FOOD TESTING

APPLICATION NOTEBOOK

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

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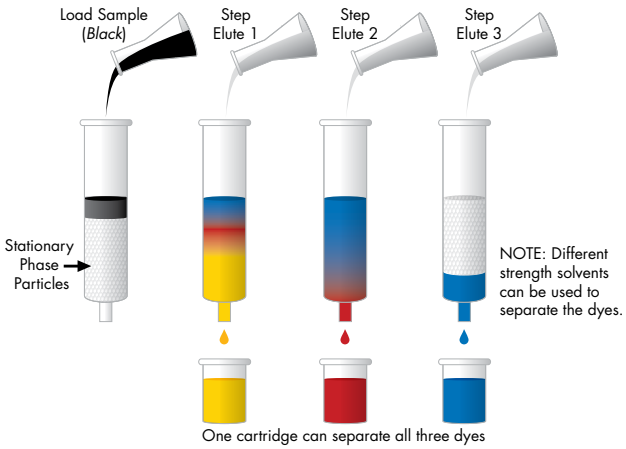
RETENTION-CLEANUP-ELUTION STRATEGY

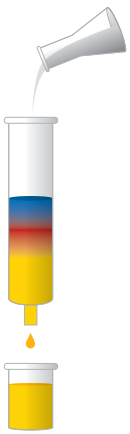
As the sample is loaded onto the cartridge, the analytes of interest are retained by the sorbent. If needed, an optimized series of washes are used to remove matrix interference from the cartridge. A strong solvent is used to elute the analytes from the cartridge. Sample enrichment results when the final elution volume is smaller than the load volume.



PASS-THROUGH CLEANUP STRATEGY

Pass-through cleanup methods optimize matrix retention while the analytes of interest pass-through the cartridge unretained. No sample enrichment occurs during the solid-phase extraction (SPE) step.

1. Sample is passed through sorbent and collected.
   - No sample enrichment.
2. Matrix interferences are retained on sorbent.



Pass-Through

SPE PROCEDURE STEPS

The following section describes the steps involved in a complete solid-phase extraction procedure:

1. PRETREATMENT

Solid samples (soil, tissue, etc.)

- Shake, sonicate, or use soxhlet extraction.
  - Extract sample with polar organic solvent (methanol, acetonitrile) for polar analytes.
  - Extract sample with organic solvent and drying agent (dichloromethane, acetone) for non-polar analytes and multi-residue extraction.

Non-aqueous liquid

- If the sample is soluble in water, dilute it with water for reversed-phase SPE.
- If the sample is soluble in hexane, dilute it with hexane for SPE.
- Alternatively, evaporate the solvent and exchange to hexane.

Wastewater

- Filter or centrifuge as necessary.

2. CONDITION

For reversed-phase sorbents, preconditioning of the sorbent with an organic solvent, such as methanol, acetonitrile, isopropanol, or tetrahydrofuran, is usually necessary to obtain reproducible results. Without this step, a highly aqueous solvent cannot penetrate the hydrophobic surface and wet the sorbent. Thus, only a small fraction of the sorbent surface area would be available for interaction with the analyte. For the same reason, it is important not to let silica-based SPE Cartridges dry out between the solvation step and the addition of the sample.
A complete preconditioning of a reversed-phase cartridge includes the solvation step and an equilibration with a low-strength solvent, such as water or buffer.

3. LOAD

When the analytes of interest are not retained by the sorbent, this is called analyte breakthrough. For some methods, such as pass-through cleanup, analyte breakthrough is desirable and is maximized for those specific methods. However, in all other cases, analyte breakthrough is unwanted and contributes to poor recovery and method reproducibility. Breakthrough occurs when:

- There is too high an organic concentration in the load solution for very polar analytes. Dilute sample at least 1:1 with water or buffer prior to loading.
- The analytes are bound to proteins, they may pass through the sorbent. Ensure that analytes are not bound to proteins by acidifying or basifying the sample.
- Sorbent is overloaded by the matrix component. Therefore, it is important to choose the correct sorbent mass (see Tables 1 and 2).
- The flow rate of the load step is too fast. There is not enough contact time between the analytes and the sorbent. Look at the drops and adjust the vacuum so that you see discrete droplets, not a stream of liquid.

Table 1. Choice of Oasis® Cartridges Based on Sample Size

Sample Size	Oasis Cartridge
1–10 mL	1 cc/30 mg or 3 cc/60 mg
10–100 mL	3 cc/60 mg or 6 cc/200 mg
100–500 mL	6 cc/200 mg or 6 cc/500 mg (LP*)
500–1000 mL	6 cc/500 mg (LP) or 12 cc/1 g (LP)

** LP=large particules (60 µm)*

4. WASH

The wash steps are designed to remove unwanted matrix components that remain from the loading step. The ideal wash solvent removes only the matrix while keeping the analytes bound to the sorbent. For complex samples this is impossible, so the wash steps are optimized using pH, solvent strength, and solvent polarity to remove as much matrix as possible while maintaining acceptable analyte recovery.

5. ELUTE

Once the interferences are washed off the cartridge, a strong solvent is introduced to elute the analytes of interest. The volume and flow rate of the eluting solvents should be precisely controlled as in the load step to ensure reproducible results. Refer to Table 3 for guidelines on various types of separation mechanisms and recommended solvents.

Table 2. Choice of Sep-Pak® Cartridges Based on Sample Size

Sample Size	Sep-Pak Cartridge
10–100 mL	3 cc/200 mg or 6 cc/500 mg
100–500 mL	3 cc/200 mg or 6 cc/500 mg
500–1000 mL	6 cc/500 mg (LP) or 6 cc/1 g

Table 3. Guidelines on the Various Types of Separation Mechanisms

	Reversed Phase	Normal Phase	Ion Exchange
Analyte	Low to moderate polarity/ hydrophobic	Moderate to highly polar/uncharged	Charged or ionized
Matrix	Aqueous	Non-polar organic solvent	Aqueous/low ionic strength
Condition/Equilibrate	1. Solvate polar organic 2. Water	Non-polar organic	Low ionic strength buffer
Wash	Aqueous/buffer	Non-polar	Low ionic strength buffer
Elute	Increase polar organic content in steps	Increase moderate to high polarity organic content in steps	Stronger ionic strength buffers or pH to neutralize the charge

Sep-Pak®

Sample Extraction Products

- Traditional SPE phases
- Many product formats
- Many literature references and validated methods available
- Ultra low extractables from Certified Sep-Pak Cartridges
- Reduced interferences and increased sensitivity using Certified Sep-Pak Cartridges

The convenient format and features of Sep-Pak Cartridges overcome many of the procedural difficulties of traditional column liquid-solid extraction and allow the enormous benefits of solid-phase extraction to be realized. Adsorbent and packed bed quality, reproducibility, versatility, and ease-of-use are assured through intelligent design, production control, and quality testing.

