Using Liquid-Liquid Extraction (LLE)?

There's a Cleaner Way!

phenomenex®
. . . breaking with tradition℠

www.phenomenex.com/ReplaceLLE

Road to Success

To our LLE users,

Replace LLE and find the way to cleaner samples. Both destinations will increase throughput, save time, improve selectivity, and lead to more consistent results.

So where are you headed?

Destination: Cleaner

Supported Liquid Extraction (SLE)

- Solid support mimics LLE
- No emulsions
- Reduces solvent waste
- 2 simple steps: load and elute

Destination: Cleanest

Solid Phase Extraction (SPE)

- Targeted specifically for analytes of interest and to remove matrix inferences
- Consistently high recoveries
- Process small or limited sample volumes
- Increased concentration

Confused? Lost? Frustrated?

Let our team navigate for you!

Email us: SamplePrepSpecialist@phenomenex.com

LiveChat us: www.phenomenex.com/livechat

guarantee

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

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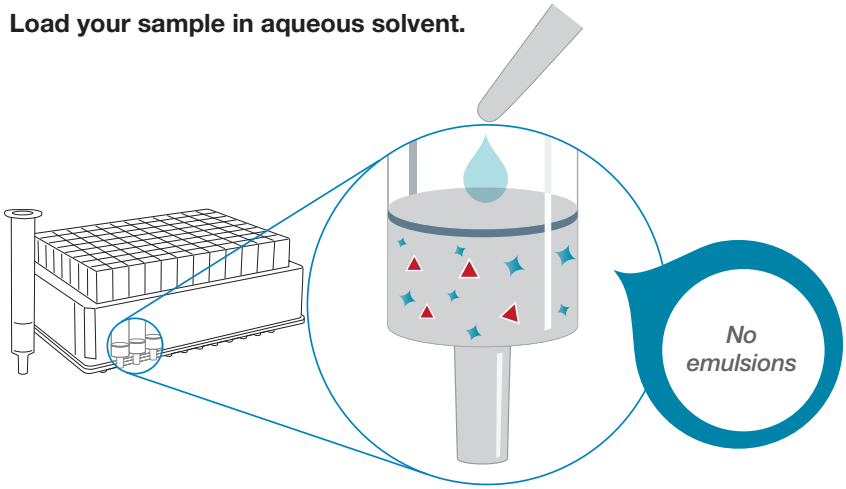
Supported Liquid Extraction (SLE)

SLE produces more reproducible results, increased accuracy, and higher throughput possibilities than Liquid-Liquid Extraction by utilizing a solid support to mimic LLE. Phenomenex offers two types of SLE sorbent: diatomaceous earth (Strata® DE) and an exclusive synthetic sorbent (Novum™). With very little method development, both SLE options remove unwanted matrix interferences to provide cleaner samples than LLE.

Two Simple Steps for a Cleaner Extraction:

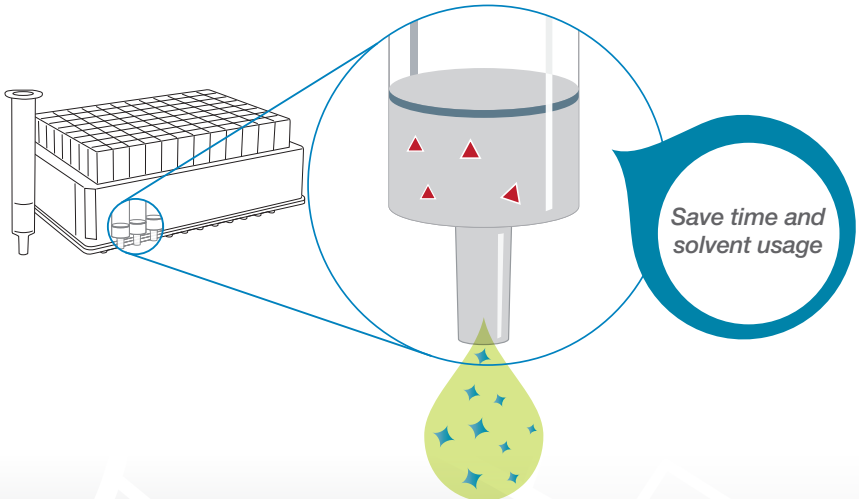
STEP 01 **Load your sample in aqueous solvent.**



▲ Interferences *(i.e. phospholipids, proteins, salts, etc.)*

✦ Target Analytes

STEP 02 **Collect target analytes in water immiscible solvent for analysis.**



Supported Liquid Extraction (SLE)

Select Your SLE Sorbent!

View the differences in our sorbent options:

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

Still need help?

SLE sorbent selections are dependent on extraction solvents, sample volumes, and analytes being extracted. To learn which SLE product is right for your extraction method:

Call us or **Live Chat**
www.phenomenex.com/LiveChat

Destination: Cleaner

SLE Application: Corticosteroid Extraction From Plasma

Introduction

A method was established 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%, displaying the high recovery capabilities available when using SLE over LLE, 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%. By using a simple method with Strata DE SLE, **high recoveries and low variability** between samples was achieved.

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:	300 µL pre-treated sample onto plate (apply vacuum or positive pressure to pull/push sample into sorbent if necessary)
Wait:	5 minutes
Elute:	3 x 600 µL Dichloromethane (DCM) or 3 x 600 µL Ethyl Acetate
Apply:	Vacuum or apply positive pressure at 5-10" Hg for 10 seconds
Dry:	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 C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Guard Cartridge: SecurityGuard™ ULTRA C18
Guard Part No.: AJ0-8782
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

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

Recovery Values and % RSD

Elution Solvent	Dichloromethane		Ethyl Acetate	
	% Recovery	% RSD (n=4)	% Recovery	% RSD (n=10)
β-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*

Destination: Cleaner

SLE Application: Comprehensive Drug Research Panel From Urine

Introduction

To determine whether Strata® DE is a viable alternative to Biotage® ISOLUTE® SLE+, drugs of abuse were extacted from urine and then analyzed by LC-MS/MS. The recovery values and % CVs for both Strata DE SLE and Biotage ISOLUTE SLE+ are in **Table 1**. While recoveries vary slightly between different analytes, both products exhibit > 85% recovery for all analytes included. For this sample set the Strata DE maintains an average % CV of 9%, while the Biotage ISOLUTE SLE+ plate has a slightly higher average % CV of 10%. The data displays that Strata DE is comparable to an industry standard diatomaceous earth SLE product and exhibits **consistently high recoveries with excellent separation** in a comprehensive drug research panel.

Pre-treatment

Combine 100 µL of spiked urine, 15 µL Campbell β-Glucuronidase (Part No. DR2102), 35 µL 100 mM Ammonium Acetate (pH 4), and 150 µL of 100 mM Ammonium Bicarbonate (pH 10).

