Sample Preparation Method Development for Complex Matrices Such as Foods by QuEChERS and Solid Phase Extraction (SPE)

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Sample preparation is an integral step in any analysis. The lack of proper sample preparation in food analysis can lead to major instrumentation and analytical challenges downstream. *However, a substantial amount of time may be required to develop or optimize a sample preparation method. In this technical note, we will evaluate the use of two sample preparation techniques commonly used in food analysis, QuEChERS and Solid Phase Extraction (SPE). Additionally we will discuss tips and considerations to streamline the method development and optimization process.*

Introduction

With the increasing demand for food safety and quality testing, sample preparation of matrices such as fruits, vegetables, dairy, and meats followed by downstream HPLC/UHPLC, LC/MS/MS, or GC/MS analysis is common practice. As regulations evolve to become more stringent, analysts are challenged to develop robust analytical methods that can reach even lower quantitation and detection levels. Sample preparation becomes significant in this analytical process due to two primary reasons: 1. food testing assays tend to include many analytes with varying chemical properties and 2. food sample matrices are complex and can often contain compounds that can largely interfere with analysis. In this technical note, we will evaluate the two most effective sample preparation techniques that are typically used in food testing, providing proper procedures for method development and tips and considerations for method optimization.

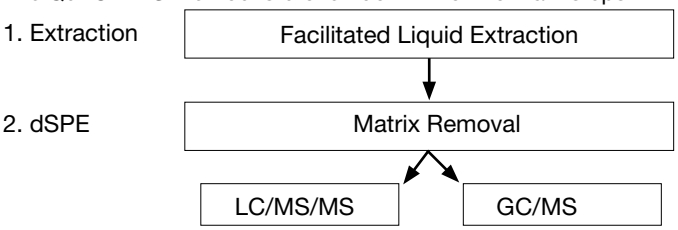
Sample Preparation Techniques

Higher Selectivity (Cleaner)	Solid Phase Extraction (SPE)
	QuEChERS
	Liquid-Liquid Extraction (LLE)
	Filtration
	Centrifugation
	Settle and Decant
	Homogenization
Lower Selectivity (Dirtier)	Dilution (dilute and shoot)
	Weighing

QuEChERS Technique

QuEChERS, an acronym for "Quick Easy Cheap Effective Rugged Safe", is a sample preparation approach that was introduced in 2002 at the European Pesticide Residues Workshop in Rome. Developed by Lehotay, et al., QuEChERS was established to extract and analyze multi-residue pesticides from food samples. The method was published in the Journal of AOAC in 2003.[1] The main advantage of QuEChERS is its ability to remove a large quantity of unwanted interferences from a large variety of food matrices in a Quick, Easy, Cheap, Effective, Rugged, and Safe process.

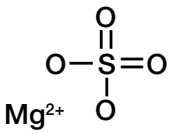
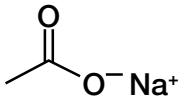
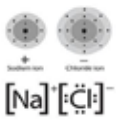
The QuEChERS method is broken down into two main steps.