Sep-Pak Cartridge Separation Guidelines

Chromatographic Mode	Normal Phase	Reversed Phase	Ion Exchange
Separation Characteristic	Silica, Florisil, Alumina, Diol, NH_2, CN	C_{18}, tC_{18}, C_8, Diol, PoraPak® RDX, NH_2, CN	Accell Plus QMA, Accell Plus CM, NH_2
Packing Surface Polarity	High	Low	High
Typical Solvent Polarity Range	Low to medium	High to medium	High
Typical Sample Loading Solvent	Hexane, toluene, dichloromethane	Water with low ionic strength	Water, buffers
Typical Elution Solvent	Ethyl acetate, acetone, acetonitrile	Methanol, acetonitrile, dichloromethane	Buffers, salt solutions with high ionic strength
Sample Elution Order	Least polar sample components first	Most polar sample components first	Most weakly ionized sample components first
Solvent Change Required to Elute Retained Compounds	Increase solvent polarity	Decrease solvent polarity	Increase ionic strength or increase pH (anion exchange) or decrease pH (cation exchange)

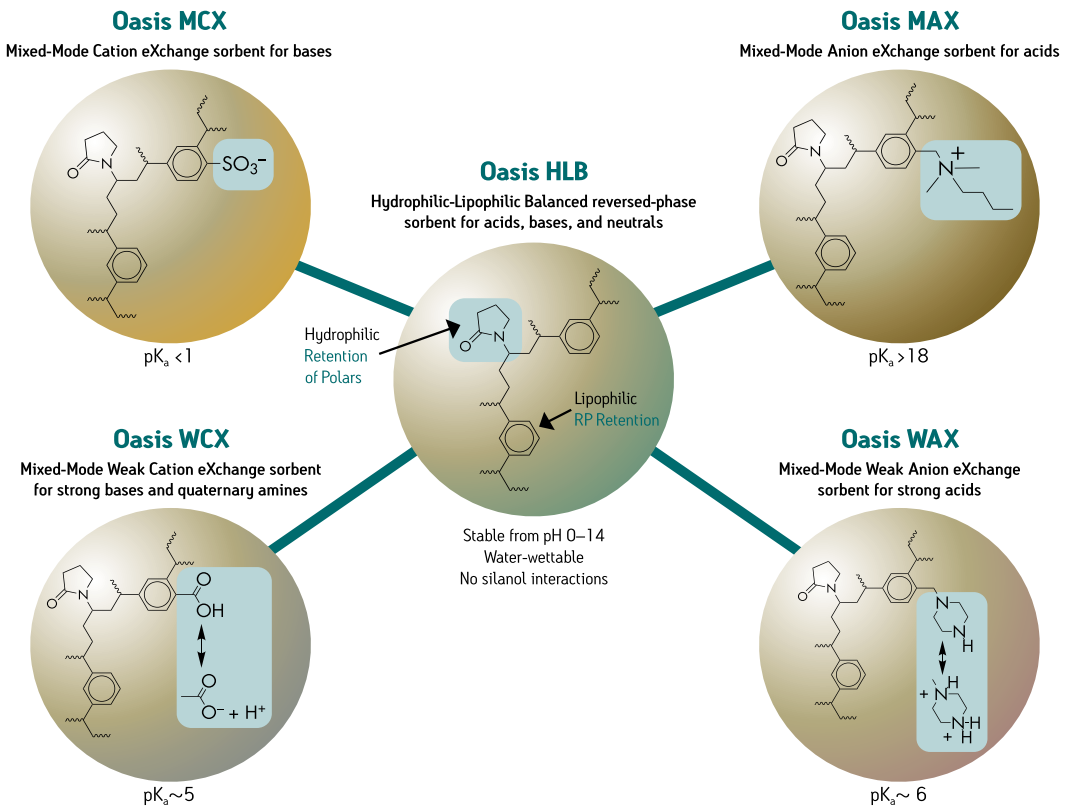
OASIS®
SAMPLE EXTRACTION PRODUCTS

- Waters premium brand for SPE
- Cartridges, plates, and µElution technology
- Co-polymer, water wettable, reproducible
- Outperforms C_{18} for polar bases

OASIS 2x4 METHOD—THE FASTEST, SIMPLEST, AND CLEANEST APPROACH TO SPE METHOD DEVELOPMENT

- Characterize your analyte (acid, base, pKa)
- Choose 1 of 5 Oasis sorbents

HLB: Hydrophilic-Lipophilic-Balanced reversed-phase sorbent for acids, bases and neutrals
MCX: Mixed-mode Cation eXchange sorbent for bases
MAX: Mixed-mode Anion eXchange sorbent for acids
WCX: Mixed-mode Weak Cation eXchange sorbent for strong bases and quaternary amines
WAX: Mixed-mode Weak Anion eXchange sorbent for strong acids



DisQuE™
Dispersive Sample Preparation

- Easy and straightforward method to implement, requiring little training
- Conforms to the AOAC and CEN official methods for determining pesticide residues in fruits in vegetables
- Cost effective
- Reliable, high quality product in a simple kit format

DisQuE DISPERSIVE SAMPLE PREPARATION KITS

Dispersive sample preparation, commonly referred to as "QuEChERS", is a simple and straightforward sample preparation technique suitable for multi-residue pesticide analysis in a wide variety of food and agricultural products. Waters DisQuE™ Dispersive Sample Preparation Kit contains conveniently packaged centrifuge tubes with pre-weighed sorbents and buffers designed for use with AOAC and European Committee for Standardization (CEN) official methods. DisQuE dispersive sample preparation is a well proven, high throughput sample preparation method for a wide array of pesticide in produce samples.

FILTERS

Filtration provides immediate protection for analytical system components and minimizes downtime. In partnership with Pall Life Sciences, Waters offers filtration products that are Certified for Compliance, which means they have been designed and developed to comply with regulatory and quality objectives.

CERTIFIED VIALS

Sample vials are a critical part to sample preparation. Ensure that the vials you use do not introduce unwanted contaminants and interferences. Waters provides a wide selection of certified vials, including the TruView® LCMS Certified Vials, tested to maximize sensitivity and improve detection limits for LC/UV/MS and LC/MS analysis. Do not compromise your test results; avoid ghost peaks, dislodged septa, and damaged needles.

ANALYTICAL STANDARDS AND REAGENTS

Waters understands the importance of high quality analytical standards and reagents in ensuring continuous analytical instrumentation advancements and workflow success. That is why Waters now offers standards and reagents that are pure, precisely formulated, reproducible, and traceable to exact specifications. From system performance standards to application specific standards, you can rely on Waters, the leading innovator of analytical instrumentation.

AccQ•Tag™ Ultra

UPLC® Amino Acid Analysis

The UPLC® Amino Acid Analysis Solution consists of:

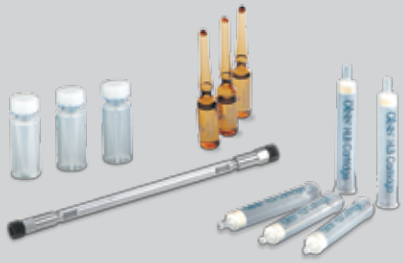
- Waters ACQUITY UPLC® System and tunable UV detector
- Full system and application level support documentation
- Application-specific performance qualification
- Connections INSIGHT® remote, intelligent services
- Empower® 2 software's pre-configured projects, methods, and report formats
- AccQ•Tag™ Ultra derivatization c hemistry including column, reagents, and eluents

AMINO ACID ANALYSIS

Amino acid composition is a critical component of the nutritional value of foods and feeds. Qualitative and quantitative amino acid analysis is used to determine the concentration and identity of a protein, or to confirm the origin of natural products based on the free amino acid content of a particular commodity. When used for food safety testing, amino acid analysis can determine protein deficiencies in processed food and to detect food adulteration that masks true protein content.

CARBAMATE ANALYSIS KIT

Containing a Waters Carbamate column, Oasis HLB Cartridges, Vials, and Reference Standards, this kit is optimized to simplify your analysis while increasing your confidence in the results.

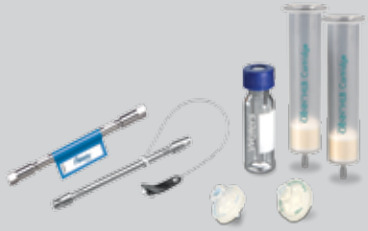


BEVERAGE ANALYSIS KIT

The kit analyzes soft drink formulations containing acesulfame-k, saccharin, caffeine, benzoate, sorbate, and aspartame and is designed to increase laboratory productivity, improve data quality, minimize cost, and enhance product consistency. Available for use with HPLC and UPLC systems.

MELAMINE ANALYSIS PACKAGES

Based on United States Food and Drug Administration (US FDA) Laboratory Information Bulletin No. 4422, these packages offer a comprehensive solution for screening Melamine and Melamine-related compounds in foods, including infant formula and dairy products. Available in both HPLC and UPLC formats.



