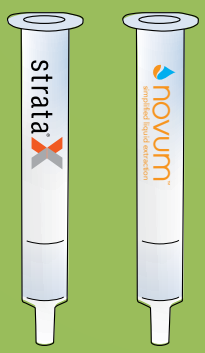
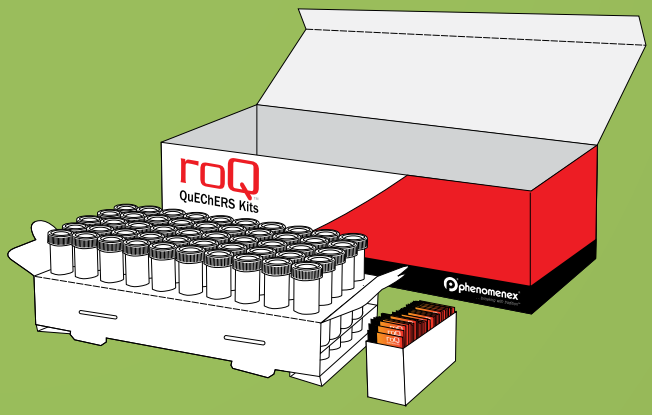
Improve Prep Time
Eliminate Matrix Effects
Cleaner Samples

FOOD TESTING
SAMPLE PREPARATION
PRODUCT SELECTION & APPLICATION GUIDE





phenomenex®
. . . breaking with tradition℠

www.phenomenex.com/FOODSP

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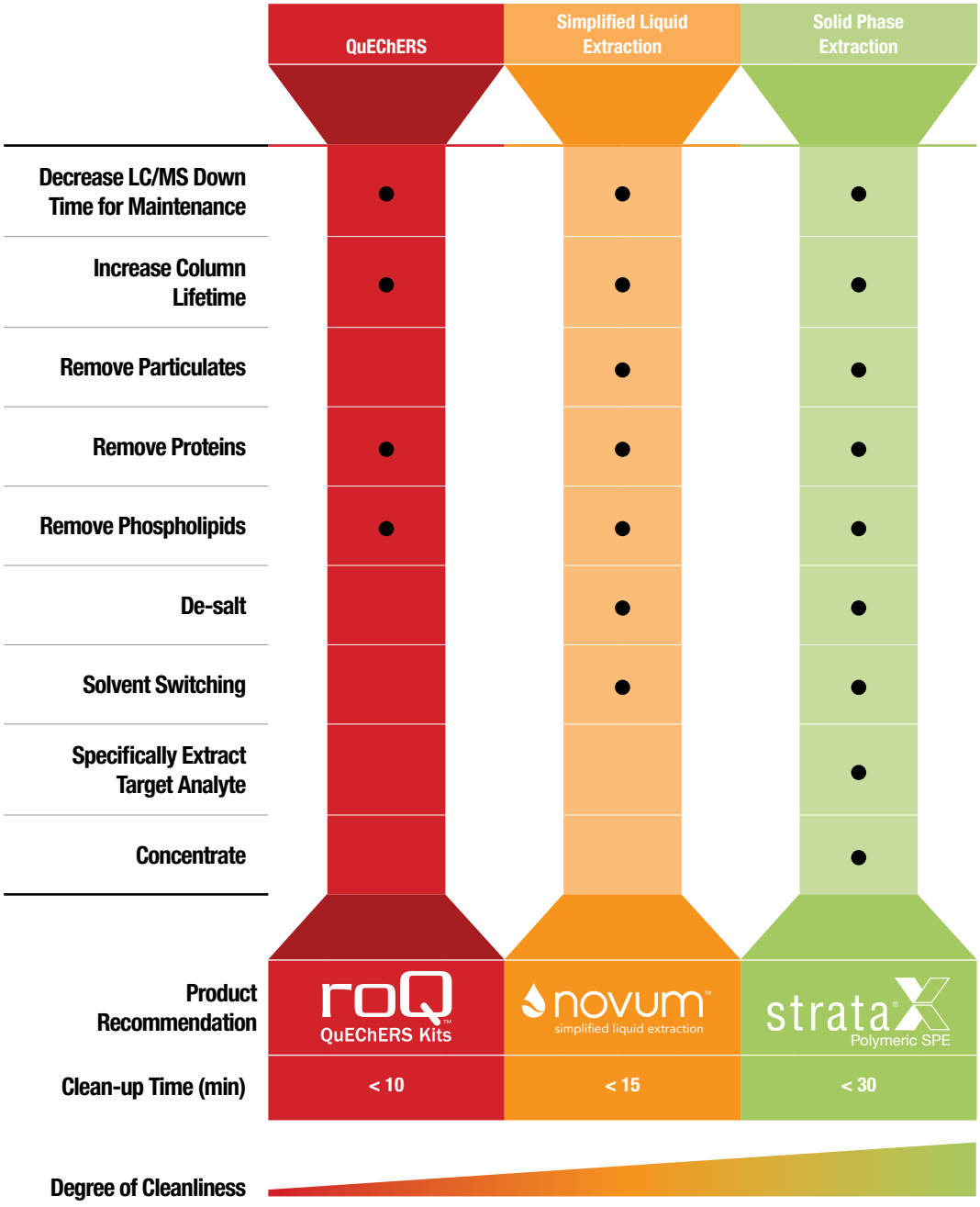
guarantee

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

Select the Appropriate Sample Prep Technique for Your Key Requirements



Explore Technique Options >>

What is QuEChERS?

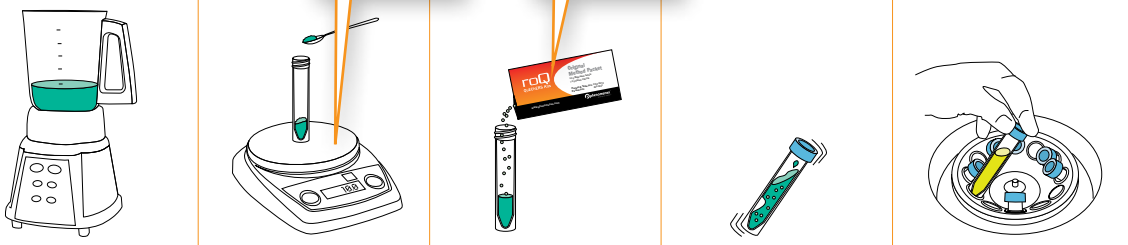
roQ™ QuEChERS Kits

A Sample Preparation Technique:

- For complex sample matrices
- Wide range of compounds
- Is Quick Easy Cheap Effective Rugged and Safe

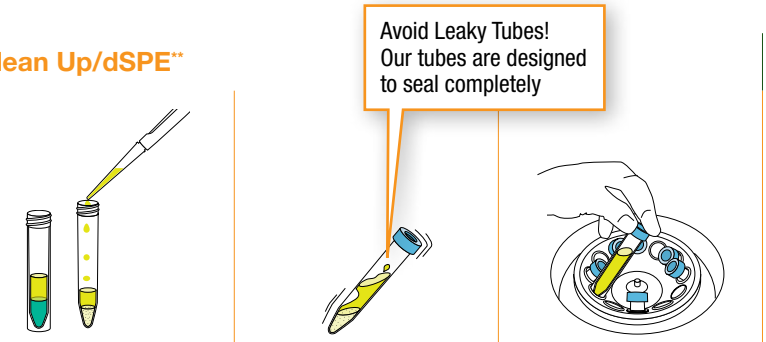
How does roQ QuEChERS Work?

STEP 01 Extraction*



Blend fruits or vegetables.	Weigh blended sample.	Add salts and acetonitrile.	Shake tube for 1 minute.	Centrifuge tube for 5 minutes.

STEP 02 Clean Up/dSPE**



Add supernatant from extraction procedure into a roQ dSPE tube.	Shake dSPE tube for 30 seconds.	Centrifuge dSPE tube for 5 minutes.

How Does It Help Me?

QuEChERS is a simplified 2 step sample preparation process that:

- Significantly reduces sample prep time
- Is simple, inexpensive, easy to use, effective, and rugged at extracting and cleaning samples for a wide range of compounds

Choose your roQ QuEChERS Kit

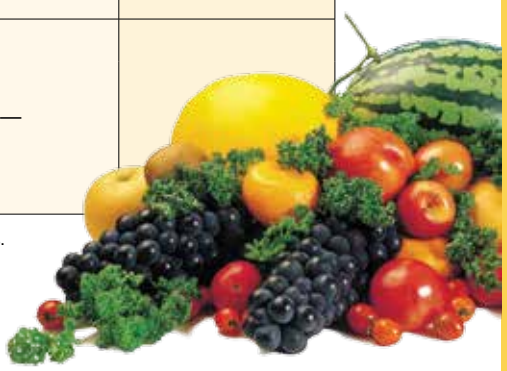
STEP 01 Extraction*

AOAC	ORIGINAL	EN
AOAC 2007.01 Method 6.0 g $MgSO_4$, 1.5 g NaOAc **KS0-8911**	**Non-Buffered Method** 4.0 g $MgSO_4$, 1.0 g NaCl **KS0-8910** 6.0 g $MgSO_4$, 1.5 g NaCl **KS0-8912**	**EN 15662 Method** 4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS **KS0-8909**

STEP 02 Clean Up/dSPE**

	AOAC 2007.01		EN 15662	
	1 mL	8 mL	1 mL	6 mL
General 	150 mg $MgSO_4$ 50 mg PSA **KS0-8920**	1200 mg $MgSO_4$ 400 mg PSA **KS0-8928**	150 mg $MgSO_4$ 25 mg PSA **KS0-8916**	900 mg $MgSO_4$ 150 mg PSA **KS0-8924**
Fats and Waxes 	150 mg $MgSO_4$ 50 mg PSA 50 mg C18E **KS0-8918**	1200 mg $MgSO_4$ 400 mg PSA 400 mg C18E **KS0-8926**	150 mg $MgSO_4$ 25 mg PSA 25 mg C18E **KS0-8913**	900 mg $MgSO_4$ 150 mg PSA 150 mg C18E **KS0-8921**
Pigmented 	150 mg $MgSO_4$ 50 mg PSA 50 mg GCB **KS0-8919**	1200 mg $MgSO_4$ 400 mg PSA 400 mg GCB **KS0-8927**	150 mg $MgSO_4$ 25 mg PSA 2.5 mg GCB **KS0-8914**	900 mg $MgSO_4$ 150 mg PSA 15 mg GCB **KS0-8922**
Highly Pigmented 	—	—	150 mg $MgSO_4$ 25 mg PSA 7.5 mg GCB **KS0-8915**	900 mg $MgSO_4$ 150 mg PSA 45 mg GCB **KS0-8923**
Pigments and Fats 	150 mg $MgSO_4$ 50 mg PSA 50 mg GCB 50 mg C18E **KS0-8917**	1200 mg $MgSO_4$ 400 mg PSA 400 mg GCB 400 mg C18E **KS0-8925**	—	

*All roQ Extraction kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes.
**All roQ dSPE kits contain pre-weighed sorbents/salts inside 2 mL or 15 mL centrifuge tubes



Simplified Liquid Extraction

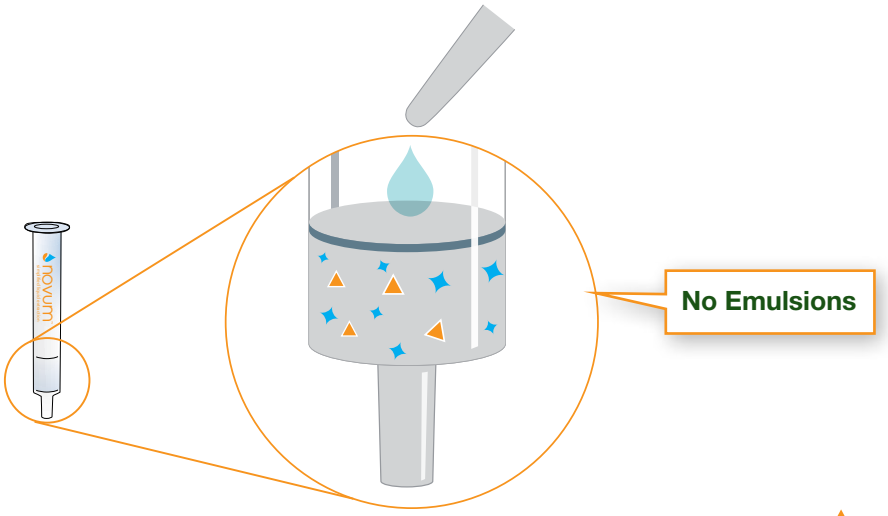
novum™ simplified liquid extraction
PATENT PENDING

Simplified Liquid Extraction (SLE) is a FASTER, EASIER, and MORE RELIABLE way to perform liquid-liquid extractions.