Step 1 - Extraction

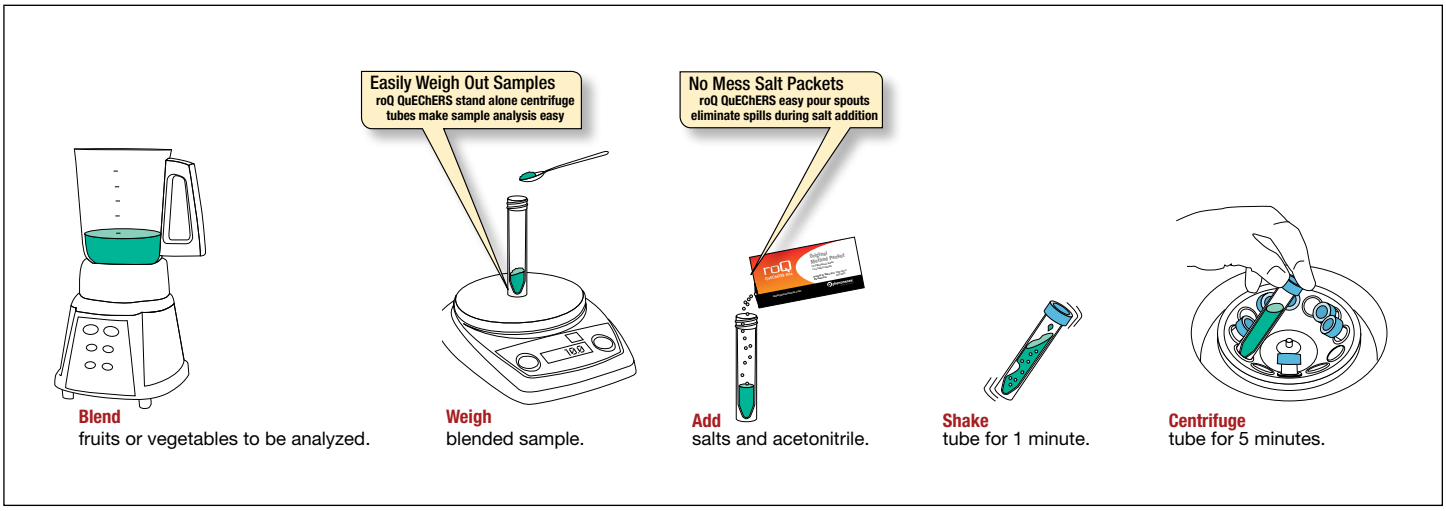
The purpose of the extraction step is to extract analytes from any given sample matrix by using a combination of magnesium sulfate (to induce phase separation and LLE partitioning), extraction solvents, and buffering salts (to stabilize base sensitive analytes). Analytes of interest will partition into the organic solvent and physical matrix interferences will partition into the aqueous solvent which will then be discarded. Sample matrices can be solids, semi-solids, small volumes of liquid, or viscous liquids.

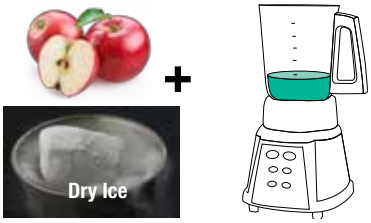
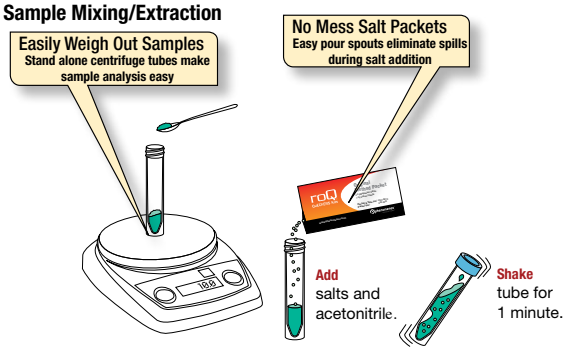
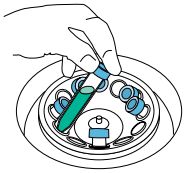
Salts and Buffers used in roQ™ Extraction Kit

dSPE Sorbent	Details
Magnesium Sulfate ($MgSO_4$) 	Induces phase separation between water content in sample and acetonitrile layer
Sodium Acetate (NaOAc) 	Buffers the sample to stabilize pH. Used for AOAC 2007.01 method.
Sodium Chloride (NaCl) 	Induces phase separation between water content in sample and acetonitrile layer. Used for Original Non-Buffered method and EN 15662 method.
Sodium Citrate Tribasic Dihydrate (SCTD)	Buffers the sample to stabilize pH. Used for EN 15662 method.
Sodium Citrate Dibasic Sesquihydrate (SCDS)	Buffers the sample to stabilize pH. Used for EN 15662 method.

The following events take place during the extraction steps:

1. Sample is homogenized
2. Sample is transferred to an extraction tube
3. Organic solvent and salts are added, the sample is then shaken by hand
4. Extraction tube is centrifuged to pellet homogenate
5. Top layer of solvent is extracted and is further cleaned up during the dSPE step



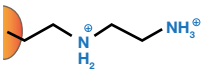
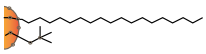
Extraction Steps	Tips and Considerations
Sample Homogenization 	**Sample Comminution Process** • It is essential prior to extraction to homogenize your sample thoroughly to achieve good extraction efficiency. • It is recommended to freeze sample with dry ice or liquid nitrogen and blend until a powdery consistency is achieved. • Use of dry ice/liquid nitrogen during homogenization greatly improves sample homogeneity. • The increase in surface area from homogenized frozen sample enhances extraction efficiency. • As an added benefit, the frozen, powdery sample is easier to handle than unfrozen samples.
Sample Mixing/Extraction 	• Longer shaking times can provide better extraction. • Water can be added to facilitate extraction for samples with low water content. • Extraction solvents can be modified to include a stronger solvent such as chloroform or toluene to improve analyte partitioning. • The buffered kits work well for base sensitive analytes. For stable analytes, the original non-buffered kit will suffice.
Centrifugation 	Centrifuge tube to a pellet homogentate and transfer organic layer to be used during the dSPE step