COLUMN SELECTION GUIDE

Waters is committed to material sciences and, with our ongoing research into HPLC and UPLC column chemistries, we continue to develop ground-breaking column technologies. As scientific challenges evolve, Waters meets these changing needs with new column innovations.

XSelect®

C_{18}	**Selectivity Features:** General purpose reversed-phase column that offers excellent pH stability and rapid mobile-phase re-equilibration for method development. Charged Surface Hybrid (CSH™) Technology enables superior peak shape and increased loading capacity for basic compounds. **Bonding:** Trifunctional C_{18} ligand, fully end-capped, bonded to a CSH particle substrate.
CSH Phenyl-Hexyl	**Selectivity Features:** General purpose alternative selectivity ligand that provides pi-pi interactions with polyaromatic compounds, while maintianing excellent reproducibility at pH extremes. CSH Technology enables superior peak shape and increased loading capacity for basic compounds.. **Bonding:** Trifunctional C_6 Phenyl ligand, fully end-capped, bonded to a CSH particle substrate.
CSH Fluoro-Phenyl	**Selectivity Features:** General purpose column that provides a very high degree of analyte selectivity, especially when using low-pH mobile phases. CSH Technology enables superior peak shape and increased loading capacity for basic compounds. **Bonding:** Trifunctional propyl fluorophenyl ligand, non-endcapped, bonded to a CSH particle substrate.
HSS C_{18}	**Selectivity Features:** High performance C_{18} chemistry, increased retention, superior peak shape, resists acid hydrolysis at low pH. Designed for UPLC separations where silica-based C_{18} selectivities are desired. **Bonding:** High coverage trifunctional C_{18}, fully endcapped, bonded to High Strength Silica (HSS) HPLC particle substrate.
HSS C_{18} SB	**Selectivity Features:** Unique, non-endcapped C_{18} chemistry designed specifically for method development scientists. Offers unique Selectivity for Bases (SB) when operating under low pH conditions and transferability between UPLC and HPLC separations. **Bonding:** Intermediate coverage trifunctionally bonded C_{18}, no endcapping, bonded to HSS HPLC particle substrate.
HSS T3	**Selectivity Features:** Aqueous mobile-phase compatible HPLC column designed for extreme retention. Combines polar compound retention with transferability between UPLC and HPLC separations. **Bonding:** T3 (C_{18}) bonding and endcapping, bonded to HSS HPLC particle substrate.

XBridge®

C_{18}	**Selectivity Features:** General purpose column ideally suited for method development due to extreme pH stability and applicability to the broadest range of compound classes. **Bonding:** Trifunctional C_{18}, fully endcapped, bonded to Ethylene Bridged Hybrid (BEH) substrate.
Shield RP18	**Selectivity Features:** Alternate selectivity as compared to straight chain C_{18}, particularly with phenolic analytes. Compatible with 100% aqueous-phase composition. **Bonding:** Monofunctional embedded polar C_{18}, fully endcapped, bonded to substrate.
C_8	**Selectivity Features:** General purpose column ideally suited for method development due to extreme pH stability and applicability to the broadest range of compounds classes. **Bonding:** Trifunctional C_8, fully endcapped, bonded to BEH substrate.
Phenyl	**Selectivity Features:** Excellent method development column for alternate selectivity, particularly for polyaromatic compounds. Unique level of pH stability for a phenyl-bonded phase. **Bonding:** Trifunctional C_6 phenyl, fully endcapped, bonded to BEH substrate.
HILIC	**Selectivity Features:** Excellent for retention of very polar, basic, water-soluble analytes. Specifically designed and tested for HILIC separations using mobile phases containing high concentrations of organic solvent. **Bonding:** Unbonded BEH substrate.

XBridge continued	
Amide	**Selectivity Features:** Rugged HILIC stationary phase designed to separate a wide range of very polar compounds. Especially good at separating carbohydrates (saccharides) using high concentrations of organic modifier, elevated temperature and high pH. Compatible with all modern detectors including MS, ELSD, UV and Fluorescence. **Bonding:** Trifuncional amide bonded to BEH substrate.

Atlantis® Columns

Atlantis®	
T3	**Selectivity Features:** Retention of polar compounds, compatible with 100% aqueous mobile phases, superior stability under low pH conditions. Specifically designed for enhanced retention of polar analytes. **Bonding:** T3 (C_{18}) bonding and endcapping, bonded to high purity silica substrate.
HILIC	**Selectivity Features:** Excellent for retention of very polar, basic, water-soluble analytes. Specifically designed and tested for HILIC separations using mobile phases containing high concentrations of organic solvent. **Bonding:** Unbonded high purity silica substrate.
C_{18}	**Selectivity Features:** Retention of polar compounds. Designed for compatibility with 100% aqueous mobile phases. **Bonding:** Difunctional C_{18} bonding, fully endcapped, bonded to high purity silica substrate.

SunFire® Columns

SunFire®	
C_{18}	**Selectivity Features:** General purpose method development column. Very high loading capacity, particularly for basic analytes in low pH mobile phases. Ideally suited for purification and impurity profile assays. **Bonding:** Difunctional C_{18}, fully endcapped, bonded to high purity silica substrate.
C_8	**Selectivity Features:** General purpose method development column. Very high loading capacity, particularly for basic analytes in low pH mobile phases. Less hydrophobic, therefore, less retentive than C_{18} for most analytes. **Bonding:** Difunctional C_8, fully endcapped, bonded to high purity silica substrate.

Acquity UPLC®

ACQUITY UPLC®	
CSH C_{18}	**Selectivity Features:** General purpose reversed-phase column that offers excellent pH stability and rapid mobile-phase re-equilibration for method development. Charged Surface Technology (CSH) Technology enables superior peak shape and increased loading capacity for basic compounds. **Bonding:** Trifunctional C_{18} ligand, fully end-capped, bonded to a CSH particle substrate.
CSH Phenyl-Hexyl	**Selectivity Features**: General purpose alternative selectivity ligand that provides pi-pi interactions with polyaromatic compounds, while maintaining excellent reproducibility at pH extremes. CSH Technology enables superior peak shape and increased loading capacity for basic compounds. **Bonding:** Trifunctional C_6 phenyl ligand, fully end-capped, bonded to a CSH particle substrate.
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BEH C_{18}	**Selectivity Features:** General purpose column ideally suited for method development due to extreme pH stability and applicability to the broadest range of compound classes. **Bonding:** Trifunctional C_{18}, fully endcapped, bonded to Ethylene Bridged Hybrid (BEH) substrate.
BEH Shield RP18	**Selectivity Features:** Alternate selectivity as compared to straight chain C_{18}, particularly for phenolic analytes. Compatible with 100% aqueous-phase composition. **Bonding:** Monofunctional embedded polar C_{18}, fully endcapped, bonded to BEH substrate.