- Eliminates interferences from your analysis
- Remove unwanted interferences such as proteins and phospholipids from difficult matrices without performing extensive method development
- Provides consistent, reliable results from lot-to-lot

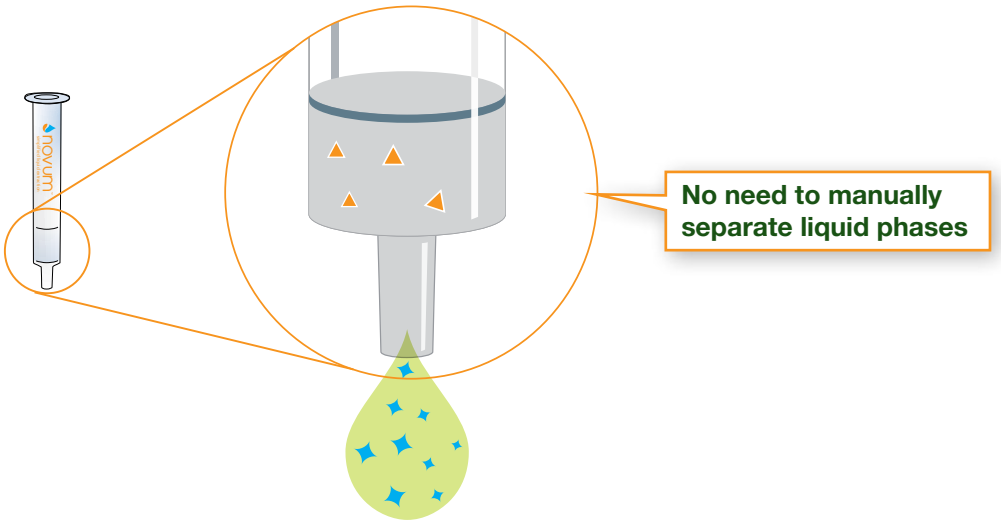
An Easy, Automatable Procedure

STEP 01 Load Your Sample in Aqueous Solvent



▲ Interferences (i.e. phospholipids, proteins, salts, etc.)
✦ Target Analytes

STEP 02 Collect Your Target Analytes in Water Immiscible Solvent



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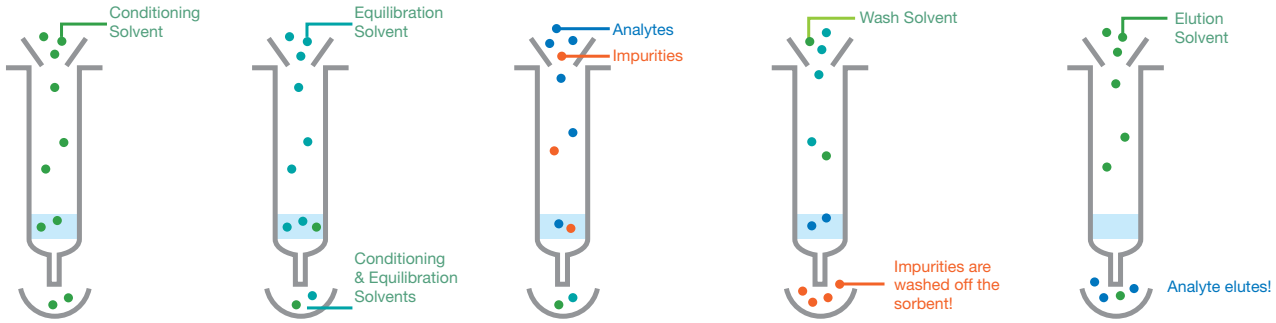
Solid Phase Extraction (SPE)

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

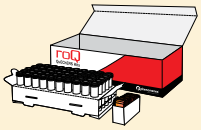
Basic Compounds

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

www.phenomenex.com/SPE

Application Highlights

Sample matrix effects can lead to poor chromatography as well as instrumentation drawbacks, decreasing your sample throughput and increased analysis time. Explore some of our solutions using various sample preparation techniques to address common challenges in food testing sample preparation.



roQ™ QuEChERS

Novum™ Simplified Liquid Extraction (SLE)

Solid Phase Extraction (SPE)

Find more Sample Prep applications
Visit: ***www.phenomenex.com/Sampleprep***

Pesticide Residues in Spinach

Using roQ™ QuEChERS AOAC Kits by LC/MS/MS and GC/MS

Introduction

QuEChERS (an acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe) has grown over the past decade to become a commonly used sample preparation technique that is employed in most multi-residue pesticide testing laboratories. The method was first introduced at the European Pesticide Residues Workshop in Rome, May 2002. Since the publication of the original method, the format and theory of QuEChERS has been adapted and modified accordingly to improve method performance for many different sample matrices and to expand the capacity of the multi-residue method by analyzing more compound classes.

Experimental Conditions

Sample Preparation

Spinach was chopped into 2-4 cm pieces, placed into a zip-lock bag, and stored in a -80 °C freezer for at least 24 hours prior to further processing. The spinach was first immersed in liquid nitrogen and homogenized in a blender to generate a powdery consistency.

QuEChERS Extraction

Liquid-Liquid Partitioning

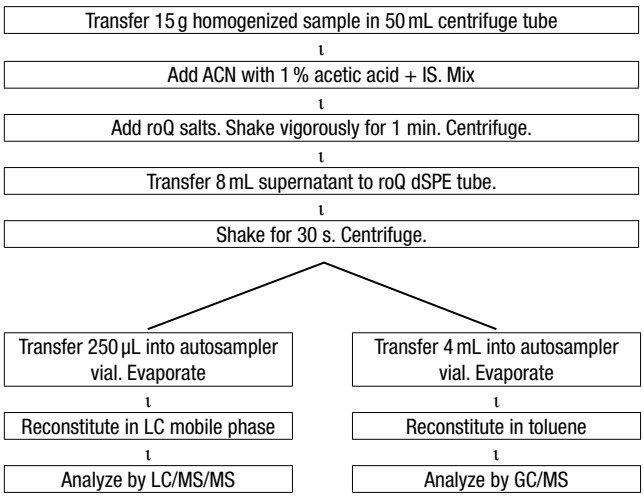
15 g of pretreated sample was weighed in a 50 mL centrifuge tube (provided in the roQ extraction kit). Two sets of QC samples were spiked at 80 ng/g and 200 ng/g using 30 µL and 75 µL of stock solution (40 µg/mL) respectively. An aliquot of 15 mL of 1 % acetic acid in ACN was added to the samples and 75 µL of d10-parathion and d6-α-BHC were added as internal standards. A roQ salt packet containing a blend of 6.0 g $MgSO_4$ and 1.5 g NaOAc provided in roQ extraction kits (p/n KS0-8911) was dispensed into each tube. The tubes were first shaken by hand for 1 minute and then centrifuged at 3500 rpm for 2 minutes. An aliquot of 8 mL of supernatant was transferred into a roQ QuEChERS dSPE tube containing 1.2 g $MgSO_4$, 0.4 g PSA and 0.4 g GCB (p/n KS0-8927).

roQ dSPE Cleanup

roQ QuEChERS dSPE tubes were shaken by hand for 30 seconds and then centrifuged at 3500 rpm for 1 minute. Supernatant (250 µL) was transferred into a vial for LC/MS/MS solvent exchange and 2 mL of supernatant was transferred into 15 mL centrifuge tubes for GC/MS solvent exchange **(Figure 1)**.

QuEChERS Procedure Outline

Figure 1.
Flow chart summary for AOAC Official Method 2007.01 for pesticide residues in foods. The final extracts were split for LC/MS/MS and GC/MS analyses.



LC/MS/MS Sample Preparation

Appropriate standard solutions were added to the samples. Extracts were evaporated to dryness under a slow stream of nitrogen and reconstituted in 200 µL of 5 mM formic acid in methanol. After sonicating and vortexing, 800 µL of 5 mM formic acid in deionized water was added. The samples were centrifuged prior to transferring into vials with low volume inserts.

GC/MS Sample Preparation

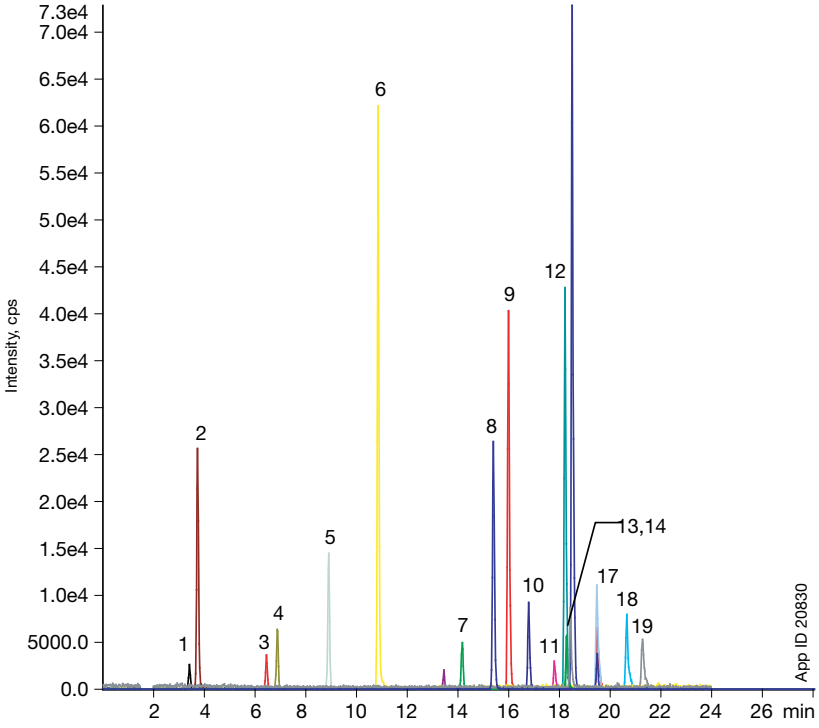
500 µL of toluene was added to each sample in 15 mL centrifuge tubes. Samples were evaporated under a slow stream of nitrogen at 50 °C until approximately 0.1 mL of volume was left. The appropriate standard solutions and toluene were added to reach the 0.5 mL mark. After adding $MgSO_4$ to each sample to remove excess water, the samples were centrifuged at 3500 rpm for 1 minute. Samples were then transferred to amber autosampler vials containing inserts for GC/MS analysis.

Pesticide Residues in Spinach

Using roQ™ QuEChERS AOAC Kits by LC/MS/MS and GC/MS *(cont'd)*

Chromatographic Conditions

Figure 2.
MRM chromatogram of spinach extract spiked at 200 ng/g.



Column: Luna® 3 µm C18(2)
Dimensions: 150 x 3.0 mm
Part No.: 00F-4251-Y0
Mobile Phase: A: 5 mM Formic acid in Water
B: 5 mM Formic acid Methanol

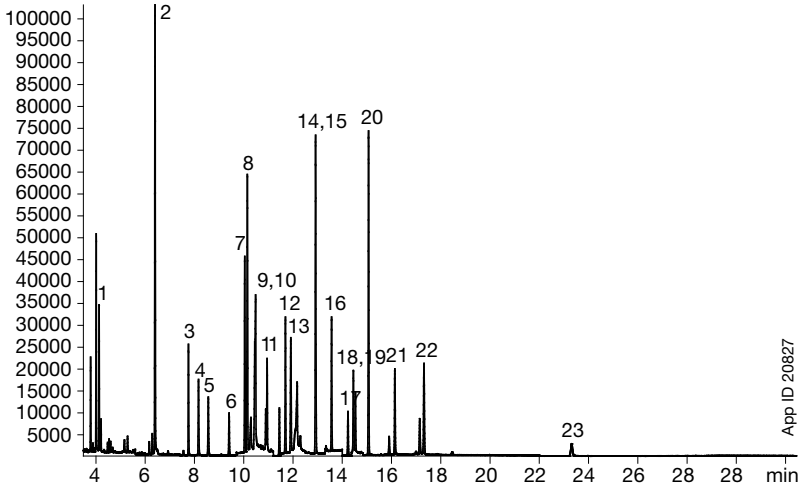
Gradient:

Time (min)	% B
0.00	25
15.00	90
30.00	90
30.01	25
40.00	25

Flow Rate: 0.3 mL/min
Temperature: 25 °C
Detection: MS (ambient)
Sample:

1. Pymetrozine
2. Methamidophos
3. Thiabendazole
4. Methomyl
5. Imidacloprid
6. Imazalil
7. Carbaryl
8. Atrazine
9. Azoxystrobin
10. Linuron
11. Cyprodinil
12. d10-Parathionl
13. Kresoxim- methyl
14. Tolyfluanid
15. Triphenylphosphate
16. Tebuconazole
17. Chlorpyrifos- Methyl
18. Ethion
19. Chlorpyrifos

Figure 3.
GC/MS of spinach extract spiked at 200 ng/g.