Step 2 - Dispersive Solid Phase Extraction (dSPE)
The goal of the dSPE step is to remove undesired chemical matrix interferences such as lipids, organic acids, sugars, and pigments. These chemical interferences are damaging to instrumentation and can cause signal enhancement or suppression leading to inaccurate results. Typically, end-capped C18 (C18-E), primary secondary amine (PSA), and graphitized carbon black (GCB) SPE sorbents are used to remove these interferences.

The following events take place during the dSPE step:

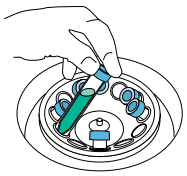
1. Solvent extracted from Extraction step is added to a dSPE tube which contains a combination of dSPE sorbents and salts
2. Tube is shaken by hand and centrifuged
3. Supernatant is ready for analysis by GC or LC/MS/MS

Salts and Sorbents used in roQ™ dSPE Tubes

dSPE Sorbent	Structure/image	Details
Magnesium Sulfate ($MgSO_4$)		Removes excess water from sample
Primary/Secondary Amine (PSA)		Removes organic acids, fatty acids, sugars, and anthrocyanin pigments from sample. 50 mg per mL of extract for AOAC 2007.01 method. 25 mg per mL of extract for EN 15662 method.
Endcapped C18 Sorbent (C18E)		Removes fats, sterols, and non-polar interferences. 50 mg per mL of extract for AOAC 2007.01 method. 25 mg per mL of extract for EN 15662 method
Graphitzed Carbon Black (GCB)		Removes chlorophyll and other pigments. 50 mg per mL of extract for AOAC 2007.01 method. 2.5 or 7.5 mg per mL of extract for EN 15662 method.

roQ QuEChERS dSPE Kit Selection Chart

Sample	dSPE Kit
High Water Content Lightly Pigmented, Contains No Fats (Example-apples, oranges, lettuce)	$MgSO_4$ / PSA
Rich in Fats and Waxes Lightly Pigmented (Example- coconuts, avocados nuts, seeds)	$MgSO_4$ / PSA / C18
Pigmented, Contains Little Fat (Example-spinach, berries, peppers)	$MgSO_4$ / PSA / GCB
Rich in Both Pigments and Fats (Example-chocolate, black olives)	$MgSO_4$ / PSA / GCB / C18

dSPE Sorbent	Tips and Considerations
Sample addition to dSPE  Add Sample	• dSPE kits are selected based on the nature of your sample. • 1 mL dSPE kits are appropriate for laboratories using large volume injection for GC/MS. • 8 mL kit requires concentration of the final extract and solvent exchange to toluene for GC/MS in order to achieve 10 ng/g detection of the pesticides. • PSA sorbent can retain acidic analytes. If this is a concern, the dSPE step can be omited and supernatant can be directly analyzed from the extraction step. • It is best to compare results with and without dSPE using PSA to decide whether a PSA cleanup is appropriate for your sample. • Bulk sorbent can be added to pre-weighed dSPE kits to create various sorbent combinations that can best clean up the sample.
Sample mixing  Shake tube for 1 minute.	
Centrifugation 	

Method Development Flow

Step	Description
1.	What is your sample and what is your target matrix?
2.	Select roQ Extraction kit based on analyte stability
3.	Select roQ dSPE kit based on interferences present in matrix
4.	Perform the two-step QuEChERS procedure
5.	Evaluate results and optimize

For assistance in selecting the appropriate roQ Extraction and dSPE kits for your analysis, please visit www.phenomenex.com/roq or contact your local Phenomenex Sample Preparation Specialist.

Solid Phase Extraction (SPE)

SPE is one of the most selective sample preparation techniques used in food safety and quality testing. SPE is a technique in which a chemically modified solid stationary phase is used to selectively interact, retain, and extract target analytes from a sample, resulting in the removal of contaminants and concentration of the analytes. SPE addresses the 3 primary goals of sample preparation: analyte extraction, concentration, and solvent switching.