ACQUITY UPLC continued	
BEH C_8	**Selectivity Features:** General purpose column ideally suited for method development due to extreme pH stability and applicability to the broadest range of compounds classes. **Bonding:** Trifunctional C_8, fully endcapped, bonded to BEH substrate.
BEH Phenyl	**Selectivity Features:** Excellent method development column for alternate selectivity, particularly in regard to polyaromatic compounds. Unique level of pH stability for a phenyl-bonded phase. **Bonding:** Trifunctional C_6 phenyl, fully endcapped, bonded to BEH substrate.
BEH HILIC	**Selectivity Features:** Excellent for retention of very polar, basic, water-soluble analytes. Specifically designed and tested for HILIC separations using mobile phases containing high concentrations of organic solvent. **Bonding:** Unbonded BEH substrate.
BEH HSS C_{18}	**Selectivity Features:** Ultra performance C_{18} chemistry, increased retention, superior peak shape, resists acid hydrolysis at low pH. Designed for UPLC separations where silica-based C_{18} selectivities are desired. **Bonding:** High coverage trifunctional C_{18}, fully endcapped, bonded to High Strength Silica (HSS) UPLC particle substrate.
BEH Amide	**Selectivity Features:** Rugged HILIC stationary phase designed to separate a wide range of very polar compounds. Especially good at separating carbohydrates (saccharides) using high concentrations of organic modifier, elevated temperature and high pH. Compatible with all modern detectors including MS, ELSD, UV and Fluorescence. **Bonding:** Trifunctional amide bonded to BEH substrate.
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HSS C_{18} SB	**Selectivity Features:** Unique, non-endcapped C_{18} chemistry designed specifically for method development scientists. Offers unique Selectivity for Bases (SB) when operating under low pH conditions. **Bonding:** Intermediate coverage tri-functionally bonded C_{18}, no endcapping, bonded to HSS UPLC particle substrate.
HSS T3	**Selectivity Features:** Aqueous mobile-phase compatible UPLC column designed for extreme retention. Combines polar compound retention with UPLC efficiencies and performance. **Bonding:** T3 (C_{18}) bonding and endcapping, bonded to HSS UPLC particle substrate.

FOOD TESTING SPECIALITY COLUMNS

In addition to a complete selection of UPLC and HPLC column chemistries, Waters also provides columns optimized for specific food testing analysis. These columns are ideal for fermentation analysis, organic acids, alcohols, and carbohydrates, triglycerides and cholesterol analysis, and fatty acid analysis.

GUARD COLUMNS

VanGuard™ Pre-columns, Sentry™ Guard Columns, and Guard-Pak™ Inserts prolong column lifetime by removing contaminants from the sample, giving you enhanced reproducibility and performance. They are packed with the same high performance stationary phases used in Waters analytical columns.

VETERINARY DRUGS IN FOOD

Veterinary medicines are given to animals to prevent or treat diseases, promote recovery from illness or injury and can also be used for the purposes of growth promotion. The presence of these medicines or their metabolites in meat, milk, or other products destined for human consumption poses serious health risks. For example, residues of chloramphenicol can result in aplastic anemia in susceptible individuals. There are also concerns surrounding the development of antibiotic resistant bacteria when low levels of antibiotics are consumed over time. Some of the applications in this section cover the analysis of antibiotics that are banned in certain foods. Others are permitted for use but subject to withdrawal periods to ensure that food is safe to consume. Also included in this section are compounds used for growth promotion and multi-residue analysis of different classes of veterinary medicines.

INTRODUCTION

β2-Agonists, veterinary drugs such as albuterol, are used to force pigs to mature faster with a higher amount of lean meat. Trace levels of β2-Agonists can cause palpitation, headaches, and even death in heart patients. β2-Agonists have been banned as growth promoters in pork production.

PRETREATMENT

1. Add 8 mL 0.2 M sodium acetate (pH 5.2) to 2 g of sample. Homogenize and take out supernatant. Add 50 µL β-Glucuronidase/arylsulfatase and hydrolyze at 37 °C overnight.
2. Shake the hydrolysate for 15 minutes. Centrifuge at 5000 rpm for 10 minutes and take out 4 mL supernatant.
3. Add 100 µL of 10 ng/mL standards (clenbuterol-D9, salbutamol-D3) and mix.
4. Add 5 mL 0.1 M perchloric acid and adjust pH to 1 ± 0.3.
5. Centrifuge at 5000 rpm for 10 minutes.
6. Collect supernatant and add 10 M sodium hydroxide to adjust pH to 11.
7. Add 10 mL saturated sodium chloride and 10 mL isopropanol-ethyl acetate (60:40, v/v).
8. After centrifugation, take organic layer and evaporate to dryness at 40 °C under nitrogen gas.
9. Dissolve residue in 5 mL 0.2 M sodium acetate (pH 5.2).

SPE PROCEDURE

Oasis® MCX, 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 2 mL methanol
B. 2 mL water

LOAD:
5 mL sample

WASH:
A. 2 mL water
B. 2 mL (2:98, v/v) formic acid in water

Dry cartridge by vacuum

ELUTE:
2 mL 5% ammonia solution in methanol

Evaporate to dryness at 40 °C under nitrogen gas

Add 200 µL 0.1% formic acid in methanol (5:95, v/v), ultrasonicate

Centrifuge at 15000 rpm for 10 minutes

LC CONDITIONS

System:	Alliance® HPLC 2695
Column:	Atlantis® dC_{18}, 5 µm, 2.1 x 150 mm
Guard column:	Atlantis dC_{18}, 5 µm, 2.1 x 10 mm
Flow rate:	0.2 mL/min
Mobile phase A:	0.1% formic acid
Mobile phase B:	0.1% formic acid in acetonitrile

Gradient:

Time (min)	A%	B%
0.00	96	4
2.00	96	4
8.00	77	23
21.00	77	23
22.00	5	95
25.00	5	95
25.50	96	4

Injection volume:	20 µL
Column temp.:	35 °C

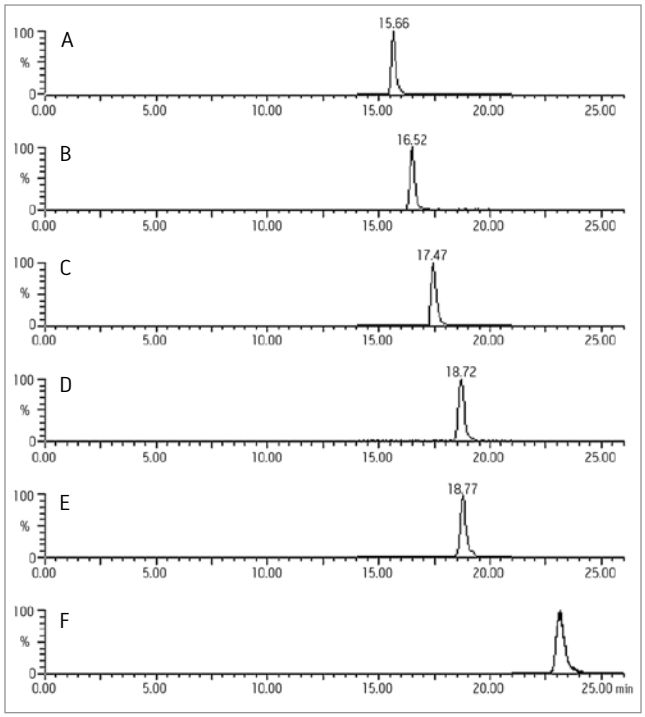
MS CONDITIONS

MS System:	Waters Quattro Premier™
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

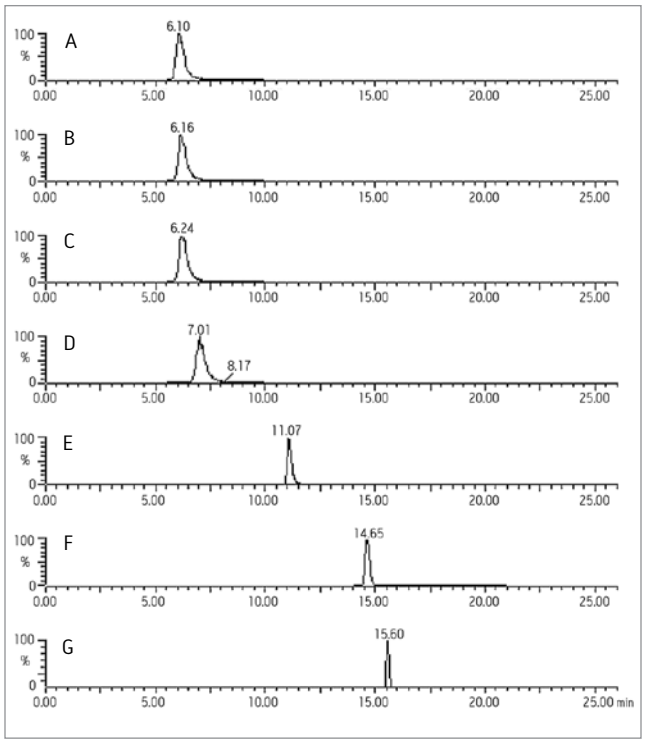
Analyte	MRM for Quantification	MRM for Confirmation
Salbutamol	240→148	240→222
Terbutaline	226→152	226→125
Cimaterol	202→160	202→143
Cimbuterol	234→160	234→143
Ractompamine	302→164	302→284
Clenbuterol	277→203	277→259
Bromclenbuterol	323→249	323→168
Bromobuterol	367→293	367→349
Isoxsuprine	302→150	302→284
Mabuterol	311→237	311→293
Mapenterol	325→237	325→217
Clenbuterol-D_9 (IS)	286→204	286→204
Salbutamol-D_3 (IS)	243→151	243→151

MRM method parameters.