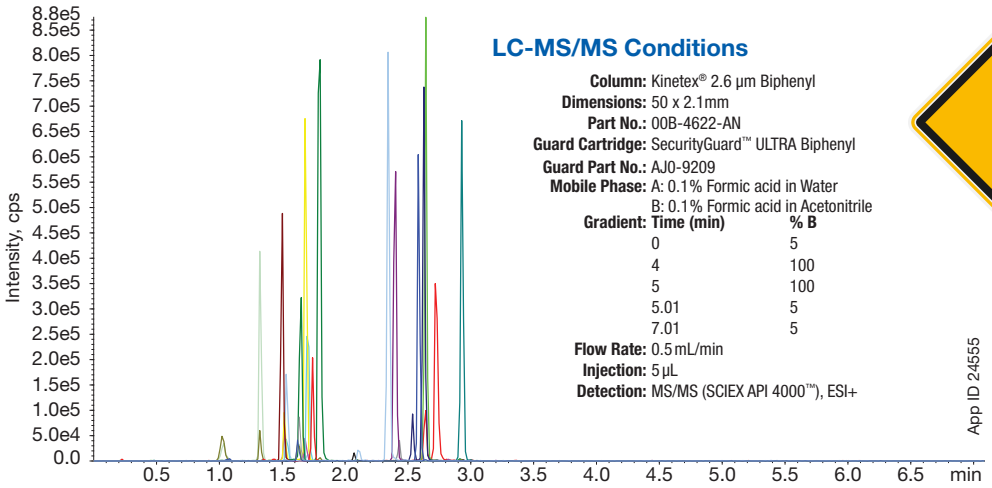
SLE Protocol

96-Well Plate:	Strata DE SLE 400 µL 96-Well Plate Biotage ISOLUTE SLE+ 400 µL 96-Well Plate
Part No.:	8E-S325-5GB (Strata DE)
Load:	300 µL pre-treated sample onto plate (apply vacuum or positive pressure to pull/push sample into sorbent if necessary)
Wait:	6 minutes
Elute:	3 x 600 µL Dichloromethane/IPA (95:5)
Apply:	Vacuum or apply positive pressure at 5-10" Hg for 10 seconds
Dry:	Sample under slow stream of Nitrogen at 30 °C
Reconstitute:	100 µL 0.1% Formic acid/Methanol (4:1) with internal standard

Table 1.
Recovery Values and % CVs: Strata DE vs. Biotage ISOLUTE SLE+

Elution Solvent	Strata DE		Biotage ISOLUTE SLE+	
	% Recovery	% CV (n=8)	% Recovery	% CV (n=8)
6-MAM	98	9	88	16
Alprazolam	104	10	98	11
Benzoylecgonine	88	6	98	11
Buprenorphine	93	7	102	15
Codeine	99	12	93	9
Diazepam	107	7	104	6
Fentanyl	85	5	94	8
Hydrocodone	104	11	93	11
Hydromorphone	95	9	93	11
Lorazepam	94	8	98	8
Methamphetamine	92	16	102	8
Morphine	98	12	94	12
Norbuprenorphine	101	11	92	11
Nordiazepam	100	9	92	8
Norfentanyl	113	7	110	11
Oxycodone	97	5	93	11
PCP	90	7	98	6

Extracted Chromatogram on Strata DE SLE



LC-MS/MS Conditions

Column: Kinetex® 2.6 µm Biphenyl
Dimensions: 50 x 2.1mm
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	5
4	100
5	100
5.01	5
7.01	5

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

App ID 24555

Strata DE is a cost effective alternative to other SLE products!

Comparative separations may not be representative of all applications.

Destination: Cleaner

SLE Application: Determination of Sterols in Olive Oil

Introduction

Due to frequent adulteration, a reliable and efficient method was developed to determine the concentration of sterols in olive oil, which can confirm the classification of oil. Presented is a modified International Olive Council (IOC) method for sterol determination improved upon by replacing LLE with a SLE protocol using diatomaceous earth, Strata® DE, for a quick and accurate extraction and further clean-up of the sample by Solid Phase Extraction (SPE) to remove hydrocarbons as well as more polar interferences from the solution. The isolated sterols and triterpene alcohols are then derivatized as the trimethylsilyl ethers prior to GC-FID analysis. The result is an improved method for determining sterols, erythrodiol and uvaol in olive oil by utilizing **faster and more accurate** extraction techniques.

Sample Preparation

Internal Standard Preparation

Add 40 µL of 1 mg/mL cholestanol in chloroform to a clean, dry 20 mL screw-top test tube and evaporate to dryness under a nitrogen flow.

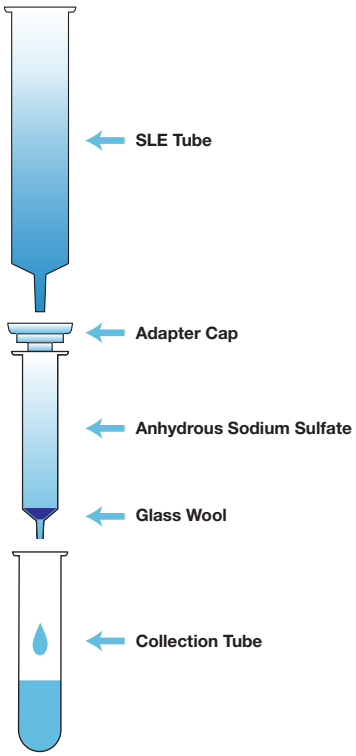
Saponification

1. Add 200 mg of olive oil sample to the test tube containing the internal standard.
2. Add 1.5 mL of 2M Potassium hydroxide in 95% Ethanol.
3. Cap the tube and heat in an 80 °C oven for 25 minutes.
4. Mix sample gently to ensure homogeneity (sample should appear as a clear solution) and continue heating for an additional 25 minutes.
5. After heating, add 13.5 mL of deionized water and mix. The entire diluted volume is now ready to load onto the SLE cartridge.

SLE Protocol

Cartridge:	Strata DE SLE cartridge, 20 mL loading capacity, 60 cc Tube
Part No.:	8B-S325-VFF
Load:	Diluted sample (from saponification step 5) plus 2 x 1 mL DI water rinse (17 mL total volume, gravity flow)
Wait:	15 minutes
Extract:	3 x 15 mL Diethyl Ether (gravity flow)
Evaporate:	Dry under N_2 at 40 °C (greenish-yellow, oily residue)
Reconstitute:	5 mL of Hexane

SLE setup with sodium sulfate drying tube attached to an SLE column (Gravity Flow)



Anhydrous sodium sulfate used to dry sample.