The power and versatility of SPE comes from the users' ability to choose a sorbent chemistry that selectively interacts with the analytes of interest or with the matrix interferences. By adjusting the chemical nature of the sorbent and the buffer conditions used during the loading, washing, and elution steps, we can develop a method that can be very selective to clean and isolate our target analytes from complex sample matrices.

SPE Procedure Steps:

1. Condition sorbent to prepare for interaction with sample.
2. Load pre-treated sample onto SPE sorbent (target analyte will be retained on the sorbent).
3. Wash sorbent to remove unwanted interferences that are not retained on the sorbent.
4. Elute target analytes (using a combination of organic strengths and buffers).
5. Analyze clean eluent by GC or HPLC.

Method Development Steps

1. Sample Pre-treatment

Pre-treat sample to maximize designated interactions with SPE sorbent (via LLE, homogenization, buffering, etc.)

Reproducible, high efficiency SPE requires that the sample be made liquid prior to loading onto a SPE device. The SPE sample should meet the following conditions:

- Liquid of low viscosity (to pass through the cartridge).
- Low solids or particulate contaminants (to prevent clogging).
- Solvent composition that is suitable for retention (each mechanism has different matrix solvent composition requirements for proper retention).

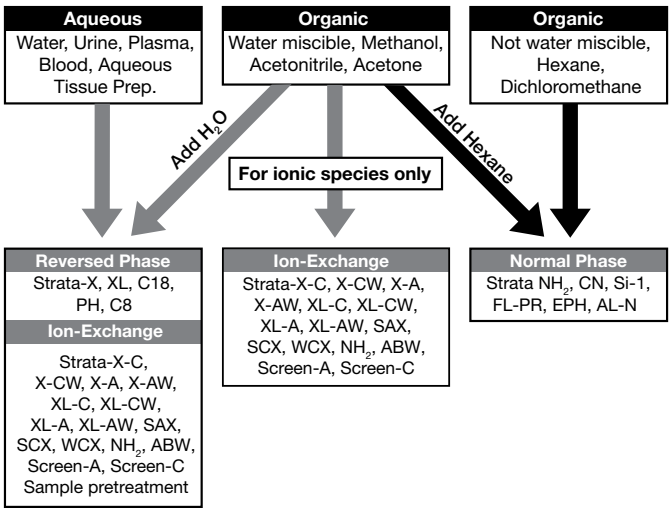
Sample Pre-treatment Recommendations

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.
Water	
Soil, Sludge	Homogenize with organic or aqueous solvent depending upon analyte solubility. Settle, decant and filter supernatant; perform Soxhlet extraction.
Fruit, Vegetable, Herbs	Homogenize with organic or aqueous solvent depending upon analyte solubility and filter supernatant. Use appropriate SPE mechanism for the dissolution solvent (hexane = polar mechanism; aqueous = non-polar mechanism; methanol/ACN = either non-polar or polar after proper dilution).

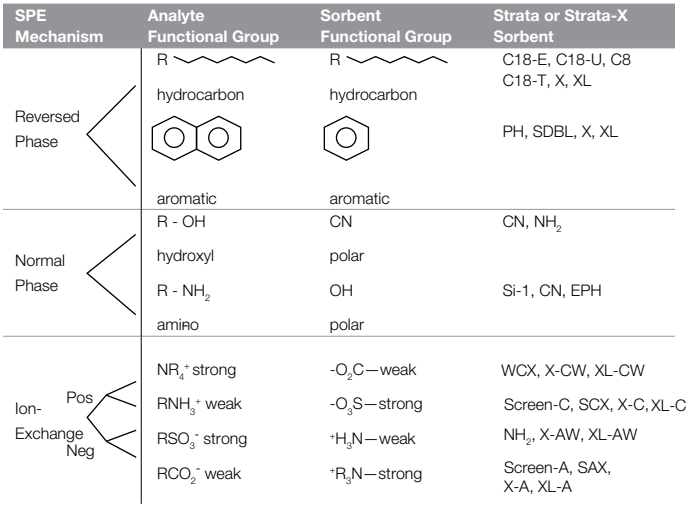
2. Extraction Mechanism

Choose appropriate extraction mechanism and SPE sorbent based on sample matrix and analyte properties (online method development resources are available such as the Phenomenex online SPE Method Development tool, www.phenomenex.com/MDTool)

- Determine the best SPE retention mechanism: Reversed Phase (RP), Ion-Exchange (IEX) or Normal Phase (NP). The sample solvent composition will guide you towards an appropriate SPE mechanism.