RESULTS



6 β_2-agonists by multiple reaction monitoring (MRM) scan mode (A) clenbuterol (B) bromclenbuterol (C) bromobuterol (D) isoxsuprine (E) mabuterol (F) mapenterol.



7 β_2-agonists by multiple reaction monitoring (MRM) scan mode (A) salbutamol-d_3 (B) salbutamol (C) terbutaline (D) cimaterol (E) cimbuterol (F) ractompamine (G) clenbuterol-D_9.

Pig liver tissues were spiked with 11 β_2-agonists standard mixture of concentrations 0.5 ng/g, 1 ng/g and 2 ng/g respectively. The SPE recoveries are between 89.4% and 110.5%, RSD are between 1% and 2.8%.

ORDERING INFORMATION

Description	Part Number
Oasis MCX, 3 cc/60 mg, 30 µm, 100/box	186000254
Atlantis dC_{18}, 5 µm, 2.1 x 150 mm	186001301
Atlantis dC_{18}, 5 µm, 2.1 x 10 mm	186001379
Sentry™ 2.1 mm Guard Holder	WAT097958
Qsert™ Vials, LCGC Certified Combination Packs	186001126C

Ref: The determination of β_2-agonists by LC/MS-MS JIN Yu-E[1], GUO De-Hua[2], ZHENG Ye[3], WANG Guo-Quan[1] (Shanghai Municipal Center for Disease Control and Prevention; Shanghai Entry-Exit Inspection and Quarantine Bureau; Shanghai University)

INTRODUCTION

Aminoglycoside antibiotics are commonly used in veterinary medicine for the treatment of animals bred for meat and milk production. As these commodities are consumed by humans, an effective method to detect residues of these antibiotics is required.

A quick and simple procedure for the extraction of these compounds from bovine meat and milk was developed. An LOQ of 10 ppb was demonstrated for milk and an LOQ of 50 ppb was seen for meat tissue.

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY® HSS PFP, 1.7 µm, 2.1 x 100 mm
Injection volume:	30 µL
Column temp.:	35 °C
Mobile phase A:	20 mM HFBA in water
Mobile phase B:	20 mM HFBA in acetonitrile
Flow rate:	0.50 mL/min
Gradient:	20% B initial, linear gradient to 80% B in 7 minutes, hold for 8 minutes, back to 20% B for 8.1 minutes. Hold and re-equilibrate for 10 minutes.

MS CONDITIONS

MS System:	ACQUITY TQD
Mode:	Positive electrospray (ES+)
Capillary:	3.0 kV
Extractor:	3.0 V
Source temp.:	130 °C
Cone gas:	20 L/h
Desolvation temp.:	450 °C
Desolvation gas:	900 L/h
Collison gas:	Argon at 0.20 mL/min

SAMPLE PREPARATION

Extraction Buffer (10 mM NH_4OOCH_3/0.4 mM Na_2EDTA/ 1% NaCl/2% TCA)

Place 0.77 g of ammonium acetate (NH_4OOCH_3) into a 1-L volumetric flask. Add approximately 900 mL of reagent water and dissolve. Adjust pH to 4.0 with 1 N HCl or 1 N NaOH. Add 0.15 g disodium ethylenediamine tetraacetate ($Na_2EDTA.2H_2O$), 5 g of sodium chloride (NaCl), and 20 g of trichloroacetic acid (TCA). Mix well to dissolve and bring to the mark with reagent water.

Initial extraction

Place 2 g homogenized bovine tissue or 10 mL milk into a 50 mL centrifuge tube. Add 20 mL extraction buffer, vortex for 10 seconds, then shake well for 1 minute. Centrifuge the sample at 4000 RPM for 5 minutes, and collect the supernatant. Adjust the pH of the supernatant to 6.5±0.5 using diluted HCl or NaOH as needed.

SPE cleanup

An Oasis® HLB 96-well Plate (30 mg) was used in this study. A 1-cc, 30-mg cartridge can be used if desired. Condition the well or cartridge with 1.5 mL methanol, followed by 1.5 mL water. Set the flow rate at 1 mL/min or less. Load the pH-adjusted supernatant obtained from the initial extraction; a 1-mL aliquot is loaded for tissue samples, a 1.5-mL aliquot for milk samples. Wash with 1 mL water. Elute with 0.5 mL 10:5:85 formic acid/isopropanol/ water. Add 1.5 µL HFBA and analyze using UPLC/MS/MS.

Aminoglycoside	MRM	Cone (V)	Collision (eV)
Streptomycin	582.3 > 246.1 582.3 > 263.2	70 70	28 28
Dihydrostreptomycin	584.3 > 246.0 584.3 > 263.2	60 60	26 26
Gentamicin C1a	450.4 > 160.1 450.4 > 322.4	35 35	25 15
Gentamicin C1	478.5 > 157.2 478.5 > 322.1	35 35	30 15
Gentamicin C2C2a	464.5 > 160.1 464.5 > 322.4	35 35	20 15
Neomycin	615.4 > 161.1 615.4 > 293.1	55 55	28 25

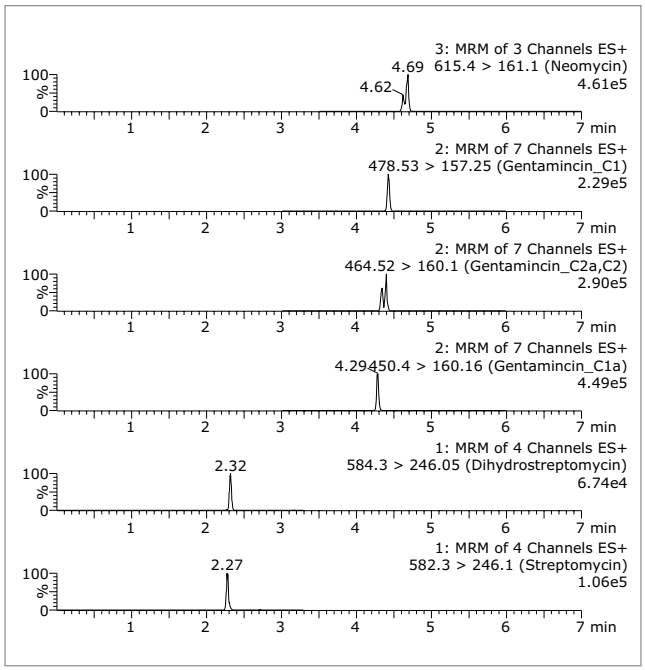
Cone and collision parameters and MRM transitions used for this study.

Aminoglycoside	% Recovery	% RSD	% Recovery	% RSD
	n=6		n=6	
	10 ppb		200 ppb	
Streptomycin	77.7	12.2	81.9	13.1
Dihydrostreptomycin	93.4	3.0	81.9	14.0
Gentamicin C1a	79.4	12.0	70.4	10.0
Gentamicin C1	88.0	4.9	79.6	7.1
Gentamicin C2C2a	78.1	7.4	86.8	9.2
Neomycin	75.5	11.6	78.3	10.1

Summary of recovery data for aminoglycosides spiked into bovine milk.