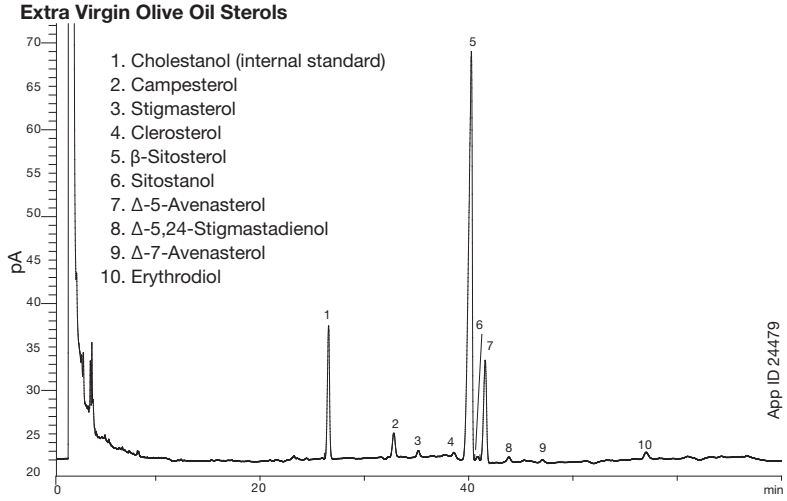
SPE Protocol and Derivatization

Cartridge:	Strata Si-1 (1 g/6 mL) tube
Part No.:	8B-S012-JCH
Condition:	1. 2 x 6 mL Hexane 2. 1 mL 0.2M Potassium hydroxide in 95% ethanol
Equilibrate:	5 mL Hexane (immediately after potassium hydroxide elution)
Load:	Reconstituted SLE extract (5 mL) followed by 2 x 1 mL Hexane rinses
Wash:	85 mL Hexane/Diethyl ether (98:2) under 3" Hg vacuum, flow rate of 2 mL/min*
Elute:	10 mL Hexane/Diethyl ether (60:40)
Dry:	Dry under N_2 at 50 °C. After evaporating to dryness, add 3-4 drops of acetone and then re-evaporate under N_2 to remove any occluded water. Place in 100 °C oven for 10 minutes
Derivatization:	250 µL Pyridine/BSTFA (3:1) at 80 °C for 30 minutes

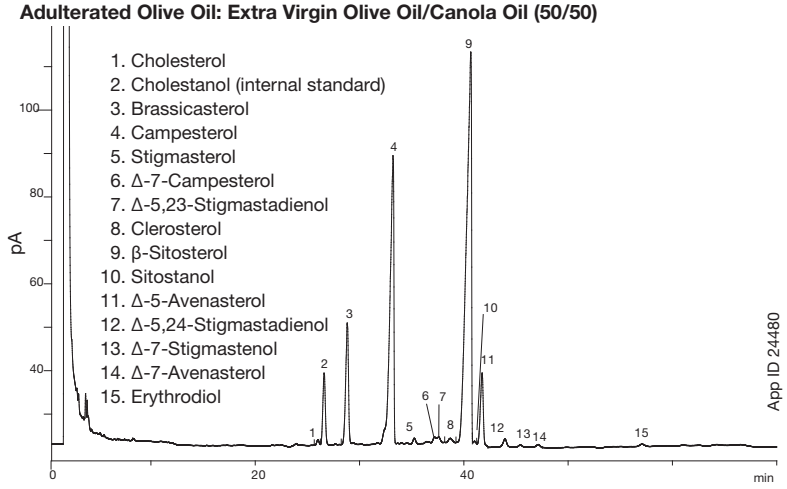
*To handle the large volume of eluant, a 60 mL empty reservoir tube was attached to the 6 mL SPE tube.

SLE Application: Determination of Sterols in Olive Oil *(cont'd)*

Extra Virgin Olive Oil Sterols



Adulterated Olive Oil: Extra Virgin Olive Oil/Canola Oil (50/50)



GC-FID Conditions

Conditions for both separations:

Column: Zebron™ ZB-5*PLUS*™
Dimensions: 30 m x 0.25 mm x 0.25 µm
Part No.: 7HG-G032-11
Recommended Liner: Zebron PLUS Single Taper Z-Liner™ (for Agilent® systems)
Liner Part No.: AG2-0A13-05
Injection: Split 5:1 @ 280 °C, 1 µL
Carrier Gas: Helium @ 0.9 mL/min (constant flow)
Oven Program: 260 °C for 70 min
Detector: FID @ 300 °C
Samples: Analytes were derivatized with Pyridine/BSTFA (3:1)

IOC Sterol Criteria for Virgin Olive Oil Classification

Standard Name	IOC Standard Criteria for Virgin Olive Oil	Extra Virgin Olive Oil		Adulterated Olive Oil	
		% Recovery	% RSD (n=3)	% Recovery	% RSD (n=2)
Apparent β-Sitosterol*	≥ 93.0% of total sterols	94.6%	0.3	60.3%	1.2
Cholesterol	≤ 0.5% of total sterols	not detected	-	0.3%	13.3
Brassicasterol	≤ 0.1% of total sterols	not detected	-	8.7%	0.7
Campesterol	≤ 4.0% of total sterols	3.8%	6.8	29.1%	1.3
Stigmasterol	≤ Campesterol (≤ 4.0% of total sterols)	1.0%	9.0	0.6%	45.0
Δ-7-Stigmastenol	≤ 0.5% of total sterols	not detected	-	0.7%	2.9
Uvaol + Erythrodiol	≤ 4.5% of total sterols	1.8%	31	0.3%	1.0
Total Sterols	**≥ 1000 mg/kg**	**1324 mg/kg**	**6**	**4221 mg/kg**	**1.0**

*Apparent β-sitosterol = β-sitosterol + Δ-5-avenasterol + Δ-5,23-stigmastadienol + clerosterol + sitostanol + Δ-5,24-stigmastadienol. Total sterols = cholesterol + 24-methylene cholesterol + brassicasterol + campesterol + campestanol + stigmasterol + Δ-7-campesterol + Δ-5,23-stigmastadienol + apparent β-sitosterol + Δ-7-avenasterol.

Destination: Cleaner

SLE Application: Acid, Neutrals, and Bases from Urine

Introduction

In most LLE method, extracting multiple pH's is not feasible. We will demonstrate 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 using Novum™ SLE from a urine matrix containing β-glucuronidase followed by two LC-MS/MS methods. This method exhibits the **versatility and effectiveness** using Novum SLE.

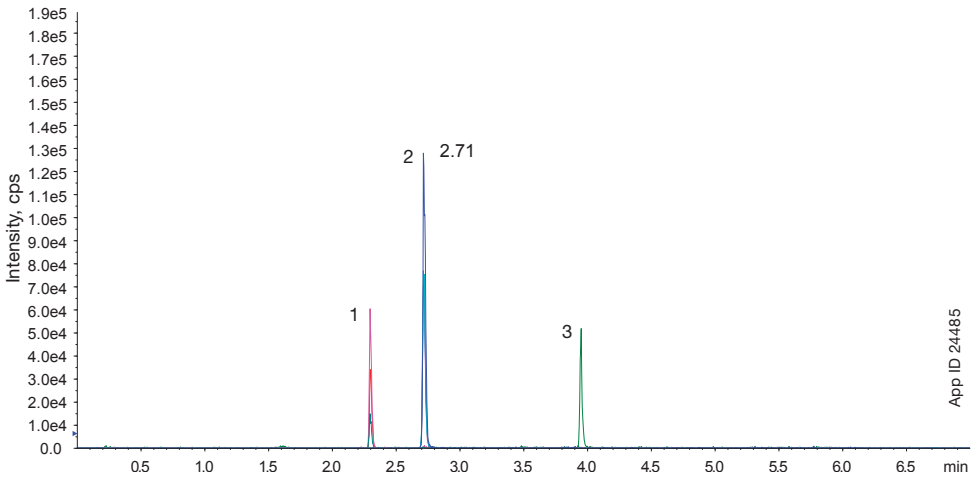
Pre-treatment

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.