Column: Zebron™ ZB-5MSi Guardian™
Dimension: 30 m x 0.25 mm x 0.25 µm
Part No.: 7HG-G018-11-GGA
Injection: Splitless @ 250 °C, 2 µL
Carrier Gas: Helium @ 1 mL/min (constant flow)
Oven Program: 100 °C to 150 °C @ 25 °C/min to 280 °C @ 10 °C/min for 10 min then to 340 °C @ 25 °C/min for 5 min
Detector: MS @ 350 °C
Sample:

1. Dichlorvos
2. O-phenylphenol
3. Trifluarlin
4. d6-α-HCH
5. Atrazine
6. Chlorothalonil
7. Chlorpyrifos-methyl
8. Carbaryl
9. d10-Parathion
10. Chlorpyrifos
11. Cyprodnil
12. Tolyfluanid
13. Procymidone
14. o,p-DDD
15. Kresoxim-methyl
16. Ethion
17. Endosulfan Sulfate
18. Tebuconazole
19. TPP
20. Bifenthrin
21. L-Cyhalothrin
22. Permethrins
23. Azoxystrobin

Results

The quick, easy, cheap, effective, rugged and safe (QuEChERS) method offers a relatively simple solution for the determination of a wide range and extensive list of pesticide compounds from many different matrices. Results for extraction from spinach using Phenomenex roQ QuEChERS PSA/GCB dSPE kits are within acceptable AOAC criteria **(Figure 6)**.

Fresh spinach is a common leafy vegetable in our diet because it is available year round. It is an excellent source of vitamins and minerals. The naturally occurring dark green color, along with the nutrients in spinach makes sample matrix clean up a very challenging task. Despite this challenge, using the roQ PSA/GCB dSPE kit, the final extracts were visibly clear after the dSPE step **(Figure 4 & 5)** indicating the majority of the pigment was removed by GCB.

The pesticide mix consisted of pesticides with a large variety of properties, including polars and semi-polars such as methamidophos and kresoxim-methyl, respectively. Retention times ranged from 3.4 to 21.3 minutes.

The Luna® column proved to be very reproducible and demonstrated ruggedness when faced with a difficult sample matrix such as spinach. Although co-eluting matrix interferences can be an issue in GC/MS analysis, compound specific matrix effects do not apply. GC/MS analysis demonstrates high sensitivity and reproducibility, especially for polar and non-polar pesticides. Baseline resolution was achieved for all 21 pesticides in the mix and 2 internal standards.

Conclusion

Phenomenex roQ™ QuEChERS extraction and PSA/GCB dSPE kits successfully extracted 18 pesticides of different classes from spinach while providing benefits such as effective removal of pigment and other matrix interferences. This method produced acceptable recoveries and reproducibilities per the official AOAC 2007.01 method **(Figures 6 & 7)**. The roQ QuEChERS PSA/GCB dSPE kit can be used for other heavily pigmented matrices using this method if analytes of interest do not include planar pesticides. This method with modification, i.e. addition of toluene in the dispersive step, can be used if the list of analytes includes planar pesticides.

Figure 4.
Spinach extracts after liquid partitioning step with 1 % acetic acid in acetonitrile and magnesium sulfate. The organic phase was heavily pigmented in dark green.

Figure 5.
Spinach extracts after dSPE cleanup. GCB removed a majority of the pigment from the sample matrix and the extracts were clear with a light green tint.

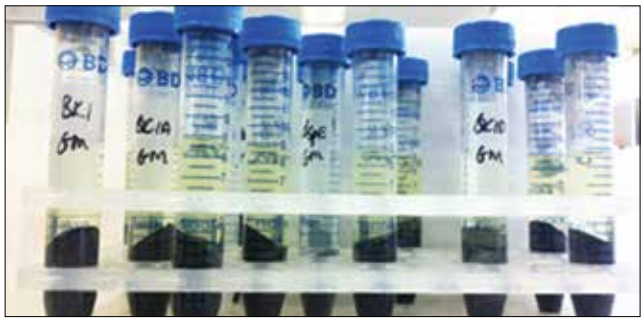


Figure 6.
LC/MS/MS recovery data of samples spiked at 80 ng/g and 200 ng/g.

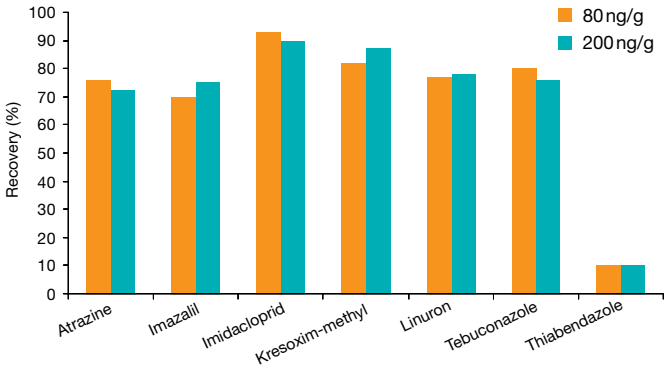
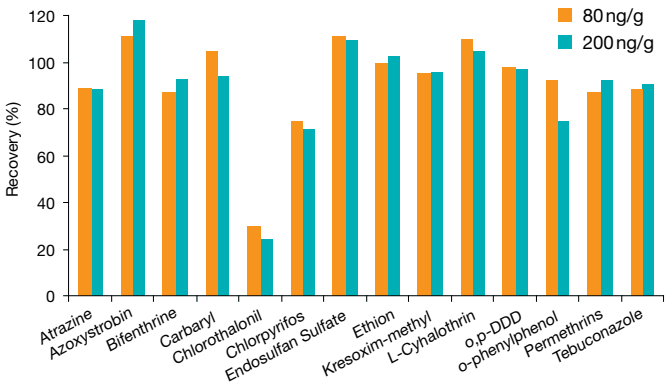


Figure 7.
GC/MS recovery data of samples spiked at 80 ng/g and 200 ng/g.



Extraction and Screening of Mycotoxins

From Cereal Products using QuEChERS and LC/MS/MS

Introduction

Mycotoxins are secondary metabolites produced by fungi that can contaminate edible crops, such as grains or coffee, during harvest or storage. Exposure to mycotoxins through consumption, inhalation or dermal routes can result in a variety of health effects including immunosuppression, mutagenicity, and cancer. Further interest in mycotoxins has resurfaced because of recent discussions on climate change and food safety.

Although aflatoxins and ochratoxins are of major significance, the FDA is also actively concerned with other mycotoxins, including fumonisins, trichothecenes, patulin, and zearalenone. Depending on the intended use of the product, the FDA has established mycotoxin action levels in the parts per billion range; 20 ppb for aflatoxins in all products intended for human consumption, except milk. As such, a specific and sensitive method is necessary to proactively monitor mycotoxins in food.

In this study, we demonstrate a rapid sample preparation and LC/MS/MS method for screening all major mycotoxins from corn-meal using roQ™ QuEChERS kits and a Kinetex® 2.6 µm XB-C18 core-shell HPLC column.

Experimental Conditions

Sample Preparation

Extraction from Ground Corn

1. Homogenize sample using a blender or similar apparatus
2. Weigh and transfer 5 g of ground corn-meal to a 50 mL roQ QuEChERS extraction tube
3. Add 10 mL of water and 10 mL of acetonitrile with 1.0 % formic acid
4. Dispense contents of the included roQ QuEChERS extraction packet (p/n KS0-8909) into the 50 mL tube containing homogenized sample
5. Shake vigorously by hand for 1 minute
6. Centrifuge for 5 minutes @ 4000 rpm, making sure that the solid material is at the bottom of the tube and a liquid layer forms on top of the solid material

Clean up using dispersive Solid Phase Extraction (dSPE)

1. Transfer the supernatant from Step 6 of the extraction process into a roQ QuEChERS 15 mL centrifuge tube containing 900 mg $MgSO_4$ and 150 mg PSA (p/n KS0-8924)
2. Shake vigorously by hand for 30 seconds
3. Centrifuge for 5 minutes at 4000 rpm to separate solid material from the liquid layer

Transfer the supernatant to a vessel for evaporation

Reconstitution

2 mL of supernatant from the dSPE step was evaporated to dryness under a stream of nitrogen at 60 °C to dryness. The sample was reconstituted in 0.25 mL of 0.5 % Acetic acid/Methanol with 0.5 % Acetic acid (95:5) for analysis.

LC/MS/MS Conditions

Column: Kinetex 2.6 µm XB-C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4496-AN
SecurityGuard Cartridge: AJ0-8782
Mobile Phase: A: 5 mM Ammonium acetate with 0.5 % Acetic acid
B: 5 mM Ammonium acetate in Methanol with 0.5 % Acetic acid
Flow Rate: 0.45 mL/min
Gradient:

Time (min)	% B
0.0	5
2.0	5
5.0	80
5.2	98
8.0	98

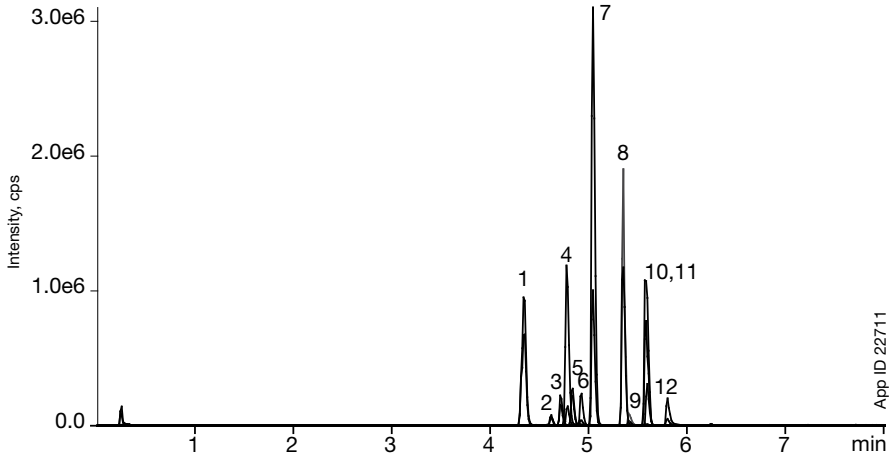
Injection Volume: 25 µL
Temperature: 70 °C
Detection: API 5000™ (SCIEX) Tandem Mass Spec (MS/MS)

Results

Figure 1 and 2 show representative ion chromatograms. All analytes eluted in less than 6 minutes with a total run time of only 8 minutes, including column equilibration.

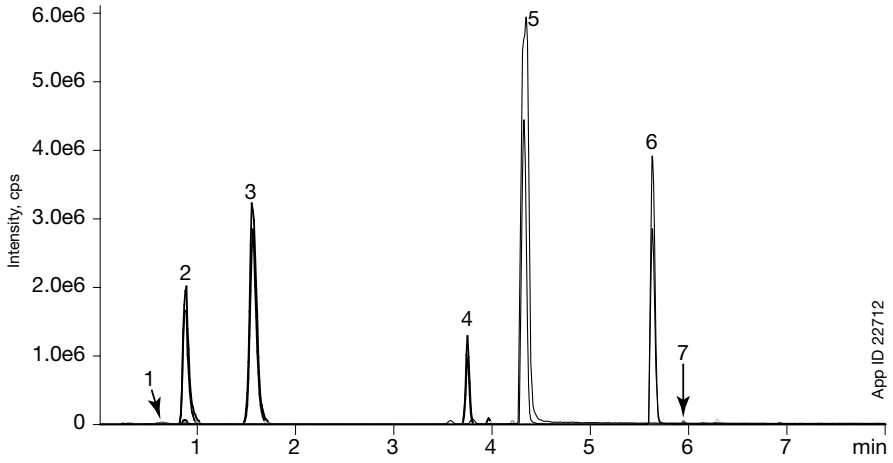
Sample extraction and cleanup using roQ QuEChERS extraction kits (KS0-8909) and dSPE Kits (KS0-8921) containing PSA/C18E successfully removed interferences from the corn-meal matrix resulting in good recoveries. **Table 1** shows recoveries between 66-118 % for most mycotoxins except fumonisins (FB1 and FB2).