Aminoglycoside	% Recovery	% RSD	% Recovery	% RSD
	n=6		n=6	
	50 ppb		1600 ppb	
Streptomycin	102.9	11.7	97.3	4.0
Dihydrostreptomycin	88.4	5.9	89.7	6.1
Gentamicin C1a	83.6	9.6	95.3	13.3
Gentamicin C1	93.0	5.5	85.8	9.9
Gentamicin C2C2a	94.9	10.9	89.1	13.8
Neomycin	86.3	3.0	83.6	11.3

Summary of recovery data for aminoglycosides spiked into bovine milk.



UPLC/MS/MS chromatogram obtained from bovine milk spiked at 10 µg/kg (ppb).

ORDERING INFORMATION

Description	Part Number
ACQUITY HSS PFP, 1.7 µm, 2.1 x 100 mm	186005967
Oasis HLB 96-well Plate, 30 mg	WAT058951
Atlantis dC_{18}, 5 µm, 2.1 x 10 mm	186001379
Sentry™ 2.1 mm Guard Holder	WAT097958
Qsert™ Vials, LCGC Certified Combination Packs	186001126C

Ref: Waters Application Note 720004512EN

INTRODUCTION

The sensitive analysis of avermectins in food products such as milk and meat can be challenging due to their complex sample matrices. Sample preparation using QuEChERS allows for fast throughput and high sensitivity analysis of food products. Although QuEChERS is commonly used for multi-residue pesticide analysis in fruits and vegetables, it is also applicable in the analysis of veterinary drugs in livestock products. In this application note, milk and ground beef are prepared and analyzed for avermectins at the ppb level using QuEChERS methodology and LC/MS/MS.

LC CONDITIONS

System:	ACQUITY UPLC
Column:	XSelect® CSH C_{18} ***XP***, 2.5 µm, 2.1 x 100 mm
Part number:	186006103
Injection volume:	5 µL
Temp.:	50 °C
Mobile phase A:	5 mM ammonium acetate in water
Mobile phase B:	5 mM ammonium acetate in methanol
Flow rate:	0.40 mL/min
Gradient:	70% B initial, linear gradient to 97% B in 5 minutes, hold until 8 minutes, back to 70% B at 8.1 minutes. Hold and re-equilibrate until 10 minutes
Sample vials:	Maximum Recovery Vial
Part number:	600000670CV

MS CONDITIONS

MS System:	Xevo® TQ-S
Ionization mode:	Electrospray positive (ESI+)

SAMPLE PREPARATION

Initial Extraction (QuEChERS)

Place 10 mL whole milk (pasteurized) into a 50 mL centrifuge tube, or for meat, place 8 g ground beef (80% lean) and 2 mL water into a 50 mL centrifuge tube. Add 10 mL acetonitrile and shake the tube vigorously for 1 minute. Add the contents of DisQuE™ Pouch salts for European Committee for Standardization (CEN) QuEChERS (P/N: 186006813) and shake vigorously for 1 minute. Centrifuge for 15 minutes at 4000 rpm and take a 1 mL aliquot of the supernatant (top layer) for d-SPE cleanup.

d-SPE Cleanup

Transfer the 1 mL aliquot of supernatant to a 2 mL d-SPE cleanup tube that contains 150 mg magnesium sulfate and 50 mg C_{18} sorbent and shake vigorously for 1 minute. Centrifuge for 5 minutes at 12000 rpm and take a 0.5 mL aliquot a sample for LC/MS/MS analysis.

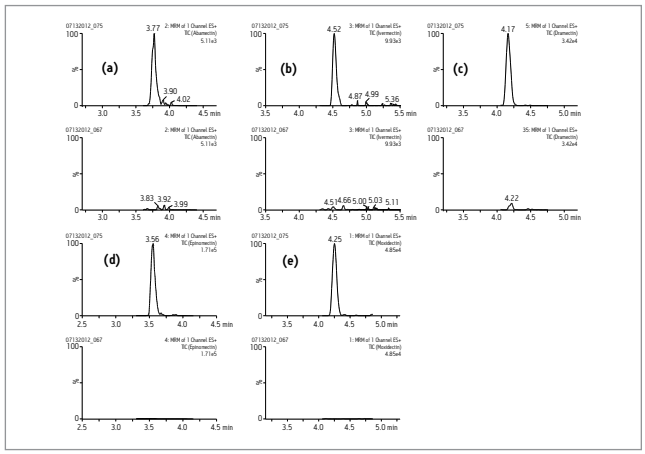


Figure 1. LC/MS/MS chromatograms of avermectins obtained from ground beef samples; the top trace is the low level spiked sample, the bottom trace is a ground beef blank. (a) abamectin, (b) ivermectin, (c) doramectin, (d) eprinomectin, (e),moxidectin.

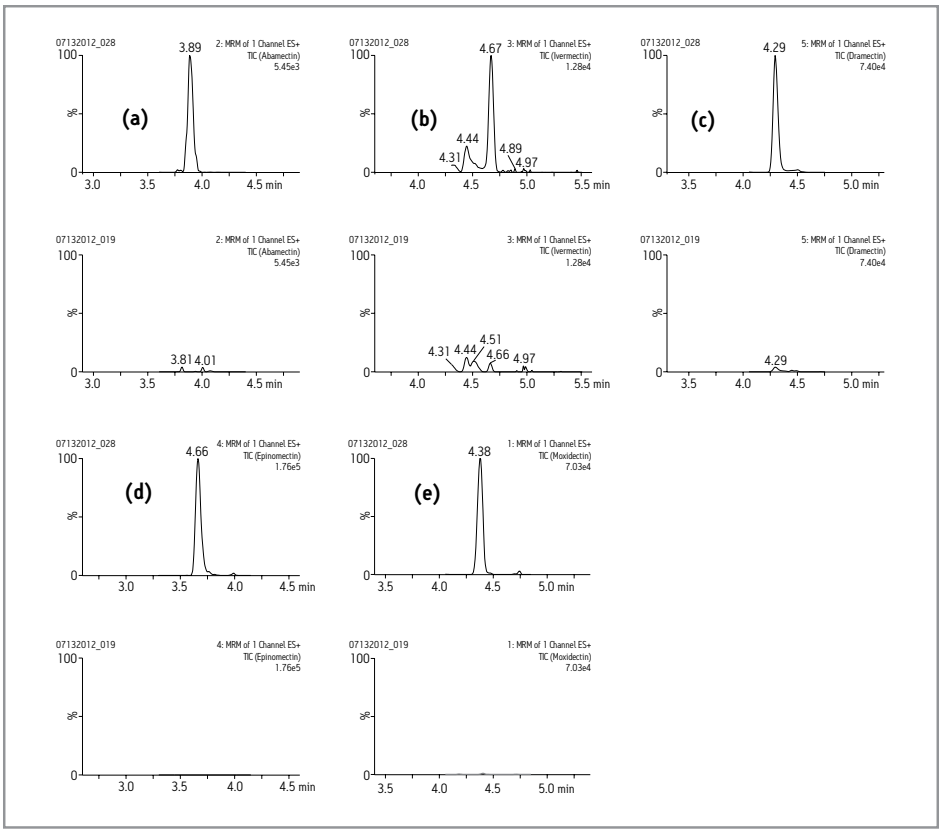


Figure 2. LC/MS/MS chromatograms of avermectins obtained from whole milk samples; the top trace is the low level spiked sample, the bottom trace is a whole milk blank. (a) abamectin (b) ivermectin (c) doramectin (d) eprinomectin (e) moxidectin.