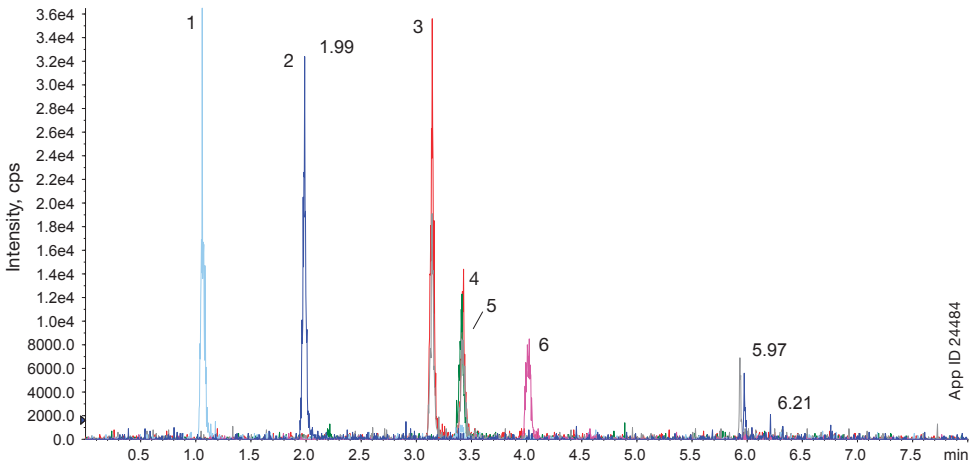
SLE Protocol

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:	2 x 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:	Reconstitute in 100 µL of Methanol/Water (1:4) with 100 ng/mL of COOH-THC-D3, 250 ng/mL of Ammobarbital-D5 and 100 ng/mL of Morphine-D6

ESI+ Chromatogram (Buprenophrine/Norbuprenorphine/THC-COOH)



ESI- Chromatogram (Barbiturates Mix)



Positive Mode LC-MS/MS Conditions

Column:	Kinetex® 2.6 µm Biphenyl 100Å
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 5 5 100 5.1 5 7 5
Flow Rate:	0.5 mL/min
Injection:	4 µL
Temperature:	Ambient
Detection:	MS/MS (SCIEX API 4000™)
Sample:	1. Norbuprenorphine 2. Buprenorphine 3. THC-COOH

Negative Mode LC-MS/MS Conditions

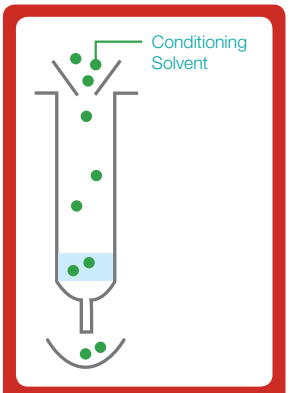
Column:	Kinetex 2.6 µm EVO C18 100Å
Dimensions:	50 x 2.1 mm
Part No.:	00B-4725-AN
Guard Cartridge:	SecurityGuard ULTRA EVO C18
Guard Part No.:	AJ0-9298
Mobile Phase:	A: 10 mM Ammonium bicarbonate, pH 9 B: Acetonitrile
Gradient:	**Time (min) %B** 0 5 2 15 5 20 5.01 60 6 60 6.1 5 7.5 5
Flow Rate:	0.5 mL/min
Injection:	4 µL
Temperature:	Ambient
Detection:	MS/MS (SCIEX API 4000)
Sample:	1. Phenobarbital 4. Amobarbital 2. Butalbital 5. Amobarbital-D5 3. Pentobarbital 6. Secobarbital

Solid Phase Extraction (SPE)

SPE is the most targeted form of sample preparation. It can involve an automatable approach to concentrate samples, clean up matrix effects, and can be used for solvent exchange. SPE offers a variety of key technical advantages and economic benefits that LLE cannot match.

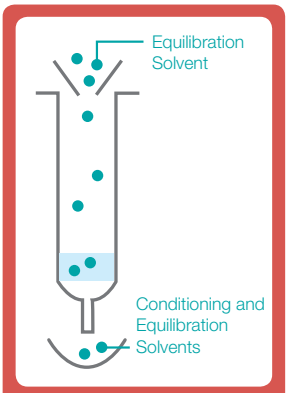
SPE General Protocol

1. CONDITION



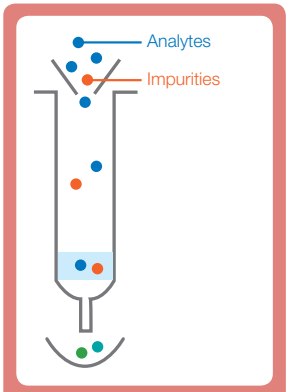
A conditioning step activates the SPE sorbent.

2. EQUILIBRATE



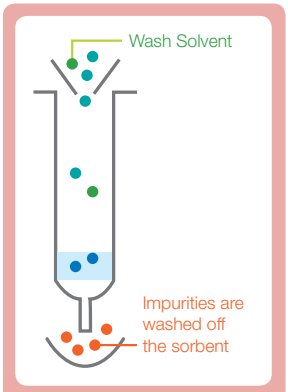
An equilibration solvent prepares the sorbent for interaction with your sample.

3. LOAD SAMPLE



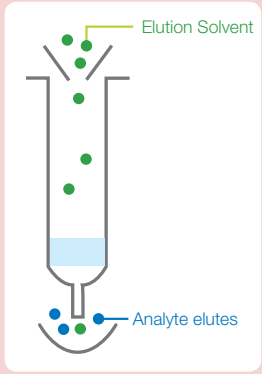
Target analytes are loaded onto sorbent and selectively targeted to bind to the SPE sorbent.

4. WASH IMPURITIES



Interferences are washed away.

5. ELUTE ANALYTE



An elution solvent breaks the bond between the SPE sorbent and your target analytes, allowing them to be collected for further analysis.

Your sample is now clean and concentrated!

Product Recommendation Based on Target Analytes	
Strong Acids (pK_a < 2)	**Strata®-X-AW**
Weak Acids (pK_a 2-4)	**Strata-X-A**
Neutral Compounds	**Strata-X**
Weak Bases (pK_a 8-10)	**Strata-X-C**
Strong Bases (pK_a > 10)	**Strata-X-CW**

See how SPE compares to LLE pp. 12-16

Destination: Cleanest

SPE Application: Increase Recovery of Pharmaceutical Drugs

Introduction

SPE has an improved specificity towards particular analytes and has allowed analysts to improve recovery and reproducibility of their samples. This method explores the distinct differences between SLE and LLE for the isolation of diclofenac, a slightly acidic drug compound, from plasma using a water matrix as the control. It was found that SPE provides **cleaner extracts, higher recoveries, and better reproducibility** which can greatly improve results.

Materials and Methods

The plasma pre-treatment step was the same for SPE and LLE and was comprised of filtration through a gauze cloth. Afterwards, 500 µL of diclofenac, which was dissolved in 5% Methanol, was added to 500 µL of plasma, and the solution mixture was then acidified with 600 µL of 1M Phosphoric acid.