Figure 1.
Sample Extract Ion Chromatogram - Positive Mode



Sample:
1. 15-Acetyldeoxynivalenol (15DNV)
2. Aflatoxin G2 (AFG2)
3. Aflatoxin G1 (AFG1)
4. Unknown Interference
5. Aflatoxin B2 (AFB2)
6. Aflatoxin B1 (AFB1)
7. Diacetoxyscirpenol (DAS)
8. HT2 Toxin
9. Fuminosin B1 (FB1)
10. T2 Toxin
11. Ocratoxin A (OTA)
12. Fuminosin B2 (FB2)

Figure 2.
Sample Extract Ion Chromatogram - Negative Mode



Sample:
1. Patulin
2. Nivalenol (NIV)
3. Deoxynivalenol (DNV)
4. Fusarenone-X
5. 3-Acetyldeoxynivalenol (3DNV)
6. Zearalenone (ZEA)
7. Verruculogen

Table 1.
Recovery of Mycotoxins

Analyte	% Recovery
AFB1	73
AFB2	87
AFG1	67
AFG2	93
HT2 Toxin	96
T2 Toxin	91
DAS	90
OTA	79
15DNV	99

Analyte	% Recovery
FB1	8
FB2	14
Patulin	118
ZEA	91
DNV	80
NIV	66
3DNV	96
Fusarenone-X	83
Verruculogen	81

Conclusion

In this study, we presented a rapid and sensitive screening method for various mycotoxins, including aflatoxins and ochratoxins. Sample cleanup using roQ QuEChERS extraction and dSPE kits successfully removed interferences from the ground corn-meal matrix resulting in good recoveries. Extracts were analyzed using a Kinetex 2.6µm XB-C18 Core-shell Technology HPLC column. Extraction of samples under acidic conditions improved extraction efficiencies, though future experiments should be considered to improve fumonisin recovery. This method presented was proven to be powerful for control management and monitoring of mycotoxins.

Screening of Multi-Class Antibiotics

From Ground Meat (sausage) using QuEChERS and LC/MS/MS

Introduction

Antibiotics consist of many different classes of compounds such as sulfa drugs, penicillins, tetracyclines, and cephalosporins, etc. These agents are used to treat infectious diseases for well over 70 years in humans. This usage has also been applied to food animals to control the bacterial harmful effect. In addition to this therapeutic use in food animals, antibiotics have been proven to promote growth when administered in small daily doses.

In order to regulate this practice, a sensitive and accurate screening method is required to detect antibiotics in meat produced from food animals. In this work, we demonstrate a rapid sample preparation and LC/MS/MS method for multi-class antibiotic screening from pork sausage using roQ™ QuEChERS dSPE (dispersive) cleanup kit and Kinetex® XB-C18 2.6µm core-shell HPLC column. Limit of detection of 50ppb was achieved with excellent signal-to-noise ratios, which is the maximum residue limit for a number of antibiotics per Commission Regulation (EU) No 37/2010.

Experimental Conditions

Sample Preparation

Extraction from Ground Meat (Sausage)

2mL of 1% formic acid solution was added to 2g of ground sausage. The sample was mixed well and further homogenized using an Omni TH hand homogenizer. 8mL of methanol was added to the mixture. The sample was placed on a mechanical shaker for 30 minutes at high setting and then centrifuged at 4000g for 5 minutes.

dSPE Cleanup

5mL of supernatant from the extraction step was transferred to a roQ QuEChERS dSPE tube containing 900mg of $MgSO_4$, 150mg of PSA and 150mg of C18E (p/n KS0-8921). The sample was shaken vigorously for 1 minute and centrifuged at 4000g for 5 minutes.

Reconstitution

2mL of supernatant from the dSPE step was evaporated over a stream of nitrogen at 60°C to dryness. The sample was reconstituted in 1mL of 0.1% formic acid/acetonitrile-methanol 50:50 with 0.1% formic acid (95:5) for analysis.

LC/MS/MS Conditions

Column: Kinetex 2.6µm XB-C18
Dimensions: 100 x 2.1mm
Part No.: 00D-4496-AN
SecurityGuard Cartridge: AJ0-8768
Mobile Phase: A: 0.1% Formic acid in water
B: Methanol/Acetonitrile (50:50) with 0.1% Formic acid

Gradient:

Time (min)	% B
0.00	5
1.00	5
4.00	50
6.00	95
7.50	95
7.51	5
10.00	5

Flow Rate: 0.45mL/min
Injection Volume: 10µL
Temperature: 50°C
Detection: API 5000™ (SCIEX)

Figure 1.
Representative chromatogram of meat fortified with 50ppb antibiotic mixture

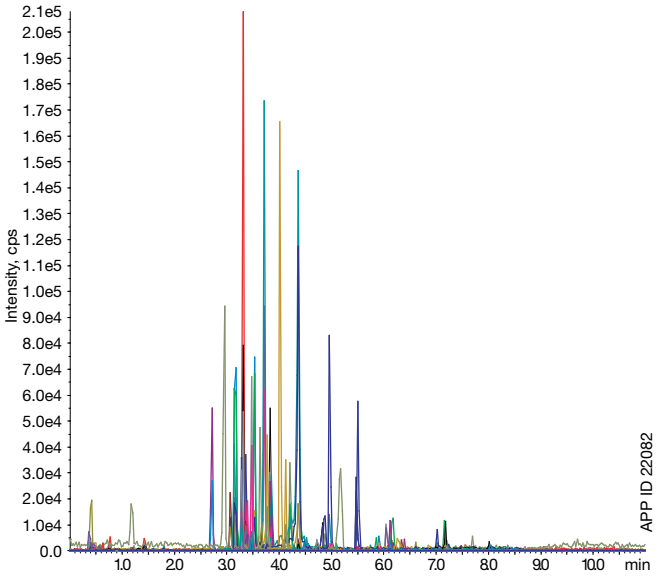
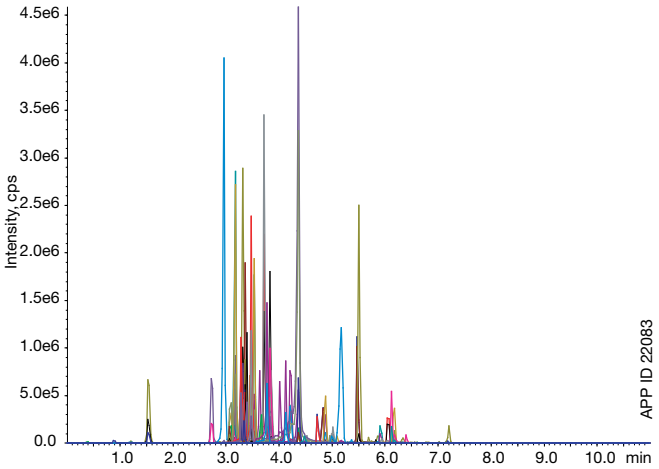


Figure 2.
Representative chromatogram of meat fortified with 800ppb antibiotic mixture



Results

In this study, a screening method for multi-class antibiotics from fatty ground meat matrix (sausage) was developed. For the pretreatment of meat, both acidic and basic pretreatment conditions were examined. However, acidic pretreatment produced higher sensitivity for a larger group of analytes. Sample cleanup using roQ QuEChERS dSPE kit (p/n KS0-8921) containing PSA/C18E successfully removed interferences from the meat matrix to furnish excellent recoveries and signal-to-noise ratios **(Table 1)**. **Figure 3** shows meat samples during various stages of sample preparation. Pretreatment under acidic conditions produced superior extraction results from meat/sausage matrix, which in turn, increased extraction efficiency for the subsequent solvent extraction using methanol **(Figure 3a)**. During dSPE cleanup, matrix interferences such as lipids and pigment were eliminated by loose SPE sorbents **(Figure 3b-c)**. The resulting extracts were visibly clear and ready for injection after solvent switching **(Figure 3d)**.

Kinetex 2.6 µm XB-C18 Core-Shell Technology column provided excellent peak shape and high efficiency. All analytes eluted in less than 7 minutes and run time was only 10 minutes, including column cleaning and re-equilibration. **Figure 1 and 2** show the extracted ion chromatograms of meat samples with antibiotics spiked at 50 and 800 ppb, respectively. High sensitivity and signal-to-noise ratios were achieved at the low spike concentration of 50 ppb **(Table 1)**. Based on this data, good signal-to-noise ratios can be expected at even lower analyte concentrations.

The current method is extremely versatile for the multi-class screening of antibiotics from meat matrices, notably a high fat content sample. Undoubtedly, similar analyses from other matrices may require slight modifications in the sample preparation step, i.e. choice of acidic or basic digestion method depending on sample, sample homogenization procedure, consideration of using different dSPE sorbents based on matrix interferences presented by sample, etc. For quantitation of specific antibiotics, a more selective method using solid phase extraction (SPE) followed by analysis with the same Kinetex 2.6 µm XB-C18 column can be employed.

Conclusion

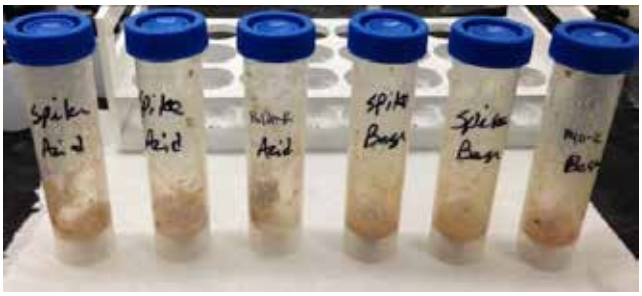
In this study, we presented a rapid and sensitive multi-class screening method for the detection of multiple classes of antibiotics in ground meat samples at maximum residue limit levels defined by Commission Regulation (EU) No 37/2010. Samples were prepared using a simple, yet effective extraction and cleanup procedure. Extracts were analyzed using a core-shell technology Kinetex 2.6 µm XB-C18 HPLC column.

Excellent signal-to-noise ratios were obtained at low spike concentration of 50 ppb and based on a small volume, 10 µm sample injection. This method was proven to be powerful for the detection of antibiotics in meat produced from food animals.

Table 1.
Analyte identification, retention time, and S/N ratio from a meat extract

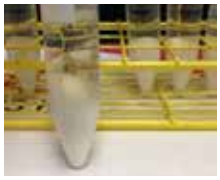
Analyte Peak Name	Analyte Retention Time (min)	Analyte Signal-To-Noise (S/N) Ratio at 50 ppb
AMOXICILLIN	1.52	236
SULFADIAZINE	2.72	1006
SULFATHIAZOLE	3.07	162
SULFAPYRIDINE	3.15	701
4-EPITETRACYCLINE	3.17	1411
TETRACYCLINE	3.47	1739
TRIMETHOPRIM	3.15	344
MARBOFLOXACIN	3.28	668
SULFAMERAZINE	3.31	649
CEFQUINOME	3.34	179
AMPICILLIN	3.39	699
CEFALONIUM	3.45	311
4-EPIOXYTETRACYCLINE	3.17	831
OXYTETRACYCLINE	3.47	530
CIPROFLOXACIN	3.50	117
CEFAPIRIN	3.55	21
DANOFLOXACIN	3.64	435
ENROFLOXACIN	3.66	405
SULFAMETHAZINE	3.72	1386
DIFLOXACIN	3.83	733
SARAFLOXACIN	3.85	118
NEOSPIRAMYCIN	3.83	1700
SPIRAMYCIN	4.07	299
SULFAMETHOXAZOLE	4.21	66
DOXYCYCLINE	4.36	1807
TILMICOSIN	4.48	161
CEFOPERAZONE	4.72	545
SULFAQUINOXALINE	4.87	558
TIAMULIN	5.10	141
TYLOSIN A	5.17	599
VALNEMULIN	5.50	2949
OXACILLIN	5.91	173
CLOXACILLIN	6.05	635
DICLOXACILLIN	6.12	408
NAFCILLIN	6.16	79

Figure 3.
Meat samples (sausage) at various stages of sample preparation
a) Prior to extraction after the initial pretreatment



b) During dSPE cleanup

c) After dSPE cleanup



d) Prior to dry down

Acrylamide from Coffee

Using Simplified Liquid Extraction (SLE) and HPLC

Introduction

Supported Liquid Extraction (or Simplified Liquid Extraction) is very popular in the clinical research industry however the technique is gaining popularity in other industries as a faster, easier, and more reliable alternative to liquid-liquid extraction. In the following we investigate an application using Novum™ SLE to clean up and extract acrylamide from both instant and brewed coffee.

According to the American Cancer society, cooking at high temperatures causes a chemical reaction between certain sugars and asparagine which causes acrylamide to form. Acrylamide is commonly found in foods that are made from plants such as potato products, grain products, and coffee whose preparation often requires longer cooking times and higher temperatures.

Below we explore how to use Novum SLE tubes to clean up a coffee matrix in order to quantitate known acrylamide levels, demonstrating that the SLE technique can be applied to a variety of compounds and sample matrices outside of the clinical research industry.

Experimental Conditions

Sample Preparation

Sample Pre-treatment

Coffee was prepared the same way that it would normally be consumed. Prepared control coffee was left on the bench to reach room temperature before further pre-treatment.

- Ground Coffee Control (40 mg/mL)
- 60 g of ground coffee was percolated with 1500 mL of boiling water
- Instant Coffee Control (8 mg/mL)
- 2 g of instant coffee was dissolved in 250 mL of boiling water

Acrylamide standard was spiked into control coffee to reach 100 ng/mL (ground coffee) and 200 ng/mL (instant coffee) by adding 20 µL Acrylamide-13C3 (4 µg/mL in water) to 800 µL of the prepared coffee.