	Concentration Range (ppb)		Average % Recovery (%RSD) n=5			
			Ground Beef		Whole Milk	
Conc. Level	Low Level	High Level	Low Level	High Level	Low Level	High Level
Abamectin	1	10	94(3.6)	88(3.6)	86(14.0)	89(3.7)
Ivermectin	1	10	98(17.7)	85(3.1)	84(5.3)	83(14.8)
Doramectin	10	100	89(4.8)	85(4.2)	101(11.7)	90(5.0)
Epinomectin	10	100	99(2.9)	91(1.5)	94(3.9)	93(3.0)
Moxidectin	10	100	90(4.2)	87(1.8)	100(2.4)	90(5.6)

Table 2. Recoveries of avermectins from ground beef and whole milk samples.

ORDERING INFORMATION

Description	Part Number
XSelect CSH C_{18} ***XP*** Column, 2.5 µm, 2.1 x 100 mm	186005275
CEN QuEChERS DisQuE Pouch	186006813
DisQuE 50 mL Centrifuge Tube	186004837
Maximum Recovery Vial	600000670CV

Ref: Waters Application Note 720004440EN

INTRODUCTION

Bee keeping and honey production is a world-wide industry. Trace levels of the antibiotic chloramphenicol have been detected in honey. The antibiotic can be detected when bee keepers apply the antibiotic on hives to control bacteria that affect bee larvae. Chloramphenicol is banned in food products.

PRETREATMENT

1. Dissolve 5 g of honey (spiked with D5-CAP) in 5 mL water.
2. Extract with 15 mL ethyl acetate and centrifuge.
3. Transfer the supernatant to a clean tube and evaporate to dryness under nitrogen at 50 °C.
4. Reconstitute the residue in 1 mL methanol and dilute with 20 mL water.

SPE PROCEDURE

Oasis® HLB, 6 cc/200 mg

CONDITION/EQUILIBRATE: A. 5 mL methanol B. 5 mL water
LOAD: 20 mL of sample at 2 drops/second
WASH: 5 mL water
ELUTE: 2 x 2.5 mL of methanol
Evaporate to dryness under nitrogen at 50 °C
Reconstitute residue with 9:1 water/methanol (500 µL)

LC CONDITIONS

System: Alliance® HPLC 2695
Column: Symmetry® C_8, 3.5 µm, 2.1 x 50 mm
Guard column: Symmetry Sentry™ C_8, 3.5 µm, 2.1 x 10 mm
Flow rate: 0.3 mL/min
Mobile phase A: water
Mobile phase B: methanol

Gradient:

Time (min)	A%	B%
0.00	90	10
8.00	10	90
10.00	10	90
10.10	90	10
15.00	90	10

Injection volume: 20 µL
Column temp.: 30 °C

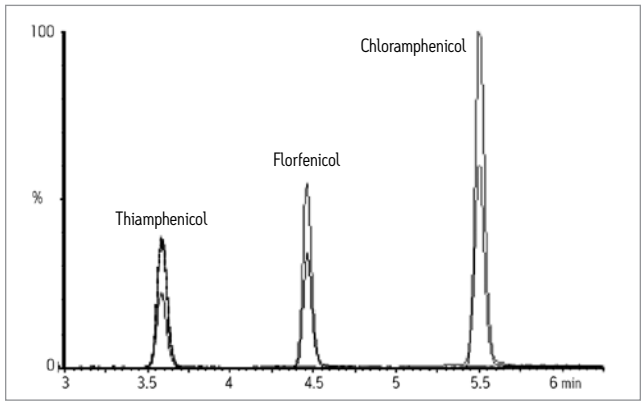
MS CONDITIONS

MS System: Waters Quattro micro™ API
Ionization mode: Negative electrospray (ESI^-)
Multiple reaction monitoring

Analyte	MRM Transition
Chloramphenicol (CAP)	321→152
	321→257
Thiamphenicol (TAP)	354→185
	354→290
Florfenicol (FP)	356→336
	356→185
Internal Standard D_5-CAP	327→157

MRM method parameters.

RESULTS



Overlays of chloramphenicol, thiamphenicol, florfenicol at 2 µg/kg for each MRM transition.

Analyte	Mean Recovery (%)	RSD (%)
Chloramphenicol	91.1	2.2
Thiamphenicol	91.9	5.9
Florfenicol	104.6	1.7

Recovery data for CAP, TAP and FP at 0.3 µg/kg (n = 5).

ORDERING INFORMATION

Description	Part Number
Oasis HLB, 6 cc/200 mg, 30 µm, 30/box	WAT106202
Symmetry C_8, 3.5 µm, 2.1 x 50 mm	WAT200624
Symmetry Sentry Guard C_8, 2.1 x 10 mm	WAT106128
Sentry 2.1 mm Guard Holder	WAT097958
Total Recovery Vials	186000750CV

Ref: Waters Application Note 720001015EN

INTRODUCTION

The European Union (EU) considers dexamethasone residues a high priority in food animals as they are synergistic with illegal growth promoters, such as beta-agonists or anabolic steroids.

PRETREATMENT

1. Add 5 g of the ground pork into 30 mL of 95:5 acetonitrile:water (v/v).
2. Shake for 20 minutes, homogenize, and shake for 10 minutes.
3. Centrifuge for 5 minutes.
4. Repeat extraction.
5. Combined both extractions and load onto Sep-Pak® Vac 3 cc (500 mg), Florisil® Cartridges and elute with 30 mL of 95% acetonitrile and bring up to 100 mL with acetonitrile.
6. Take out 20 mL of extract add 10 mL of hexane. Shake for 3 minutes and leave to stand.
7. Take acetonitrile layer and evaporate to dryness.
8. Reconstitute with 4 mL of 0.2 M sodium phosphate buffer (pH 5) to a final volume of 6 mL with water.

SPE PROCEDURE

Sep-Pak Plus C_{18}, 360 mg

CONDITION:
A. 10 mL methanol
B. 10 mL water
C. 2 mL sodium phosphate buffer (pH 5)

↓

LOAD

↓

WASH:
A. 5 mL sodium phosphate buffer (pH 5)
B. 10 mL 25% methanol in water

↓

ELUTE:
10 mL 60% acetonitrile 40% water (60:40, v/v)

↓

Reconstitute in 200 µL in 10% acetonitrile and 90% water (10:90, v/v)

LC CONDITIONS

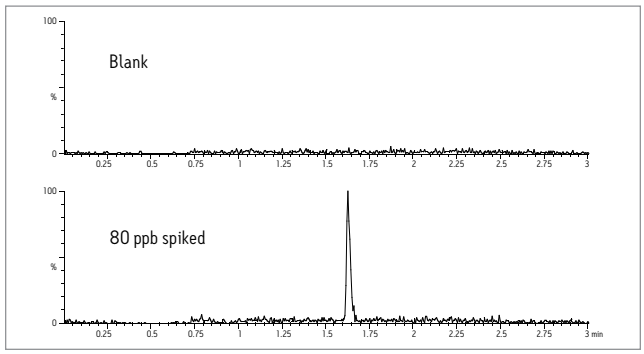
System: ACQUITY UPLC®
Column: ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Flow rate: 400 µL/min
Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in acetonitrile
Gradient:

Time (min)	A%	B%
0.00	70	30
3.00	10	90
3.10	70	30
4.00	70	30

MS CONDITIONS

MS System: Waters Quattro Premier™ XE
MRM transitions: 1. 393.00 → 373.00
2. 393.00 → 393.00
Ionization mode: Positive electrospray (ESI^+)
Multiple reaction monitoring

RESULTS



50 ppb dexamethasone spiked in pork muscle.

Compound Name: Dexamethasone 393→393	RT	Area
50 ppb-spiked	1.64	333.30
50 ppb-spiked	1.63	322.59
50 ppb-spiked	1.63	548.12
50 ppb-spiked	1.63	506.48
50 ppb-spiked	1.63	386.79
RSD (%)	24.43	-
Recovery (%)	90.09	-

Compound Name: Dexamethasone 393→393	RT	Area
50 ppb-spiked	1.64	270.38
50 ppb-spiked	1.64	250.18
50 ppb-spiked	1.63	503.14
50 ppb-spiked	1.63	404.46
50 ppb-spiked	1.63	327.81
RSD (%)	29.59	-
Recovery (%)	89.00	-

Recovery data for 50 ppb dexamethasone spiked in pork muscle.