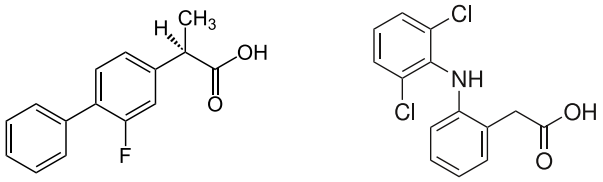
SPE Protocol

Cartridge:	Strata®-X 30 mg on a 1 mL Presston™ 100 Positive Pressure Manifold (Part No.: AH0-9342)
Part No.:	8B-S100-TAK
Condition:	1 mL Methanol
Equilibrate:	2 mL Water
Load:	1.6 mL Pre-treated plasma
Wash:	1 mL 5% Methanol
Dry:	1 minute under vacuum at 10 inches Hg
Elute:	1 mL Methanol
Dry down:	Dry down @ 53 °C under a stream of nitrogen for 20 minutes
Reconstitute:	500 µL of mobile phase

Liquid-Liquid Extraction

1. After pre-treatment, add 5 mL of Hexane/IPA (95:5) to the pre-treated solution
2. Vortex for 1 minute and then centrifuge at 2,000 rpm for 10 minutes
3. Take 4 mL of the top organic layer and transfer to a clean glass centrifuge tube
4. Evaporate to dryness under a stream of nitrogen at 53 °C for 20 minutes

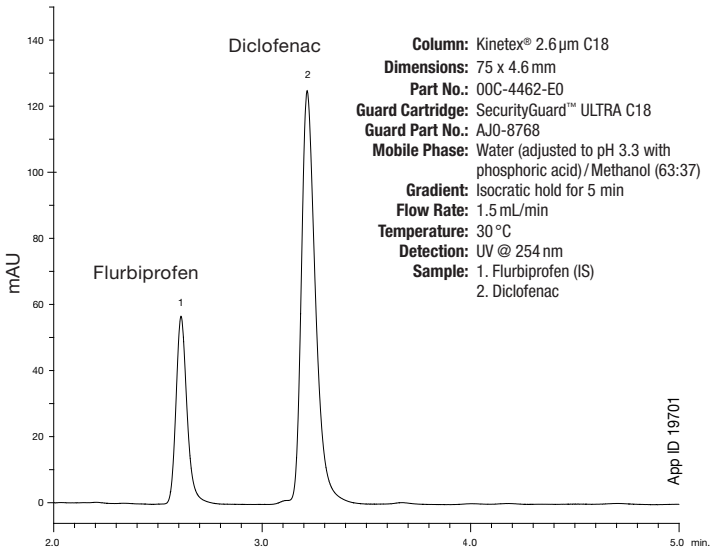
Structure of Internal Standard Flurbiprofen and Diclofenac



Flurbiprofen (pK_a = 4.2) Diclofenac (pK_a = 4.0)

LC-UV

Chromatogram of Diclofenac and IS after SPE extraction from a plasma matrix.

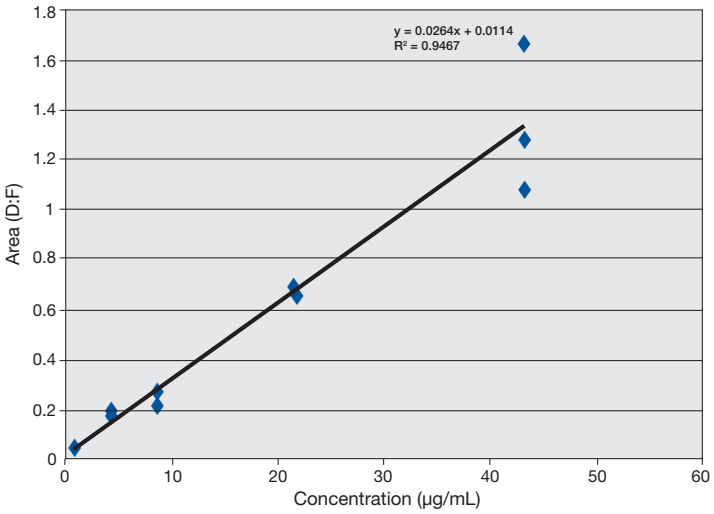


Diclofenac spiked plasma sample (50 µg/mL) after extraction with Strata-X. Flurbiprofen (IS) was added post-extraction at a concentration of 160 µg/mL.

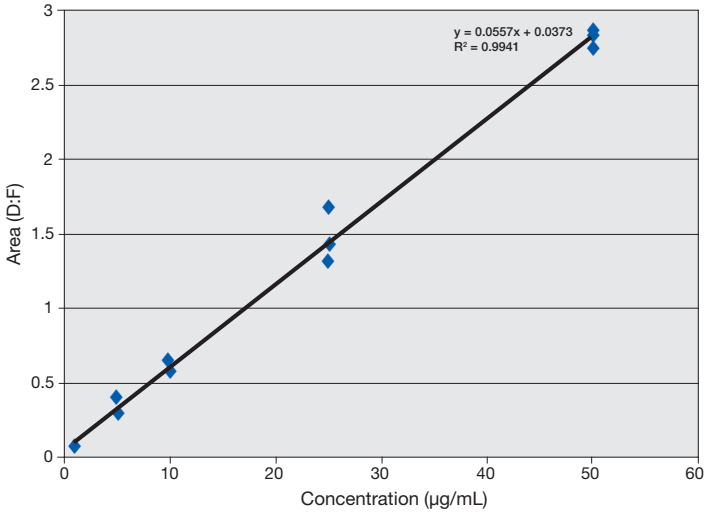
Note: the flurbiprofen was added post-blowdown, which is also post-extraction.

SPE Application: Increase Recovery of Pharmaceutical Drugs *(cont'd)*

Diclofenac Extracted Reference Curve:
Liquid-Liquid Extraction in Plasma Matrix



Diclofenac Extracted Reference Curve:
Solid Phase Extraction in Plasma Matrix



Results and Discussion

It was found that SPE on the Strata®-X sorbent yields approximately 86% absolute recovery of 15µg/mL of diclofenac in the plasma matrix as opposed to 46% for LLE (**Table 1**). This accounts for almost a two-fold decrease in recovery when using LLE. Furthermore, while this procedure for LLE involved one extraction step, in order to have obtained a greater yield, a greater amount of solvent would have been required. This would not have only increased the time required to obtain a higher extraction yield, but would also increase the total time required for the evaporation of the solvent. In addition to SPE providing a greater absolute % recovery by two-fold over LLE the Strata-X sorbent procedure shows less variability between. According to the % RSD values for SPE and LLE, SPE is more precise and reproducible than LLE for the extraction of pharmaceutical compounds.

Consequently, this data shows that SPE provides greater absolute recovery of diclofenac when compared to LLE: SPE is less time-intensive, consumes less solvent than traditional LLE procedures, and provides better reproducibility, thereby demonstrating that the extraction method of choice for pharmaceuticals, such as diclofenac, is SPE.

Table 1.
% Absolute Recovery for Diclofenac

	Spiked concentration (µg/mL)	Diclofenac (% Recovery)	Mean % RSD (n=4)
SPE	15	86	10
LLE	15	46	35

Destination: Cleanest

SPE Application: Improved Analysis of Semivolatile Environmental Pollutants

Introduction

The method for EPA 625, which tests for a wide range of semi-volatile organic pollutants in water, specifies LLE followed by GC-MS analysis. With the importance on increased productivity gains, successful implementation of the SPE technique has gained attention over the traditional LLE method for faster extraction time, reduced solvent use, increased reproducibility, and increased recovery. This method shows an improved SPE methodology that incorporates large-particle polymeric SPE cartridges (Strata®-XL-C) and improvements in drying, which is effective for EPA 625 analytes and is considerably faster and easier than LLE. Following the optimized SPE protocol, the sample is analyzed by GC-MS using a Zebron™ ZB-SemiVolatiles GC column, resulting in a rapid 17 minute run time. By utilizing the large-particle SPE method and GC method outlined here, EPA Method 625 **efficiency**, **reproducibility**, and **productivity are dramatically improved**.