Sample Loading

- Add 150 µL 2 % Ammonium hydroxide in water to the spiked samples (from Pre-treatment step), vortex for 30 seconds.
- Load the sample onto the Novum SLE 6 cc tubes and apply a short and gentle pulse of vacuum (~5-10 seconds at 5" or less of Hg) until the sample has completely entered the media.
- Wait for 5-6 minutes.

Note: Inadequate or excessive wait periods can lead to variable recoveries and poor precision.

Elution

- Dispense 2x 2.5 mL of Ethyl acetate Tetrahydrofuran (1:1) onto the Novum SLE sorbent and collect the solvent under gravity into a collection tube that contains 10 µL Ethylene glycol.
- Apply vacuum at 5" of Hg (or lower) for 20-30 seconds to complete the extraction.

Note: To reduce analyte loss due to dry down, ethylene glycol was added to the collection tube to prevent the sample from drying completely during the dry down step.

Dry Down

- Evaporate extracted samples to complete dryness under a slow stream of N2 at 45° C.
- Reconstitute sample in 300 µL of water.

HPLC Conditions

Column: Synergi™ 4 µm Hydro-RP
Dimensions: 50 x 2.0 mm
Part No.: 00B-4375-B0
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol
Flow Rate: 300 µL/min
Gradient:

Time (min)	% B
0.00	0
0.60	0
0.85	100
3.00	100
3.01	0
5.00	0

Temperature: Ambient
Detection: MS/MS, API 5000™ (SCIEX), ESI+
Injection: 5 µL

Figure 1.
Acrylamide from coffee (100 ng/mL)

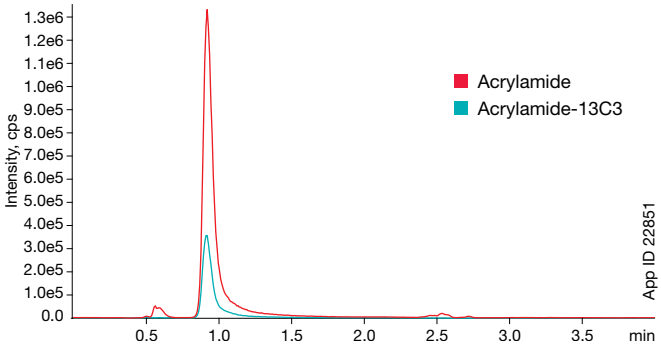


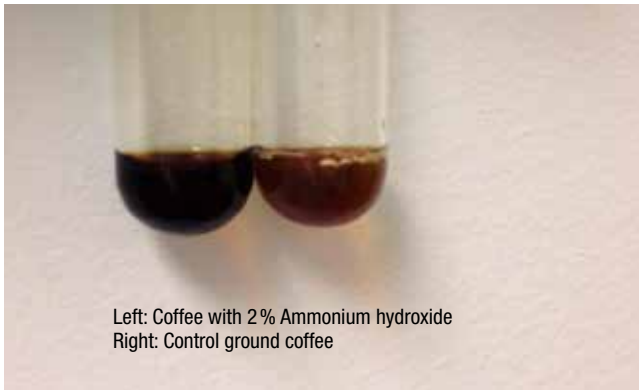
Table 1.
MRM Transitions

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	CE
Acrylamide_1	72	54.9	250	16
Acrylamide_2	72	43.9	250	18
Acrylamide-$^{13}C_3$	75	58.2	250	16

Table 2.
Recovery of Acrylamide

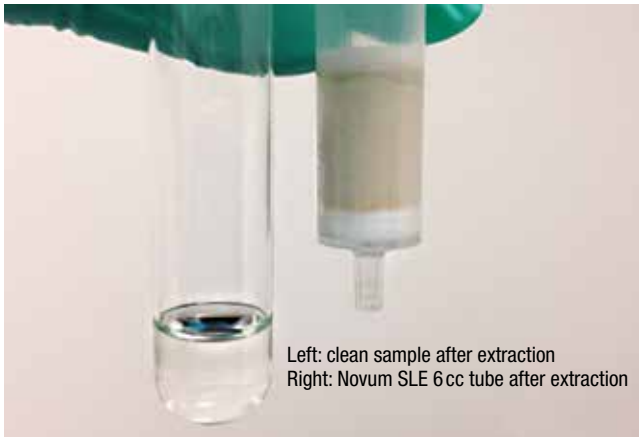
Sample ID	Ground coffee (100 ng/mL)	Instant coffee (200 ng/mL)
Mean of area ratio	1.89	3.75
STDV	0.01	0.06
CV (%)	0.78	1.61
Absolute Recovery (%)	94.9	92.8
n=	6	6

Figure 2.
Sample prior to cleanup with Novum™ SLE



Left: Coffee with 2 % Ammonium hydroxide
Right: Control ground coffee

Figure 3.
Sample after cleanup with Novum SLE



Left: clean sample after extraction
Right: Novum SLE 6 cc tube after extraction

Results

The SLE technique requires that analytes be neutral in charge prior to loading onto the SLE sorbent. This step is important because neutral analytes will partition into the organic elution more efficiently than a charged species. While acrylamide only exhibits charged behavior at extremely low pH, the pre-treated sample was diluted with 2 % Ammonium hydroxide as a way of ensuring that all of the acrylamide was neutralized. This step increases the LogD and facilitates a highly efficient partition to the organic solvent in order to maximize recovery.

After the sample was loaded onto the sorbent, the sample was allowed to soak into the sorbent for 5 minutes. This step allows the sample to disperse amongst the sorbent, creating a higher surface area for interaction with the organic elution solvent. After 5 minutes, a mixture of ethyl acetate/tetrahydrofuran (1:1) was applied to the sorbent in 2 aliquots and the eluent was collected in a collection tube that contained 10 µL of ethylene glycol. Ethylene glycol was added to the collection tube to help prevent analyte loss during the subsequent dry down step. The sample was then blown down under a stream of nitrogen and reconstituted in water.

The SLE procedure resulted in a very clean sample which is depicted in before and after pictures. **Figure 2** shows the ground coffee diluted with 2 % Ammonium hydroxide versus the undiluted ground coffee. Note the darker appearance of the coffee that was diluted with 2 % Ammonium hydroxide. **Figure 3** shows the sample after cleanup, which produced a clear sample that is visually cleaner than the original sample.

Recoveries of acrylamide were studied at 100 ng/mL (ground coffee) and 200 ng/mL (instant coffee) which are values that are in line with typical reported concentration levels in an attempt to mimic a real world sample. **Table 2** shows the absolute recoveries for acrylamide in both the instant and ground coffee which were 92.8 and 94.9, respectively. The resulting HPLC separation of internal standard and acrylamide are depicted in **Figure 1**.

Conclusion

In conclusion, the ethyl acetate/tetrahydrofuran (1:1) elution solvent proved to be an effective solvent choice in terms of both cleanup and recovery. The recovery is especially significant when you consider the polar nature of the acrylamide (LogP -0.27). In addition to high recoveries (>90 %), the low values for the standard deviation and %CV suggest that this method is reproducible. This demonstrates that Novum SLE is capable of being used for non-clinical based samples and effectively functions as a more automatable and easier to use replacement for any liquid-liquid extraction method.

Chlorinated Pesticides in Poultry Fat

Using Solid Phase Extraction and GC/ECD

Introduction

Chlorinated hydrocarbons (CHC) are persistent in the environment and are known to cause adverse health effects. Several of these compounds are endocrine disruptors and may be linked to a variety of sexual, developmental, behavioral, and reproductive problems. Many of them are known to have carcinogenic effects as well.

Traditionally under this method, glass chromatography columns are manually prepared using glass wool, alumina, and sodium sulfate which are then suspended in petroleum ether. In an effort to reduce the labor force and space needed to analyze this method, Phenomenex Strata® Alumina-N solid phase extraction (SPE) cartridges were used to develop a new extraction procedure that delivers reliable and accurate results. In this study we demonstrate a simple, rapid extraction for CHCs in poultry fat using SPE and fast GC/ECD analysis with a Phenomenex Zebron™ ZB-MultiResidue™-1 column.

Experimental Conditions

Sample Preparation

Pre-treatment:

1. Render poultry tissue pads using a microwave in 1 minute intervals ensuring the sample does not exceed 100 °C.
2. Weigh 1 gram of rendered fat into a 10 mL volumetric flask and bring to volume with hexane containing internal standards 1 and 2 for SPE.
3. Vortex or shake volumetric flasks to ensure proper mixing. Sample is now ready for SPE.

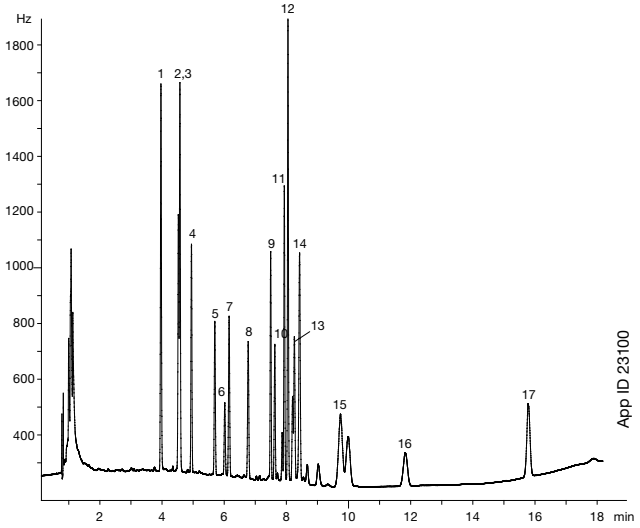
Solid Phase Extraction (SPE) Protocol

1. Before extracting samples, condition Strata Alumina-N SPE cartridges (part no. 8B-S313-KDG) by placing on vacuum chamber with pump and filling each cartridge to volume with 86/14 methanol/water solution.
2. Slowly rinse the solution through the cartridge at a rate of approximately 10 mL/minute until bedding is dry.
3. Fill cartridges to volume with petroleum ether to remove traces of methanol/water solution and drip at a rate of approximately 10 mL/minute.
4. Place glass culture tubes inside vacuum chamber to catch sample eluent and replace manifold to ensure a tight vacuum seal.
5. Pipet 1 mL sample into cartridge.
6. As sample drains through alumina bed, fill cartridge to volume with 1.5/98.5 Ethyl Ether/ Petroleum Ether solution to ensure analyte elution.
7. Once samples have been collected, transfer glass culture tubes into nitrogen evaporator and evaporate to dryness.
8. Reconstitute samples with 2 mL Hexane, vortex for 30 seconds, and transfer to autosampler vial for injection

GC/ECD Conditions

Column: Zebron ZB-MultiResidue -1
Dimensions: 30 m x 0.32 mm x 0.50 µm
Part No.: 7HM-G016-17
Injection: Splitless @ 250 °C, 1.0 µL
Carrier Gas: Helium (ramped flow) 3.4 mL/min for 6 min to 8 mL/min @ 5 mL/min, hold 12.08 min
Oven Program: 150 °C for 0.5 min to 220 °C @ 20 °C/min to 310 °C @ 6 °C/min
Detector: ECD @ 350 °C
Makeup Gas: 40 mL/min (N_2)
Analytes:
1. 2,4,5,6-Tetrachloro-m-xylene (IS 1)
2. α-BHC
3. Hexachlorobenzene
4. Lindane
5. Heptachlor
6. Dursban® (Chlorpyrofos)
7. Aldrin
8. Heptachlor Epoxide
9. p,p'-DDE
10. Dieldrin
11. o,p-DDT
12. p,p'-DDE
13. Endrin
14. p,p'-DDT
15. Methoxychlor
16. Mirex
17. Decachlorobiphenyl (IS 2)

Figure 1.
GC/ECD analysis of CHC extraction



Results

The GC method was optimized on a Zebron™ ZB-MultiResidue-1 column, which is a proprietary mid-polar phase. This phase offered high efficiency and selectivity required to resolve all 17 components in the pesticide mix. The run time was 19 minutes, with the last peak in the chromatogram, Decachlorobiphenyl, eluting at 15.59 minutes. This gave room to hold the column for 3.4 more minutes at the final temperature of 310 °C to bake the column after each run. As shown in **Figure 1**, all the peaks eluted at 310 °C, which is well below the column's maximum temperature. This aspect enhances the lifetime of the column. The MS certified, engineered self cross-linked phase not only gave enhanced separation for the pesticide mix but also provided the lowest bleed on ECD.

