free from defects in materials or workmanship. In the event that the Presston 100 Positive Pressure Manifold does not meet this warranty, Phenomenex will repair or replace defective parts. Please visit **www.phenomenex.com/Presston** for complete warranty information.

presston™ 100

Kinetex Analytical LC Columns

KINETEX® Core-Shell Technology

5 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
EVO C18	00A-4633-AN	00B-4633-AN	00D-4633-AN	00F-4633-AN	AJ0-9298
					for 2.1 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
EVO C18	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJ0-9296
F5	00B-4724-E0	00D-4724-E0	00F-4724-E0	00G-4724-E0	AJ0-9320
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207
					for 4.6 mm ID

2.6 µm Minibore Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
EVO C18	00A-4725-AN	00B-4725-AN	—	00D-4725-AN	00F-4725-AN	AJ0-9298
Polar C18	00A-4759-AN	00B-4759-AN	—	00D-4759-AN	00F-4759-AN	AJ0-9532
F5	00A-4723-AN	00B-4723-AN	—	00D-4723-AN	00F-4723-AN	AJ0-9322
Biphenyl	00A-4622-AN	00B-4622-AN	—	00D-4622-AN	00F-4622-AN	AJ0-9209
XB-C18	00A-4496-AN	00B-4496-AN	00C-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782
C18	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782
C8	00A-4497-AN	00B-4497-AN	00C-4497-AN	00D-4497-AN	00F-4497-AN	AJ0-8784
HILIC	00A-4461-AN	00B-4461-AN	00C-4461-AN	00D-4461-AN	00F-4461-AN	AJ0-8786
Phenyl-Hexyl	00A-4495-AN	00B-4495-AN	00C-4495-AN	00D-4495-AN	00F-4495-AN	AJ0-8788
						for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
EVO C18	—	00B-4725-Y0	—	00D-4725-Y0	00F-4725-Y0	AJ0-9297
Polar C18	—	00B-4759-Y0	—	00D-4759-Y0	00F-4759-Y0	AJ0-9531
F5	—	00B-4723-Y0	—	00D-4723-Y0	00F-4723-Y0	AJ0-9321
Biphenyl	—	00B-4622-Y0	—	00D-4622-Y0	00F-4622-Y0	AJ0-9208
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJ0-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	00F-4497-Y0	AJ0-8777
HILIC	00A-4461-Y0	—	—	—	00F-4461-Y0	AJ0-8779
Phenyl-Hexyl	—	00B-4495-Y0	—	00D-4495-Y0	00F-4495-Y0	AJ0-8781
						for 3.0 mm ID

Luna Omega UHPLC Columns

explore LUNA® OMEGA

1.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Polar C18	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJ0-9505
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJ0-9508
C18	00A-4742-AN	00B-4742-AN	00D-4742-AN	00F-4742-AN	AJ0-9502
					for 2.1 mm ID

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