- Once the general mechanism is identified, it will be necessary to identify the most specific Strata® or Strata™-X sorbent by matching the analyte functional groups to the sorbent functional group.



3. Sorbent Mass Selection

To select the proper sorbent mass, it is first necessary to determine the volume of sample to be extracted in order to meet method detection limits (not including buffer). Two tables are included below: Polymer-based and silica-based. This is necessary because the large surface area of polymeric sorbents such as Strata-X have a higher analyte capacity per gram than Strata silica-based sorbents.

Suggested Loading Capacity

Polymer-Based Sorbents

Strata-X, Strata-XL

Sample Matrix	Sorbent Selection Guideline	
Filtered Sample Homogenates	100 mg sorbent per 100 mg sample	50 mg sorbent per 100 mg sample
Water (particulate-free) drinking	200 mg sorbent per 100-400 mL sample	200 mg sorbent per 200 mL sample

Silica-Based Sorbents

Strata C18, C8, SCX, SAX, WCX, NH2, etc

Sample Matrix	Sorbent Selection Guideline
Filtered Sample Homogenates	50 mg sorbent/100 mg sample
Water (particulate- free) drinking	500 mg sorbent per 100 mL-500 mL sample
Soil extracts or Equivalent	1 g Sorbent per 100 g of soil extract

4. Method and Solvent Volume Selection

The volume of solvent needed for the wash and elution steps is directly related to the mass of sorbent in the SPE tube and more specifically the "bed volume" of the SPE device. Intuitively we know more sorbent requires more solvent, less sorbent requires less solvent. Typically 4 – 16 bed volumes are used in SPE methods.

Sorbent Wash and Elution Volumes*

Strata Silica-Based Sorbent Mass	Practical Minimum Wash and Elution Volume 4 bed volumes	Recommended Wash and Elution Volume 8 bed volumes	Polymer-Based Sorbent Mass*	Practical Minimum Wash and Elution Volume 4 bed volumes	Recommended Wash and Elution Volume 8 bed volumes
10 mg	60 µL	120 µL	10 mg	100 µL	200 µL
—	—	—	30 mg	300 µL	600 µL
50 mg	300 µL	600 µL	—	—	—
—	—	—	60 mg	600 µL	1.2 mL
100 mg	600 µL	1.2 mL	100 mg	1 mL	2 mL
150 mg	900 µL	1.8 mL	150 mg	1.5 mL	3 mL
200 mg	1.2 mL	2.4 mL	200 mg	2 mL	4 mL
500 mg	3 mL	6 mL	500 mg	5 mL	10 mL
1 g	6 mL	12 mL	1 g	10 mL	20 mL
2 g	12 mL	24 mL	—	—	—
5 g	30 mL	60 mL	—	—	—
10 g	60 mL	120 mL	—	—	—

*Strata-X polymeric resins have a larger surface area than Strata silica-based material, hence requiring slightly more solvent per gram for processing. The elution volumes are specific to the chemical nature of the analyte being extracted, its concentration in the sample, the chemical nature of the eluting solvent and the bed mass used. The above is a guideline. An elution study should be conducted to determine the appropriate volume to use.

5. Generic Method

Each SPE mechanism/phase has a general set of solvent conditions under which SPE may be performed. Use the solvents/pH conditions listed below to determine the best method for your extraction. Volumes as determined in Method and Sorbent Volume step.