Optimized SPE Protocol for EPA Method 625

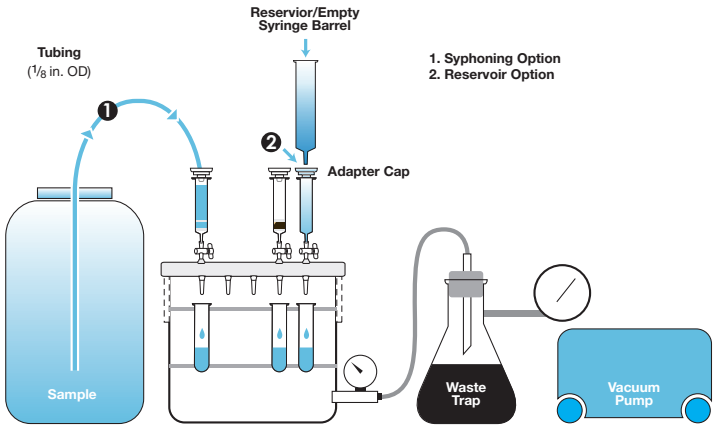
Sample Pre-Treatment

2 mL concentrated HCl was spiked in 1 L of water matrix to target a pH between 1 and 3. 20 µL of each surrogate (acid and base) was spiked at 1000 µg/mL.

SPE Protocol

Cartridge:	Strata-XL-C, 2 g/20 mL Giga™ Tube
Part No.:	8B-S044-KEG
Condition:	10 mL methanol followed by 10 mL DI water
Load:	The pre-treated 1 L water sample was loaded at 10–12 mL/min
Dry:	Reservoir tubes used for loading the sample were removed and the SPE cartridges were dried by applying vacuum (15-20" of Hg) for 4-5 minutes
Elution 1:	• 2 aliquots of 2 mL acetone • 2 aliquots of 2 mL dichloromethane/acetone (3:1) • 3 aliquots of 2 mL dichloromethane
Elution 2:	2 aliquots of 4.5 mL ethyl acetate/methanol (1:1) in 1.5% NH_4OH. (To prepare the aliquots, 9.5 mL of ethyl acetate was combined with 9.5 mL of methanol and 1 mL of 30% NH_4OH and vortexed for 30 seconds)
Water Removal:	Elution 1 and elution 2 fractions were passed through separate Strata Sodium Sulfate 10 g/20 mL cartridges separately to remove water under gravity. Concentrated elution 1 and 2 fractions were collected in two separate test tubes. To collect residual amounts of sample from the Strata Sodium Sulfate cartridges, elution with an additional 4 mL of dichloromethane per cartridge was performed. After the addition of dichloromethane, two layers were formed. The stopcock was opened to collect the bottom organic layer
Dry Down:	Samples were dried using a TurboVap® under nitrogen (no heat) until the volume of elution 1 and elution 2 was reduced to 0.5 mL. Samples were not evaporated to complete dryness to prevent analyte loss
Reconstitute:	Elution 1 and elution 2 fractions were combined (total volume ~1 mL) and reconstituted to a total volume of 4 mL with dichloromethane. 50 µL of internal standard was spiked at 1000 µg/mL

SPE accessories and set-up used for sample processing



GC Conditions

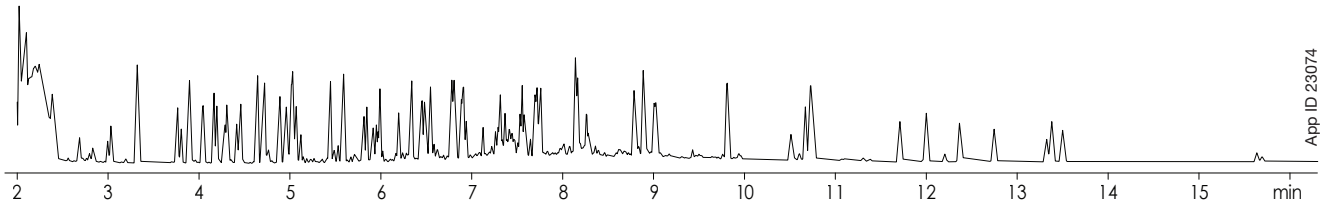
Column: Zebron ZB-SemiVolatiles
Dimensions: 30 meter x 0.25 mm x 0.25 µm
Part No.: 7HG-G027-11
Recommended Liner: Zebron PLUS Single Taper Z-Liner™ (for Agilent® Systems)
Liner Part No.: AG2-0A13-05
Injection: Splitless @ 250 °C, 1 µL
Carrier Gas: Helium @ 1.6 mL/min (constant flow)
Oven Program: 40 °C for 0.66 min to 260 °C @ 30 °C/min to 295 °C @ 6 °C/min
Detector: MSD @ 300 °C; 40-500 amu
Samples: View full list of analytes online

SPE Application: Improved Analysis of Semivolatile Environmental Pollutants *(cont'd)*

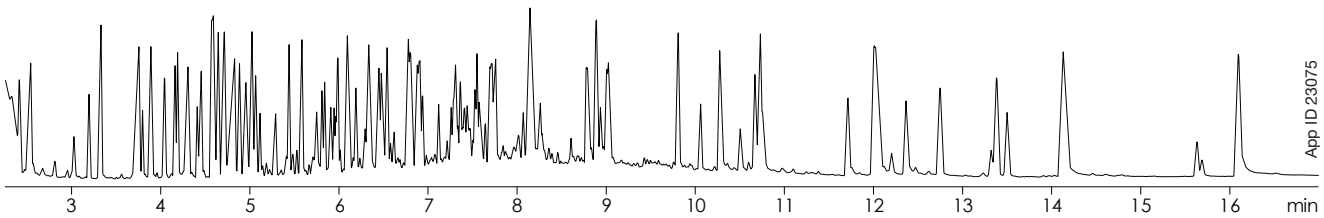
Optimized SPE Method vs. LLE

Protocol Components	Traditional LLE Method	Strata®-XL-C SPE Method	SPE Improvements
Throughput (samples/day)	20	30-35	⬆ **50-75% Increase**
Solvent Usage (mL/sample)	> 360	41	⬇ **Significant Decrease**
Glassware	~ 100 pieces (large)	< 100 test tubes (disposable)	⬇ **Significant Decrease**
Data Quality	Sufficient	Improved	⬆ **Increase**
Manual Labor	High	Very Low	⬇ **Significant Decrease**
Procedural Steps	Dozens	6	⬇ **Significant Decrease**