The presented method is selective in terms of extraction, separation, and detection: the Strata® Alumina-N SPE sorbent is a polar phase that extracts polar compounds, such as chlorinated pesticides, from food matrices; the GC analysis using the Zebron ZB-MultiResidue-1 column offers the selectivity and sensitivity necessary to separate the 17 pesticide compounds; and the ECD is selective to electronegative compounds and gives high peak response to chlorinated pesticides. The selectivity in each stage of analysis resulted in bringing the detection limits to µg/L level. The SPE sample preparation and GC analytical method were optimized by Tyson Foods, Inc. Further optimization of the method can be done based on specific analytical goals. Carrier gas in constant flow mode is recommended for quantitative analysis using ECD. To further enhance lifetime, the GC oven program can be extended for 5 minutes and a Z-Guard™ column can be used in front of the Zebron ZB-MultiResidue-1 column to prevent non-volatile impurities from attacking the analytical column.

The practical implications of this methodology revision are quite significant due to the space and labor saving aspects. With no reduction in sample volume, the residue section of the Tyson Foods, Inc. Food Safety and Research Laboratory was able to effectively cut labor by 50 percent and space required by 75 percent while maintaining compliance with program protocols. The Strata Alumina-N SPE cartridges greatly increased efficiency by providing excellent results at a fraction of the time required to manually prepare columns under the traditional method. This increase in efficiency allowed flexibility to reassign labor previously tied up for pesticide residue analysis to focus on other areas of need throughout the laboratory. Additionally, these products allow for a greener method by requiring much less organic solvents and chemicals to extract CHC residues. This contributes to a reduction in hazardous waste which impacts both safety and budgetary issues.

Conclusion

The Strata Alumina-N SPE method decreases the amount of labor and reagent that is associated with the USDA-FSIS CHC method. This analysis can be performed in an 8 foot vent hood freeing up valuable bench top space for an expanding laboratory. The combination of these findings make for decreased costs involved with reagent use and disposal as well as the labor and time associated with the analysis while providing accurate and reliable results.

Acknowledgements

We would like to provide special thanks to Tyson Foods, Inc. and WBA Analytical Laboratories for their contributions.

Tyson Foods, Inc.

WBA Analytical Laboratories

Phenylbutazone in Ground Meat

Using Solid Phase Extraction and LC/MS/MS

Introduction

Communities worldwide were outraged in 2012 when it was discovered that horse meat had been illegally incorporated into food products. As scientific laboratories raced to identify all sources of contamination, concern was also raised about the potential for veterinary drugs such as phenylbutazone (bute) to have entered the food chain. Many veterinary drugs that are safe and approved for use in animals can pose significant health risk to humans, especially children.

In Europe, horses can be classified as food producing animals and maximum residue limits have been set for pharmacologically active substances in foodstuffs of animal origin.

Phenylbutazone is a non-steroidal anti-inflammatory drug (NSAIDs) commonly prescribed for horses. Phenylbutazone can cause blood dyscrasias and is a carcinogen. Due to its toxicity, phenylbutazone is not approved for human use. In order to establish the compliance with the legislation, a robust analytical method for phenylbutazone is required. The meat matrix is a sample cleanup challenge due to its high complexity. Several most anticipated matrix interferences from meat are lipids, proteins, and carbohydrates. A highly selective extraction/cleanup method is necessary in order to eliminate matrix interferences which might generate false-positive or false-negative results.

Previously published analytical procedures require liquid extractions with a variety of solvents such as acetonitrile and methanol, followed by a multi-step solid phase extraction method using different retention modes and LC/MS/MS analysis with long run times. In this study, we demonstrate an ultra-selective single step solid phase extraction of phenylbutazone and a rapid, high resolution LC/MS/MS analysis.

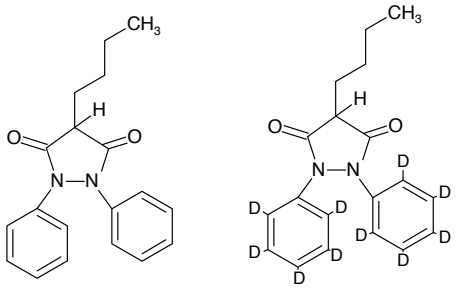
Experimental Conditions

Sample Preparation

Sample Pre-treatment

1. Add 2 mL 0.1 N NaOH and 50 µL of 4 µg/mL Int Std (phenylbutazone-D10) to 2 ±0.1 g homogenized ground beef sample in an Erlenmeyer flask (or similar).
2. Cap and shake vigorously for at least 30 seconds using a vortex.
3. Homogenize the meat sample with the Omni TH hand homogenizer machine until the sample appears as a very thick slurry, then add 8 mL of methanol to the mixture.
4. Cap and place on a mechanical shaker for 1 hour at medium high to high setting. Make sure the vessels are properly secured.
5. Transfer the mixture to a 15 mL centrifuge tube and centrifuge for 10 minutes at room temperature (18-22 °C) and 3000- 3200 rpm.
6. Add 2 mL of the resulting supernatant to 2 mL of deionized (D.I.) water for solid phase extraction.

Figure 1.
Phenylbutazone and Phenylbutazone-D10 Chemical Structures



Solid Phase Extraction (SPE) Protocol

Cartridge:	Strata®-X-A, 100 mg/6 mL
Part No.:	8B-S123-ECH
Condition:	3 mL Methanol
Equilibrate:	3 mL DI Water
Load:	4 mL of Pretreated sample
Wash 1:	2 mL D.I. Water
Wash 2:	2 mL Acetonitrile
Wash 3:	2 mL Ethyl Acetate
Dry:	5 minutes under full vacuum
Elute:	2x 1.5 mL 1 % Formic Acid in Methanol
Dry Down:	Evaporate under a stream of nitrogen gas at 50 °C to dryness
Reconstitute:	Resuspend the residue with 500 µL of 50:50-Methanol/ 0.1% Formic Acid

LC/MS/MS Conditions

Column: Kinetex® 2.6 µm XB-C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4496-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol

Gradient:

Time (min)	B (%)
0.00	50
0.50	50
2.00	95
3.50	95
3.51	50
5.00	50

Flow Rate: 450 µL/min
Column Temp: 45 °C
Detection: API 4000™ (SCIEX) triple quad
Analytes: 1. Phenylbutazone-D10 (I.S.)
2. Phenylbutazone
3. Solvent/MP impurity

Table 1.
% Recovery of Phenylbutazone from Beef Extract at 5 ppb and 75 ppb (µg/Kg) n=4

Spiked Conc.	%CV	Accuracy
5	8.02	100.7
75	5.0	90.3

Figure 2.
Linearity Curve of Phenylbutazone from Meat extract (1-200 ppb µg/Kg) Linear with 1/x weighting factor, CC=0.9991

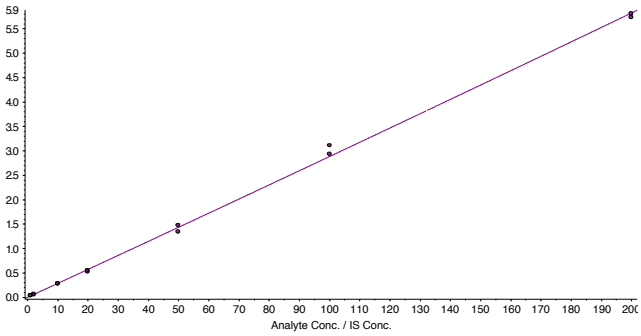


Figure 3.
Chromatogram of 10 ppb (µg/Kg) Phenylbutazone and its internal standard

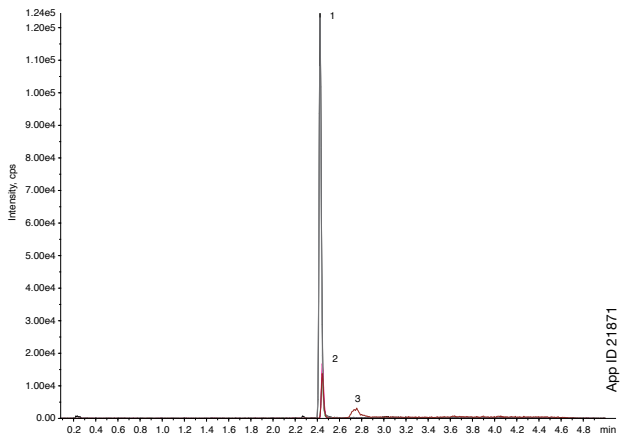
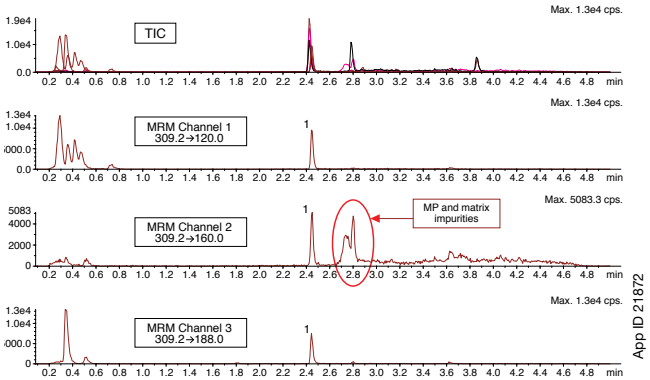


Figure 4.
A representative chromatogram of phenylbutazone extract from ground beef. Total ion chromatogram (TIC) and each individual MRM channels are displayed for clarity. Peak assignment: 1) Phenylbutazone



Results

For prohibited and unauthorized analytes, the limit of detection (LOD) must be at or less than the Minimum Required Limit (MRL) as defined in Commission Decision 2002/657/EC.

The determination of phenylbutazone from meat requires a LOD below 5 ppb. A highly sensitive detector, supported by a selective sample preparation are required in order to accurately analyze phenylbutazone at such low levels.

In this study, we employed a polymeric strong anion exchange method using Strata®-X-A. Phenylbutazone has a strongly acidic α-hydrogen that is ionized above pH ~5 **(Figure 1)**. In its ionized form, phenylbutazone can retain very strongly via ion-exchange mechanism on the Strata-X-A sorbent, which is surface-modified with quaternary amine groups. The powerful ion-exchange interactions allow for two aggressive washes— acetonitrile followed by ethyl acetate to furnish an extremely thorough cleanup of the meat matrix. The recoveries of phenylbutazone extracted by the Strata-X-A method are shown in **Table 1**. Ground beef spiked at 5 and 75 ppb were extracted and analyzed against a matrvix matched calibration curve **(Figure 2)**. The matrix-matched calibration curve of 1- 200 ppb (µg/Kg) yielded a linear fit (r=0.9991) with 1/x weighting factor, where x is the concentration. The LOD of this method is 1 ppb (µg/Kg). The 2 ppb (µg/Kg) spiked meat extract produced an average accuracy of 108.2 % (n=2) against the calibration curve and can be used as the LOQ. In this study, a minimal injection volume of 5 µL was used. A slightly larger injection volume can further improve both LOD & LOQ levels.

For LC/MS/MS analysis, a Kinetex® 2.6µm XB-C18 core-shell HPLC column was chosen. The innovative core-shell technology can provide sub-2µm performance at backpressure compatible with standard HPLC instruments. The 2.6µm particle gives backpressure similar to a 3µm particle, but the improved mass transfer kinetics of the core-shell particle significantly enhance resolving power, giving sub-2µm performance. In the current analysis, the backpressure generated was 253 bar. The chromatogram of 10ppb (µg/Kg) spiked ground beef extract is shown in **Figure 3**.

It is interesting to mention that during LC/MS/MS method development, the MRM transition 309.2→160.0 displayed a persistent broad peak eluting at a later retention time than phenylbutazone **(Figure 4)**. This may have been a mobile phase related impurity that was introduced onto the LC column during the column equilibration period and did not come from the sample or extraction procedure. In ground beef extract, the same channel also displayed a sharp peak almost co-eluting with the other impurity peak observed earlier. However, both of these peaks are well separated from phenylbutazone on the Kinetex 2.6µm core-shell particle combined with unique XB-C18 chemistry. The resolution of these impurities further allows the suitability of three MRM channels for low level determination (both screening and confirmation) of this toxic anti-inflammatory residue in meat.

Conclusion

A rapid and robust method to quantify and unequivocally identify phenylbutazone in ground meat samples was developed. The single step strong anion exchange SPE method eliminates problematic lipids, proteins, and other matrix interferences prior to a high efficiency HPLC method using a Kinetex 2.6µm XB-C18 column. This method generated a matrix-matched calibration with excellent linearity, which provided high precision and accuracy. The estimated detection and quantification limits are well below 5ppb, the method performance requirement proposed by the EU reference laboratory for NSAIDs.

The sample pretreatment procedure is flexible to incorporate an enzymatic hydrolysis step if needed, as the highly selective strong anion exchange extraction and cleanup method is able to specifically extract and concentrate phenylbutazone amidst any intricate matrices.