	Reversed Phase SPE Method		Normal Phase SPE Method		Strong Ion-Exchange SPE Method	
Sorbent	SDB-L, C18, C8, PH, CN, X, XL		Silica, Florisil®, NH_2, CN		Anion exchange: Screen-A, SAX, X-A, X-AW, XL-A, XL-AW Cation exchange: Screen-C, SCX, X-C, X-CW, XL-C, XL-CW	
	Guideline	**Example**	**Guideline**	**Example**	**Guideline**	**Example**
Analyte Properties	Low to moderate polarity (or non-polar) Hydrophobic Neutralized/uncharged	Pharmaceuticals Pesticides, herbicides	Moderate to high polarity compounds (neutralized/uncharged)	Pesticides	Ionized/charged compounds	Anion exchange: Acidic analytes Cation exchange: Basic analytes
Sample/Matrix	Aqueous, diluted with buffer	Biological fluids Water	Non-polar organic solvents or moderately polar organic solvents	Hexane, chloroform, petroleum ether, toluene or methylene chloride	Aqueous; Low ionic strength buffers (<30 mM), pH adjusted	Biological fluids plus buffer
Conditioning Step	Polar organic solvents	Methanol	Polar organic solvents (optional)	Methanol (optional)	Polar organic solvents	Methanol
Equilibration	Aqueous, buffers	Water or buffer	Sample/matrix solvent from above	Hexane or chloroform	Low ionic strength buffers, pH adjusted	25 mM Tris-OAc, pH 7
Wash Step	Aqueous buffers with 5 to 50 % polar organic solvent	Methanol: Water (5:95)	Non-polar organic solvents with a low concentration (1 to 5 %) of moderate to low polarity organic solvents	Hexane with 1-5 % THF, ethyl acetate, acetone, acetonitrile or IPA	Aqueous buffers of low salt concentrations with or without organic solvent	Anion exchange: Buffer pH 7: Methanol (50:50) Cation exchange: 1. Buffer pH 6 2. 1 M acetic acid 3. Methanol
Elution Step	Polar or non-polar organic solvent(s) with or without water, buffer and/or strong acid or base	Methanol: Acetonitrile (50:50)	Non-polar organic solvents containing higher concentrations (5 to 50 %) of moderate to high polarity organic solvents	Hexane with 10 % THF, ethyl acetate, acetone, acetonitrile or IPA	• Neutralize the charge on the weak anion or cation • Increase the ionic strength and counter ion concentration • Add a strong counter ion displacer	Methanol with 5 % Acid/Base modifier

Conclusion

Sample preparation is an integral step in any analysis. The lack of proper sample preparation in food analysis can lead to major instrumentation and analytical challenges downstream. Lack of sample preparation can lead to increased LC method development. The analysis is more straightforward if interferences are removed prior to analysis rather than performing LC method development to resolve analyte signals from interferences. Proper sample preparation should also concentrate analytes and increase method sensitivity. Knowing which sample preparation technique to use for the goals at hand is beneficial in achieving the desired outcome. In food analysis, the use of QuEChERS and SPE are the most commonly used approaches to prepare samples. No one approach is better than another. Rather, each approach has its own strengths in achieving the desired outcome of the analysis. Knowing the strengths and uses of each procedure can significantly improve your analysis.

For assistance in selecting the appropriate Sample Preparation for your analysis, please visit www.phenomenex.com/Sampleprep or contact your local Phenomenex Sample Preparation Specialist.

References

1. Lehotay, S. J., de Kok, A., Hiemstra, M., van Bodegraven, P. Validation of a Fast and Easy Method for the Determination of Residues from 229 Pesticides in Fruits and Vegetables Using Gas and Liquid Chromatography and Mass Spectrometric Detection. JAOAC Int, 2005, 88(2) 595- 614
2. Phenomenex, SPE Reference manual and users' guide, 2010