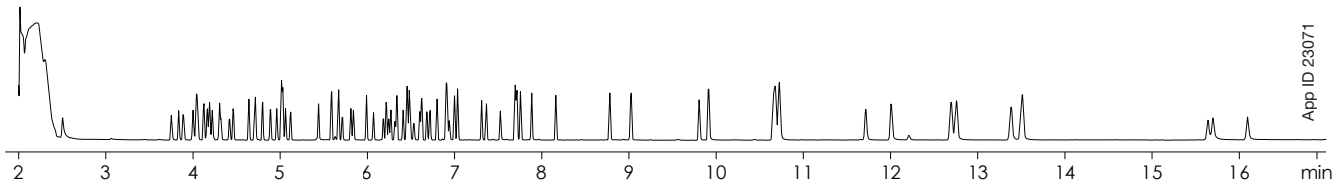
TCLP matrix extraction



Waste water matrix extraction



EPA Method 625 standard curve at 25 µg/mL



Destination: Cleanest

SPE Application: A Sensitive Extraction and Analysis of Urinary Catecholamines

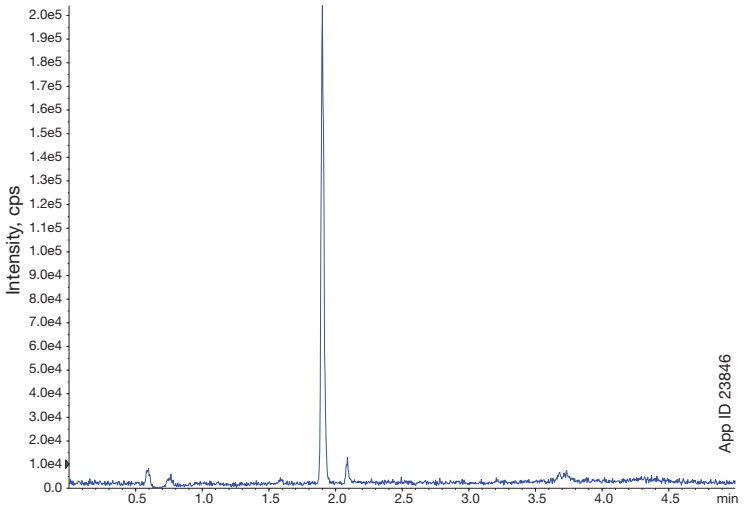
Introduction

As an additional benefit of SPE, the microelution format allows one to use small sample concentrations and still achieve high recoveries and sensitive analyses, all while skipping the dry down step and saving time. LLE uses large solvent volumes and requires high analyte concentrations in order to achieve high recovery and a dry down step that can take a significant amount of time. In this application, an interference that coelutes with 3-Methoxytyramine on a standard C18 HPLC column will be resolved using Strata®-X-CW Microelution SPE 96-Well Plates in conjunction with a Kinetex® Biphenyl HPLC column, while reaching low limits of quantification for specific urinary catecholamines, metanephrine, and normetanephrine. This method displays that microelution SPE can **clean-up and concentrate with small or limited sample volumes**.

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 pretreated sample (500 µL of urine was diluted with 500 µL of 50 mM Ammonium acetate buffer, (pH 7). Urine was pre-spiked with standards
Wash 1:	200 µL of 50 mM Ammonium acetate buffer, pH 7
Wash 2:	200 µL Acetonitrile/IPA (1:1)
Elute:	2 x 25 µL of Water/Acetonitrile/Formic acid (85:10:5)*
Dry Down:	**NOT REQUIRED**. Save 30 minutes or more!
Injection:	Dilute eluent with 100 µL of 0.1% Formic acid in water**

Chromatogram of resolved interference for 3-methoxytyramine at 1 ng/mL



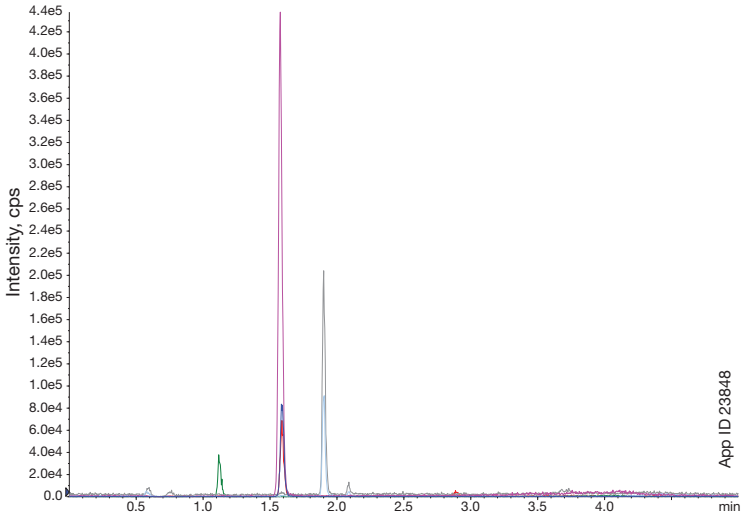
LC-MS/MS Conditions

Column:	Kinetex 5 µm Biphenyl
Dimensions:	50 x 4.6 mm
Part No.:	00B-4627-E0
Guard Cartridge	SecurityGuard™ ULTRA Biphenyl
Guard Part No.:	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
Flow Rate:	0.7 mL/min
Injection:	30 µL
Temperature:	Ambient
Detection:	MS/MS (SCIEX API 4000™)

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
3-Methoxytyramine		
10	91	3
1	89	6
0.5	95	4
0.25	86	5
0.125	87	6
0.063	92	7

Representative Chromatogram of Urinary Catecholamines



SLE Ordering Information

Diatomaceous Earth (DE) SLE

Strata® DE SLE Well Plates

Strata-DE Diatomaceous Earth SLE Well Plates		
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

Strata DE SLE Tubes

Strata-DE Diatomaceous Earth SLE Tubes		
Part No.	**Description**	**Unit**
8B-S325-KDG	Strata DE SLE 12 cc Tubes	20/pk
8B-S325-VFF	Strata DE SLE 60 cc Tubes	16/pk

Synthetic SLE

Novum™ SLE 96-Well Plates

Novum Simplified Liquid Extraction SLE Well Plates		
Part No.	**Description**	**Unit**
8E-S138-FGA	Novum SLE MINI 96-Well Plate	1/pk
8E-S138-5GA	Novum SLE MAX 96-Well Plate	1/pk

Novum SLE Tubes

Novum Simplified Liquid Extraction SLE Tubes		
Part No.	**Description**	**Unit**
8B-S138-FAK	Novum SLE 1 cc Tubes	100/pk
8B-S138-5BJ	Novum SLE 3 cc Tubes	50/pk
8B-S138-JCH	Novum SLE 6 cc Tubes	30/pk
8B-S138-KDG	Novum SLE 12 cc Tubes	20/pk

presston™ 100

guarantee

If Phenomenex SLE products do not perform as well or better than your current SLE product, return the product with comparative data within 45 days for a FULL REFUND.

Presston 100 Positive Pressure Manifold

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

The Presston 100 96-Well Positive Pressure Manifold can also process 1, 3, and 6 mL tubes using the following adapter kits

Presston 100 Tube Adapter Kits (for AH0-9334)	
Part No.	**Description**
AH0-9344	1 mL Tube Adapter Kit
AH0-9345	3 mL Tube Adapter Kit
AH0-9346	6 mL Tube Adapter Kit

Vacuum Manifold

Vacuum Manfolds		
Part No.	**Description**	**Unit**
AH0-6023	12-Position Vacuum Manifold Set	ea
AH0-6024	24-Position Vacuum Manifold Set	ea
AH0-8950	96-Well Plate Manifold, Universal with Vacuum Gauge	ea

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.