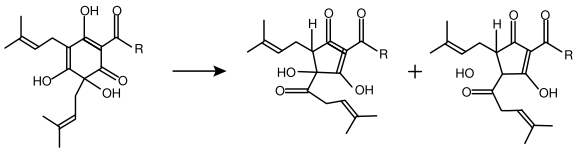
Hop Acids in Beer

Using Solid Phase Extraction and LC/MS/MS

Introduction

Iso-alpha acids, derived from hops, are the compounds responsible for beer's bitter taste. During the beer brewing process, compounds known as alpha acids are extracted from the hops and isomerized to form iso-alpha acids **(Figure 1)**. The conversion of alpha acids into iso-alpha acids takes place when the hops are added to the wort (unfinished beer) and boiled. The amount and type of iso-alpha acids formed is dependent on a number of factors including the boiling time, the variety and age of the hops, and the pH of the wort. The bitterness derived from iso-alpha hop acids is a primary flavor attribute of beer and accurate determination of beer bitterness is of great importance to the brewer. Therefore, to maintain a consistent product, brewers must carefully monitor the levels of iso-alpha acids throughout the manufacturing process and in the final beer product.

Figure 1.
Structures of Iso-Alpha Acids



R =	α-acids	trans-iso-α-acids	cis-iso-α-acids
$CH(CH_3)_2$	cohumulone	trans-isocohumulone	cis-isocohumulone
$CH2CH(CH_3)_2$	humulone	trans-isohumulone	cis-isohumulone
$CH(CH_3)_2 H_5$	adhumulone	trans-isoadhumulone	cis-isoadhumulone

Experimental Conditions

Sample Preparation

Solid Phase Extraction

Each beer sample was degassed by stirring for approximately 30 min at room temperature.

Cartridge: Strata®-X 200 mg/6 mL
Part No.: 8B-S100-FCH
Condition: 4 mL acidified methanol (1-2 mL/min)
Equilibrate: 4 mL water (1-2 mL/min)
Note: Do not let sorbent run dry
Load: 5 mL of beer degassed (1 mL/min)
Wash: 4 mL of 40 % methanol in water
Dry: >10" Hg for 5 minutes to remove residual water
Elute: 2 mL of acidified methanol (1 mL/min)
Drydown: Nitrogen gas at 55 °C
Reconstitute: 500 µL of mobile phase

HPLC Conditions

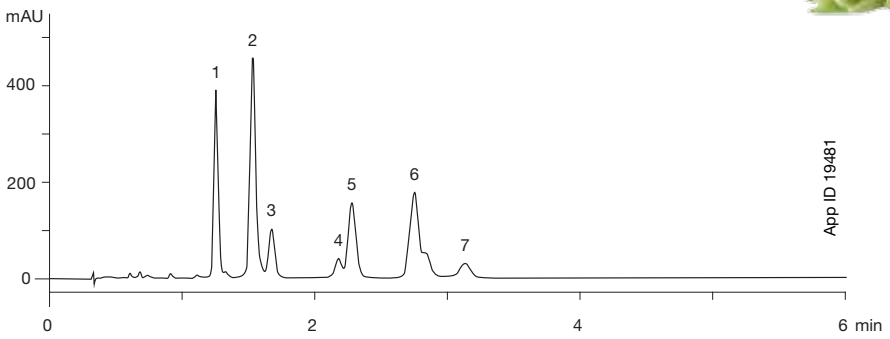
Column: Kinetex® 2.6 µm C18 100Å
Dimensions: as noted
Mobile Phase: Methanol / Water / Phosphoric acid (75:24:1, v/v/v)
Flow Rate: 1.4 mL/min
Injection Volume: as noted
Temperature: as noted
Detection: UV @ 270 nm (ambient)
Sample: as noted

Results and Discussion

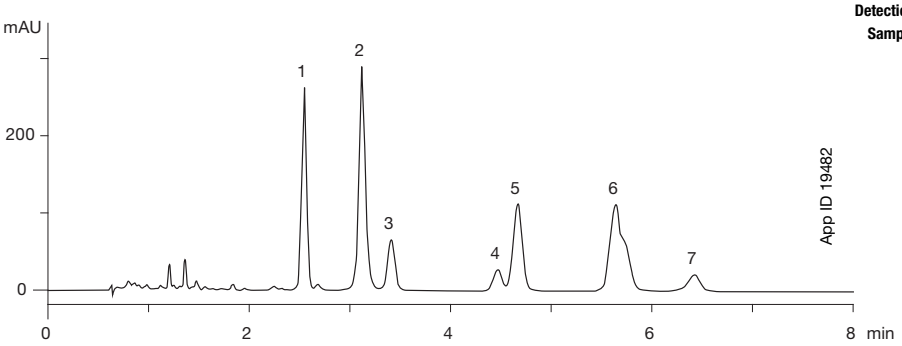
Figure 2 is a chromatogram of hop acid standards (obtained from the American Society of Brewing Chemists) run on a Kinetex 2.6 µm C18 column. All six of the iso-alpha acids that are most commonly tested for by brewers, were resolved isocratically in less than 7 min. The first three hop acids isomers (isocohumulone, isohumulone, and isoadhumulone) are derived from the naturally occurring alpha-acids in hops. The last three hop acids isomers (tetrahydroisocohumulone, tetrahydroisohumulone, and tetrahydroisoadhumulone) are specially modified reduced forms of the corresponding iso-alpha acids (tetrahydro-iso-alpha acids) and resistant to photo degradation. Both the naturally derived and the tetrahydro-iso-alpha acids have cis and trans isomers, therefore partial or even full resolution of some of the cis and trans iso-alpha acids is to be expected.

Both the 50 mm and 100 mm Kinetex columns completely separate all 6 iso-alpha acids **(Figures 2a and 2b)**. The 100 mm column provides near baseline separation for cis- and trans-tetrahydroisocohumulone (peaks 4 & 5), while the 50 mm column only partially separates these compounds. Cis- and trans-tetrahydroisohumulone coelute on both Kinetex column dimensions (peak 6), but because of the better separation of the tetrahydroisocohumulone isomers, more accurate quantitation is achieved with the longer 100 mm Kinetex column.

Figure 2.
Iso-Alpha Acid Standards
a) Kinetex 2.6 µm C18 50 x 4.6 mm



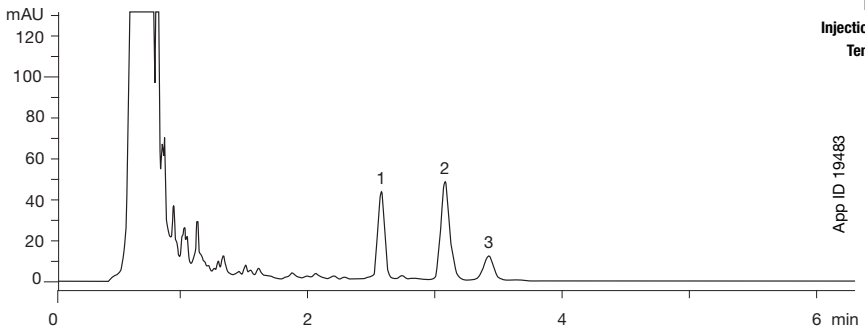
b) Kinetex 2.6 µm C18 100 x 4.6 mm



Column: Kinetex® 2.6 µm C18 100 Å
Mobile Phase: Methanol / Water / Phosphoric acid (75:24:1, v/v/v)
Flow Rate: 1.4 mL/min
Injection Volume: 1 µL
Temperature: 45 °C
Detection: UV @ 270 nm (ambient)
Sample:
1. Isocohumulone
2. Isohumulone
3. Isoadhumulone
4. trans-Tetrahydroisocohumulone
5. cis-Tetrahydroisocohumulone
6. Tetrahydroisohumulone
7. Tetrahydroisoadhumulone

Figure 3 analysis is performed on a beer sample (Red Stripe® Jamaican Lager) containing iso alpha-acids using Kinetex 2.6 µm C18 **(Figure 3)**, all of the hop acids are well resolved with excellent peak shape in less than 4 minutes.

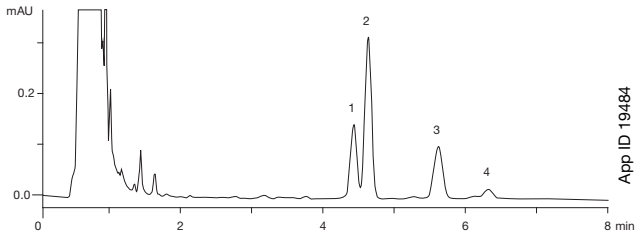
Figure 3.
Kinetex 2.6 µm C18 100 x 4.6 mm



Column: Kinetex 2.6 µm C18 100 Å
Dimensions: 100 x 4.6 mm
Part No.: 00D-4462-E0
Mobile Phase: Methanol / Water / Phosphoric acid (75:24:1, v/v/v)
Flow Rate: 1.4 mL/min
Injection Volume: 50 µL
Temperature: 22 °C
Detection: UV @ 270 nm (ambient)
Sample:
1. Isocohumulone
2. Isohumulone
3. Isoadhumulone

Figures 4 is obtained from the analysis of beer samples that contain tetrahydro-iso-alpha acids. (Miller Genuine Draft, **(Figure 4)** is analyzed using the Kinetex 2.6 µm C18 column, the tetra acids are eluted in less than 7 minutes. Note that the cis and trans isomers are partially resolved under these conditions, but quantified as a single unit.

Figure 4.
Kinetex 2.6 µm C18 100 x 4.6 mm



Column: Kinetex® 2.6 µm C18 100 Å
Dimensions: 100 x 4.6 mm
Part No.: 00D-4462-E0
Mobile Phase: Methanol / Water / Phosphoric acid (75:24:1, v/v/v)
Flow Rate: 1.4 mL/min
Injection Volume: 50 µL
Temperature: 22 °C
Detection: UV @ 270 nm (ambient)
Sample: 1. *trans*-Tetrahydroisocohumulone
2. *cis*-Tetrahydroisocohumulone
3. Tetrahydroisohumulone
4. Tetrahydroisoadhumulone

The iso-alpha acids are present in sufficient concentration to be able to be detected by simply injecting 50 µL of degassed beer straight onto the LC column without any sample preparation (other than degassing). All analytes are completely separated from any obvious matrix interferences. However, the complex nature of the beer matrix makes sample preparation worth investigating. The iso-alpha acids were successfully extracted from packaged beer using Strata®-X SPE products. A 40 % methanol wash was found to remove some sample matrix components without adversely affecting analyte recovery. Two mL of acidified methanol was sufficient for eluting the analytes from the SPE cartridge. Recoveries from two commercial beer samples are shown in **Tables 1** and **2** and the corresponding chromatograms are shown in **Figure 5**.

Conclusions

Typical HPLC methods for measuring iso-alpha acids throughout the brewing process have relied upon HPLC columns packed with fully porous particles. Converting these methods over to columns packed with high efficiency Kinetex 2.6 µm core-shell particles significantly improves chromatographic resolution while drastically reducing analysis times. While the iso-alpha acids are typically present in sufficient concentration to allow for direction analysis of the beer sample, a simple SPE cleanup was demonstrated to remove potential matrix interferences with good recovery.