Ordering Information

Tubes	3 mL (50/box)			6 mL (30/box)		
Strata® Silica-based SPE sorbents						
Phase	**100 mg**	**200 mg**	**500 mg**	**200 mg**	**500 mg**	**1 g**
C18-E	8B-S001-EBJ	8B-S001-FBJ	8B-S001-HBJ	8B-S001-FCH	8B-S001-HCH	8B-S001-JCH
C18-U	—	8B-S002-FBJ	8B-S002-HBJ	—	8B-S002-HCH	8B-S002-JCH
C18-T	—	8B-S004-FBJ	8B-S004-HBJ	—	8B-S004-HCH	8B-S004-JCH
C8	—	8B-S005-FBJ	8B-S005-HBJ	—	8B-S005-HCH	8B-S005-JCH
Phenyl	—	8B-S006-FBJ	8B-S006-HBJ	—	8B-S006-HCH	8B-S006-JCH
SCX	8B-S010-EBJ	8B-S010-FBJ	8B-S010-HBJ	—	8B-S010-HCH	8B-S010-JCH
WCX	—	8B-S027-FBJ	8B-S027-HBJ	—	8B-S027-HCH	8B-S027-JCH
SAX	8B-S008-EBJ	8B-S008-FBJ	8B-S008-HBJ	—	8B-S008-HCH	8B-S008-JCH
NH_2	—	8B-S009-FBJ	8B-S009-HBJ	—	8B-S009-HCH	8B-S009-JCH
CN	—	8B-S007-FBJ	8B-S007-HBJ	—	8B-S007-HCH	8B-S007-JCH
Si-1	—	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
Strata Polymeric SPE sorbents						
Phase	**—**	**200 mg**	**500 mg**	**200 mg**	**500 mg**	**1 g**
SDB-L	—	8B-S014-FBJ	8B-S014-HBJ	8B-S014-FCH	8B-S014-HCH	8B-S014-JCH
Strata™-X Polymeric SPE sorbents						
Phase	**60 mg**	**200 mg**	**500 mg**	**100 mg**	**200 mg**	**500 mg**
Strata-X	8B-S100-UBJ	8B-S100-FBJ	8B-S100-HBJ	8B-S100-ECH	8B-S100-FCH	8B-S100-HCH
Strata-X-C	8B-S029-UBJ	8B-S029-FBJ	8B-S029-HBJ	8B-S029-ECH	8B-S029-FCH	8B-S029-HCH
Strata-X-CW	8B-S035-UBJ	8B-S035-FBJ	8B-S035-HBJ	8B-S035-ECH	8B-S035-FCH	8B-S035-HCH
Strata-X-A	8B-S123-UBJ	8B-S123-FBJ	8B-S123-HBJ	8B-S123-ECH	8B-S123-FCH	8B-S123-HCH
Strata-X-AW	8B-S038-UBJ	8B-S038-FBJ	8B-S038-HBJ	8B-S038-ECH	8B-S038-FCH	8B-S038-HCH
Strata-XL	8B-S043-UBJ	8B-S043-FBJ	8B-S043-HBJ	8B-S043-ECH	8B-S043-FCH	8B-S043-HCH
Strata-XL-C	8B-S044-UBJ	8B-S044-FBJ	8B-S044-HBJ	8B-S044-ECH	8B-S044-FCH	8B-S044-HCH
Strata-XL-CW	8B-S052-UBJ	8B-S052-FBJ	8B-S052-HBJ	8B-S052-ECH	8B-S052-FCH	8B-S052-HCH
Strata-XL-A	8B-S053-UBJ	8B-S053-FBJ	8B-S053-HBJ	8B-S053-ECH	8B-S053-FCH	8B-S053-HCH
Strata-XL-AW	8B-S051-UBJ	8B-S051-FBJ	8B-S051-HBJ	8B-S051-ECH	8B-S051-FCH	8B-S051-HCH

Ordering Information *(cont'd)*

roQ™ Extraction Kits

Extraction kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Kits		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	KS0-8911
EN 15662 Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	KS0-8909
Original Non-buffered Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	KS0-8910
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	KS0-8912

roQ dSPE Kits

dSPE kits contain pre-weighed sorbents/salts inside 2 mL or 15 mL centrifuge tubes

Description	Unit	Part No.
2 mL dSPE Kits		
150 mg $MgSO_4$, 25 mg PSA, 25 mg C18E	100/pk	KS0-8913
150 mg $MgSO_4$, 25 mg PSA, 2.5 mg GCB	100/pk	KS0-8914
150 mg, $MgSO_4$, 25 mg PSA, 7.5 mg GCB	100/pk	KS0-8915
150 mg $MgSO_4$, 25 mg PSA	100/pk	KS0-8916
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18E, 50 mg GCB	100/pk	KS0-8917
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18E	100/pk	KS0-8918
150 mg $MgSO_4$, 50 mg PSA, 50 mg GCB	100/pk	KS0-8919
150 mg $MgSO_4$, 50 mg PSA	100/pk	KS0-8920
15 mL dSPE Kits		
900 mg $MgSO_4$, 150 mg PSA, 150 mg C18E	50/pk	KS0-8921
900 mg $MgSO_4$, 150 mg PSA, 15 mg GCB	50/pk	KS0-8922
900 mg $MgSO_4$, 150 mg PSA, 45 mg GCB	50/pk	KS0-8923
900 mg $MgSO_4$, 150 mg PSA	50/pk	KS0-8924
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18E, 400 mg GCB	50/pk	KS0-8925
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18E	50/pk	KS0-8926
1200 mg $MgSO_4$, 400 mg PSA, 400 mg GCB	50/pk	KS0-8927
1200 mg $MgSO_4$, 400 mg PSA	50/pk	KS0-8928

guarantee

If Strata, Strata-X, or roQ products do not perform as well or better than your current SPE or QuEChERS product of similar phase, mass and size, return the product with comparative data within 45 days for a FULL REFUND.

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