SPE Ordering Information

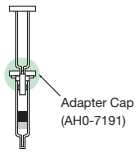
SPE Tubes

Process Multiple Samples at Once

Tubes + Positive Pressure Manifold OR Vacuum Manifold

Process Samples Manually

Syringe + Adapter Cap



Strata® Silica-Based Sorbents

Tubes	1 mL (100/box)		3 mL (50/box)			6 mL (30/box)		
Phase	50 mg	100 mg	100 mg	200 mg	500 mg	200 mg	500 mg	1 g
C18-E	8B-S001-DAK	8B-S001-EAK	8B-S001-EBJ	8B-S001-FBJ	8B-S001-HBJ	8B-S001-FCH	8B-S001-HCH	8B-S001-JCH
C18-U	—	8B-S002-EAK	—	8B-S002-FBJ	8B-S002-HBJ	—	8B-S002-HCH	8B-S002-JCH
C18-T	—	8B-S004-EAK	—	8B-S004-FBJ	8B-S004-HBJ	—	8B-S004-HCH	8B-S004-JCH
C8	—	8B-S005-EAK	—	8B-S005-FBJ	8B-S005-HBJ	—	8B-S005-HCH	8B-S005-JCH
Phenyl	—	8B-S006-EAK	—	8B-S006-FBJ	8B-S006-HBJ	—	8B-S006-HCH	8B-S006-JCH
SCX	—	8B-S010-EAK	8B-S010-EBJ	8B-S010-FBJ	8B-S010-HBJ	—	8B-S010-HCH	8B-S010-JCH
WCX	—	8B-S027-EAK	—	8B-S027-FBJ	8B-S027-HBJ	—	8B-S027-HCH	8B-S027-JCH
SAX	—	8B-S008-EAK	8B-S008-EBJ	8B-S008-FBJ	8B-S008-HBJ	—	8B-S008-HCH	8B-S008-JCH
NH_2	—	8B-S009-EAK	—	8B-S009-FBJ	8B-S009-HBJ	—	8B-S009-HCH	8B-S009-JCH
CN	—	8B-S007-EAK	—	8B-S007-FBJ	8B-S007-HBJ	—	8B-S007-HCH	8B-S007-JCH
Si-1	—	8B-S012-EAK	—	8B-S012-FBJ	8B-S012-HBJ	—	8B-S012-HCH	8B-S012-JCH
Florisil®	—	—	—	—	8B-S013-HBJ	—	8B-S013-HCH	8B-S013-JCH
EPH	—	—	—	—	8B-S031-HBJ	—	—	—
AL-N	—	—	—	—	8B-S313-HBJ	—	—	8B-S313-JCH

Mixed-mode sorbents (for drugs of abuse)

Tubes	1 mL (100/box)		3 mL (50/box)			6 mL (30/box)		
Phase	—	100 mg	100 mg	150 mg	200 mg	200 mg	500 mg	—
Screen-C	—	8B-S016-EAK	8B-S016-EBJ	8B-S016-SBJ	8B-S016-FBJ	8B-S016-FCH	8B-S016-HCH	—
Screen-A	—	8B-S019-EAK	—	—	8B-S019-FBJ	8B-S019-FCH	8B-S019-HCH	—

Polymeric sorbents

Tubes	1 mL (100/box)		3 mL (50/box)			6 mL (30/box)		
Phase	50 mg	100 mg	—	200 mg	500 mg	200 mg	500 mg	1 g
SDB-L	8B-S014-DAK	8B-S014-EAK	—	8B-S014-FBJ	8B-S014-HBJ	8B-S014-FCH	8B-S014-HCH	8B-S014-JCH

Strata-X Polymer-Based Sorbents

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

Accessories For Tubes

Adapter Caps		
Part No.	Description	Unit
AH0-7191	Adapter Caps for 1, 3, and 6 mL SPE tubes, polyethylene, with Luer tip	15/pk

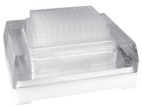
SPE Ordering Information

SPE 96-Well Plates

Process Samples with a Vacuum Manifold

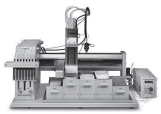
96-Well Plate | Positive Pressure Manifold | 96-Well Plate Manifold


+

OR

OR

Process Samples with a Robot

Robot



Strata®-X Polymer-Based Sorbents

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 Plates

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

Round Well Collection Plates (polypropylene)

Part No.	Well Bottom	Well Volume	Unit	Suggested Sealing Mats
AH0-7279	Round	1 mL	50/pk	AH0-8631 AH0-8632
AH0-8636	Round	2 mL	50/pk	AH0-8633 AH0-8634

Square Well Collection Plates (polypropylene)

Part No.	Well Bottom	Well Volume	Unit	Suggested Sealing Mats
AH0-7192	Conical	350 µL	50/pk	AH0-8597 AH0-8598 AH0-8199 AH0-7195
AH0-7193	Conical	1 mL	50/pk	AH0-8597 AH0-8598 AH0-8199 AH0-7195
AH0-7194	Conical	2 mL	50/pk	AH0-8597 AH0-8598 AH0-8199 AH0-7195
AH0-8635	Round-Conical	2 mL	50/pk	AH0-8597 AH0-8598 AH0-8199 AH0-7195

Strata Silica-Based Sorbents

96-Well Plates (2/Box)			
Phase	25 mg	50 mg	100 mg
C18-E	8E-S001-CGB	8E-S001-DGB	8E-S001-EGB
C18-U	—	8E-S002-DGB	8E-S002-EGB
C18-T	8E-S004-CGB	8E-S004-DGB	—
C8	8E-S005-CGB	—	—
Phenyl	8E-S006-CGB	—	8E-S006-EGB
Silica	—	8E-S012-DGB	8E-S012-EGB
NH_2	8E-S009-CGB	8E-S009-DGB	8E-S009-EGB
SAX	8E-S008-CGB	8E-S008-DGB	8E-S008-EGB
SCX	8E-S010-CGB	8E-S010-DGB	8E-S010-EGB
WCX	8E-S027-CGB	8E-S027-DGB	—
Screen-C	—	8E-S016-DGB	8E-S016-EGB
SDB-L	—	8E-S014-DGB	—

Round Well Sealing Mats

Part No.	Description	Material	Unit
AH0-8631	Pierceable, 7 mm diameter	Silicone	50/pk
AH0-8632	Pre-Slit, 7 mm diameter	Silicone	50/pk
AH0-8633	Pierceable, 8 mm diameter	Silicone	50/pk
AH0-8634	Pre-Slit, 8 mm diameter	Silicone	50/pk
AH0-7362	Sealing Tap Pad	—	10/pk

Square Well Sealing Mats

Part No.	Description	Material	Unit
AH0-8597	Pierceable	Silicone	50/pk
AH0-8598	Pre-Slit	Silicone	50/pk
AH0-8199	Pierceable	Santoprene™	100/pk
AH0-7195	Pierceable	Ethylene Vinyl Acetate (EVA)	50/pk
AH0-7362	Sealing Tap Pad	—	10/pk

guarantee

If Phenomenex SPE products do not perform as well or better than your current SLE product, return the product with comparative data within 45 days for a FULL REFUND.

Using Liquid-Liquid Extraction (LLE)?

There's a Cleaner Way!

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