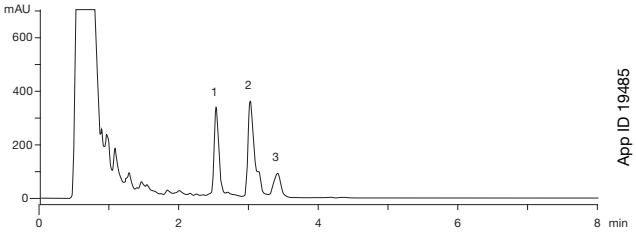
Table 1.
SPE Recoveries of Iso-alpha Acids from
Red Stripe® Jamaican Lager Beer using Strata-X

Iso-Alpha Acids	% Recovery
Isocohumulone	104
Isohumulone	104
Isoadhumulone	118

Table 2.
SPE Recoveries of Tetrahydro-Iso-alpha Acids from
Miller® Genuine Draft Beer using Strata-X

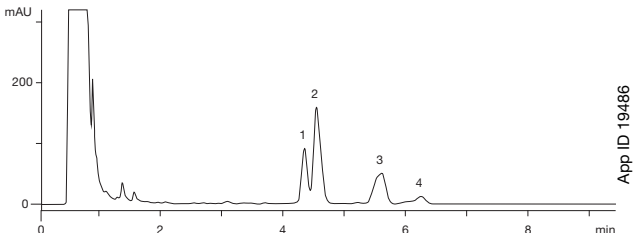
Tetra Iso-alpha Acids	% Recovery
Tetrahydroisocohumulone	80
Tetrahydroisohumulone	82
Tetrahydroisoadhumulone	69

Figure 5.
a) Iso-Alpha Acids from Red Stripe® beer following SPE



Column: Kinetex 2.6 µm C18 100 Å
Dimensions: 100 x 4.6 mm
Part No.: 00D-4462-E0
Mobile Phase: Methanol / Water / Phosphoric acid (75:24:1, v/v/v)
Flow Rate: 1.4 mL/min
Injection Volume: 50 µL
Temperature: 22 °C
Detection: UV @ 270 nm (ambient)
Sample: 1. Isocohumulone
2. Isohumulone
3. Isoadhumulone

b) Tetrahydro-iso-alpha acids from Miller® Genuine Draft beer following SPE

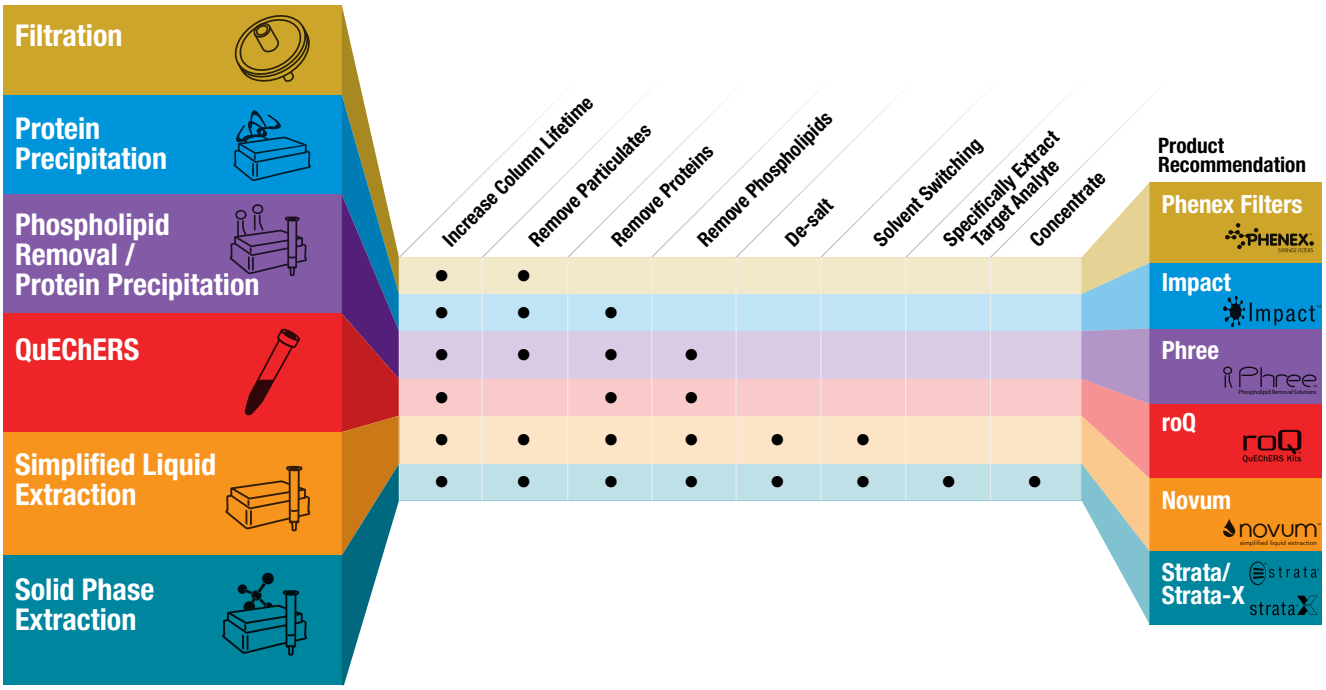


Column: Kinetex 2.6 µm C18 100 Å
Dimensions: 100 x 4.6 mm
Part No.: 00D-4462-E0
Mobile Phase: Methanol / Water / Phosphoric acid (75:24:1, v/v/v)
Flow Rate: 1.4 mL/min
Injection Volume: 50 µL
Temperature: 22 °C
Detection: UV @ 270 nm (ambient)
Sample: 1. *trans*-Tetrahydroisocohumulone
2. *cis*-Tetrahydroisocohumulone
3. Tetrahydroisohumulone
4. Tetrahydroisoadhumulone

Featured Resources and Guides

Sample preparation is crucial in achieving desired analytical results. Sample matrix effects can result in an array of interferences which can lead to poor chromatography as well as instrumentation drawbacks, hindering your approach and goal for the analysis.

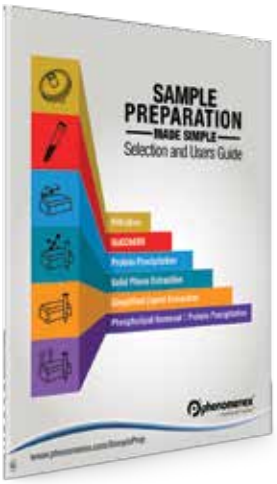
Choose Your Best Sample Preparation Solution



Sample Preparation Selection and Users Guide

Over 50 pages to assist you in selecting and using the appropriate technique. Request Your Copy:

www.phenomenex.com/SPguide



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Sample Prep Solutions

roQ™ QuEChERS Kits

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

*AOAC and EN Extraction Kits also available in traditional non-collared 50 mL centrifuge tubes, Part No.: KS0-8911-NC and KS0-8909-NC

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

roQ Extraction Salt Packets

Salt packets only. Centrifuge tubes not included.

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Packets		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	AH0-9043
EN 15662 Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	AH0-9041
Original Non-Buffered Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	AH0-9042
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	AH0-9044

Bulk roQ QuEChERS Sorbents

Phase	10 g	100 g
C18-E	—	04G-4348
GCB (Graphitized Carbon Black)	04D-4615	04G-4615
PSA	—	04G-4610

Sample Prep Solutions

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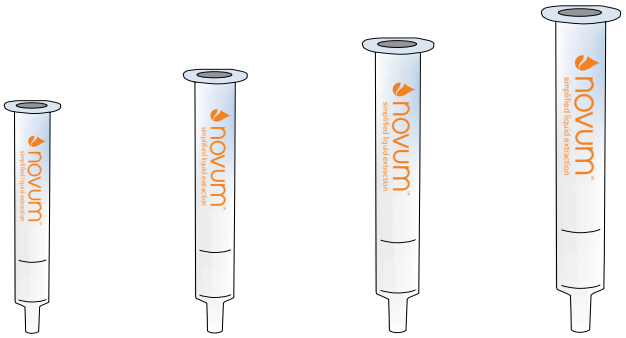
simplified liquid extraction
PATENT PENDING

Novum Simplified Liquid Extraction (SLE) Tubes

Process samples as small as 100 µL or as large as 1 mL using Novum SLE tubes.
Ideal for all types of applications including Bioanalytical, Food Testing and Environmental.



Novum SLE Tubes	1 cc	3 cc	6 cc	12 cc
Maximum Sample Volume (before dilution)	100 µL	200 µL	500 µL	1 mL
Recommended Elution Volume	1.2 mL	1.8 mL	5 mL	10 mL
Part No.	8B-S138-FAK	8B-S138-5BJ	8B-S138-JCH	8B-S138-KDG
Unit	100/pk	50/pk	30/pk	20/pk

Sample Prep Solutions

strata X Polymeric SPE

Strata-X (Reversed Phase)

Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S100-ECH	6 mL (30/box)
	200 mg	8B-S100-FBJ	3 mL (50/box)
	200 mg	8B-S100-FCH	6 mL (30/box)
	500 mg	8B-S100-HBJ	3 mL (50/box)
	500 mg	8B-S100-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S100-HDG	12 mL (20/box)
	1 g	8B-S100-JDG	12 mL (20/box)
	1 g	8B-S100-JEG	20 mL (20/box)
	2 g	8B-S100-KEG	20 mL (20/box)
	5 g	8B-S100-LFF	60 mL (16/box)

Strata-X-C (Strong Cation – Exchange)

Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S029-ECH	6 mL (30/box)
	200 mg	8B-S029-FBJ	3 mL (50/box)
	200 mg	8B-S029-FCH	6 mL (30/box)
	500 mg	8B-S029-HBJ	3 mL (50/box)
	500 mg	8B-S029-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S029-HDG	12 mL (20/box)
	1 g	8B-S029-JDG	12 mL (20/box)
	1 g	8B-S029-JEG	20 mL (20/box)
	2 g	8B-S029-KEG	20 mL (20/box)
	5 g	8B-S029-LFF	60 mL (16/box)

Strata-X-CW (Weak Cation – Exchange)

Format	Sorbent Mass	Part Number	Unit
Tube			
	200 mg	8B-S035-FBJ	3 mL (50/box)
	200 mg	8B-S035-FCH	6 mL (30/box)
	500 mg	8B-S035-HBJ	3 mL (50/box)
	500 mg	8B-S035-HCH	6 mL (30/box)
Giga™ Tube			
	1 g	8B-S035-JDG	12 mL (20/box)
	1 g	8B-S035-JEG	20 mL (20/box)
	2 g	8B-S035-KEG	20 mL (20/box)
	5 g	8B-S035-LFF	60 mL (16/box)

Strata-X-A (Strong Anion – Exchange)

Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S123-ECH	6 mL (30/box)
	200 mg	8B-S123-FBJ	3 mL (50/box)
	200 mg	8B-S123-FCH	6 mL (30/box)
	500 mg	8B-S123-HBJ	3 mL (50/box)
	500 mg	8B-S123-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S123-HDG	12 mL (20/box)
	1 g	8B-S123-JDG	12 mL (20/box)
	1 g	8B-S123-JEG	20 mL (20/box)
	2 g	8B-S123-KEG	20 mL (20/box)
	5 g	8B-S123-LFF	60 mL (16/box)

Strata-X-AW (Weak Anion – Exchange)

Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S038-ECH	6 mL (30/box)
	200 mg	8B-S038-FBJ	3 mL (50/box)
	200 mg	8B-S038-FCH	6 mL (30/box)
	500 mg	8B-S038-HBJ	3 mL (50/box)
	500 mg	8B-S038-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S038-HDG	12 mL (20/box)
	1 g	8B-S038-JDG	12 mL (20/box)
	1 g	8B-S038-JEG	20 mL (20/box)
	5 g	8B-S038-LFF	60 mL (16/box)

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HPLC and GC Options

KINETEX® Core-Shell Technology

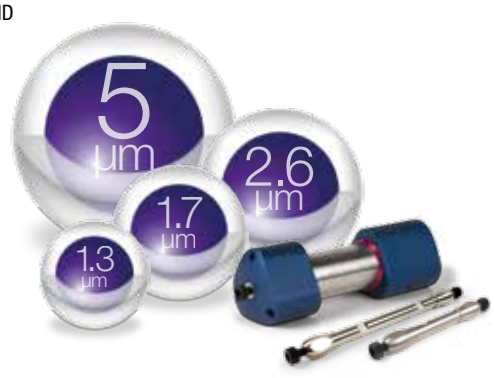
Kinetex Core-Shell HPLC Columns

5 µm Columns (mm)		SecurityGuard ULTRA Cartridges‡					SecurityGuard ULTRA Cartridges‡
Phases	50 x 2.1	3/pk	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
XB-C18	00B-4605-AN	AJ0-8782	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJ0-8768
C18	00B-4601-AN	AJ0-8782	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
		for 2.1 mm ID					for 4.6 mm ID

2.6 µm Analytical Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJ0-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768
						for 4.6 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
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
						for 3.0 mm ID

2.6 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
XB-C18	00A-4496-AN	00B-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782
					for 2.1 mm ID



Zebron™ GC Columns

Zebron ZB-MultiResidue GC columns (MR)-1

ID(mm)	df(µm)	Temp. Limits °C	Part No.
20-Meter			
0.18	0.18	-60 to 320/340	7FD-G016-08
30-Meter			
0.25	0.25	-60 to 320/340	7HG-G016-11
0.32	0.25	-60 to 320/340	7HM-G016-11
0.32	0.50	-60 to 320/340	7HM-G016-17
0.53	0.50	-60 to 320/340	7HK-G016-17

Zebron ZB-MultiResidue GC columns (MR)-2

ID(mm)	df(µm)	Temp. Limits °C	Part No.
30-Meter			
0.25	0.20	-60 to 320/340	7HG-G017-10
0.32	0.25	-60 to 320/340	7HM-G017-11
0.53	0.50	-60 to 320/340	7HK-G017-17

Note: If you need a 5 in. cage, simply add a (-B) after the part number, e.g., 7HG-G016-11-B or 7HG-G017-10-B. Some exceptions may apply. Agilent 6850 and some SRI and process GC systems use only 5 in. cages.

guarantee

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

FOOD TESTING
SAMPLE PREPARATION
PRODUCT SELECTION & APPLICATION GUIDE

Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 (0)20 2282-6668
f: +86 (0)20 2809-8130
chinainfo@phenomenex.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

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