ORDERING INFORMATION

Description	Part Number
Sep-Pak Vac, 3 cc/500 mg, Florisil Cartridges	WAT020815
Sep-Pak Plus C_{18}, 360 mg	WAT020515
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002353
Qsert™ Vials, LCGC Certified Combination Packs	186001126C

INTRODUCTION

The United States Food and Drug Administration (US FDA) banned the use of enrofloxacin for growth enhancement in poultry production. Ciprofloxacin is a degradant of enrofloxacin, therefore, both compounds need to be screened in the assay. Any detectable residue can be evidence of inappropriate poultry farming practice.

PRETREATMENT

1. Extract 1.5 g homogenized sample with 30 mL ethanol/acetic acid (99:1, v/v) and centrifuge at 4000 rpm for 5 minutes.
2. Take 10 mL aliquot of the supernatant for SPE enrichment and cleanup.
3. For muscle samples, dilute 10 mL of supernatant with 5 mL water prior to SPE; liver samples are not diluted.

SPE PROCEDURE

Cartridge I: Oasis® MCX, 6 cc/150 mg

CONDITION/EQUILIBRATE:
A. 3 mL methanol
B. 3 mL water
C. 3 mL ethanol

LOAD:
10 mL sample

WASH:
A. 3 mL 1% acetic acid/ethanol
B. 3 mL water
C. 3 mL methanol

Cartridge II: Sep-Pak® Accell™ QMA, 3 cc/500 mg

CONDITION/EQUILIBRATE:
3 mL 5% ammonia in methanol

Attach Sep-Pak Accell QMA Cartridge to outlet of MCX Cartridge.
Elute from MCX Cartridge into Sep-Pak Accell QMA Cartridge.
(6 cc cartridge on top)

ELUTE*:
3 mL (5:95, v/v) ammonia in methanol

Remove Oasis MCX Cartridge

WASH:
3 mL ethanol

ELUTE:
3 mL methanol in formic acid (98:2, v/v)

Evaporate solvent and reconstitute in 150 µL of acetonitrile in water (15:85, v/v)

**Note: As the analytes are eluted from cartridge I, they are subsequently retained by anion exchange on cartridge II. Therefore, the eluate from cartridge I becomes the load for cartridge II.*

UPLC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 1 x 50 mm
Flow rate:	0.12 mL/min
Mobile phase A:	1% formic acid in water
Mobile phase B:	Acetonitrile

Gradient:

Time (min)	A%	B%
0.00	95	5
3.00	50	50
6.50	50	50
10.50	95	5
15.50	95	5

Injection volume:	10 µL
Column temp.:	30 °C

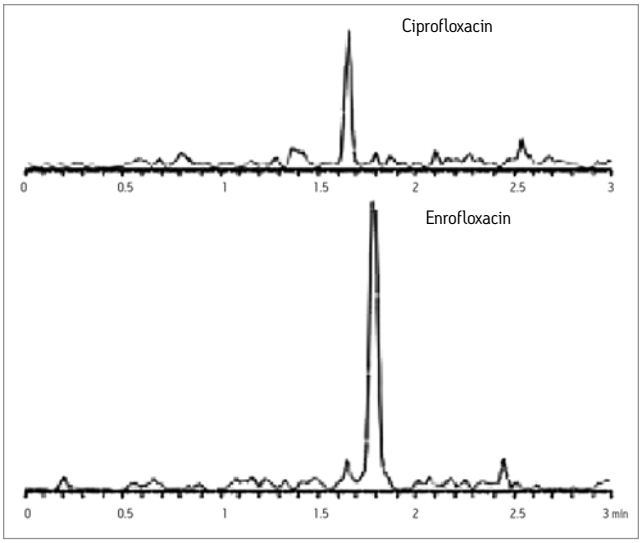
MS CONDITIONS

MS System:	Waters Quattro micro™ API
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

Analyte	MRM Transition
Enrofloxacin	360→342
	360→316
Ciprofloxacin	332→314
	332→288

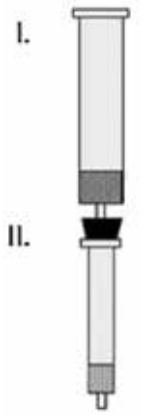
MRM method parameters.

RESULTS



Typical UPLC®/MS/MS chromatogram of chicken muscle spiked (2 ng/kg).

Recovery averaged 75% measured by comparison of results from chicken samples spiked before and after sample preparation. Precision for six replicate samples spiked at 2 µg/kg was 12%.



Oasis MCX
6 cc/150 mg SPE Cartridge

Cation-exchange retention of bases.
Neutrals or acids, are poorly retained and washed off before elution.

Sep-Pak Accell QMA
3cc/500 mg SPE cartridge

Anion-exchange retention of acids.
Neutrals or bases are poorly retained and are washed off before elution.

Tandem SPE Cartridges setup.

ORDERING INFORMATION

Description	Part Number
Oasis MCX, 6 cc/150 mg, 60 µm, 30/box	186000255
Sep-Pak Accell Plus QMA, 3 cc/500 mg, 50/box	WAT020850
ACQUITY UPLC BEH C_{18}, 1.7 µm, 1 x 50 mm	186002344
ACQUITY UPLC BEH C_{18}, 1.7 µm, 1 x 50 mm, 3/pk	176000861
Qsert™ Vials	186001126

Ref: Waters application WA43206

INTRODUCTION

Optimized sample preparation and analysis protocols were developed for tandem LC/MS determination of a wide variety of veterinary drug residues in tissue samples. Three types of muscle tissue samples (pork, chicken, and salmon) were chosen to demonstrate the suitability of the methodology. Samples are treated with an acidified acetonitrile/water solvent to precipitate proteins and to extract the veterinary drugs of interest. Then, a simple SPE cleanup is performed using a Sep-Pak® C_{18} Cartridge or 96-well plate. After evaporation and reconstitution, the sample is analyzed using tandem LC/MS. Representative compounds were chosen from major classes of veterinary drugs including tetracyclines, fluoroquinolones, sulfonamides, macrolides, beta-lactams, NSAIDS, steroids, and beta-andrenergids.

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY UPLC CSH™ C_{18}, 1.7 µm, 100 mm x 2.1 mm (i.d.)
Mobile phase A:	0.1% formic in water
Mobile phase B:	0.1% formic acid in acetonitrile
Injection volume:	7 µL
Injection mode:	Partial loop injection
Column temp.:	30 °C
Weak wash:	10:90 acetonitrile:water (600 µL)
Strong wash:	50:30:40 water:acetonitrile:IPA (200 µL)
Seal wash:	10:90 acetonitrile:water

Gradient:

Time (Min)	Flow (mL/min)	Solvent A (%)	Solvent B (%)	Curve
Initial	0.4	85	15	Initial
2.5	0.4	60	40	6
3.9	0.4	5	95	6
4.9	0.4	5	95	6
5.0	0.4	85	15	6
7.0	0.4	85	15	6

MS CONDITIONS

Detector:	Xevo® TQ
Ionization mode:	Positive Electrospray (except negative for chloramphenicol)
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas flow:	1,000 L/hr
Cone gas flow:	30 L/hr
Collision gas flow:	0.15 mL/min
Data management:	MassLynx® v4.1

SAMPLE PREPARATION

Initial Extraction/Precipitation

Place a 5 g sample of homogenized tissue into a 50 mL centrifuge tube. Add 10 mL 0.2% formic acid in 80:20 acetonitrile/water. Vortex for 30 seconds and place on mechanical shaker for 30 minutes. Centrifuge at 12000 rpm for 5 minutes.

The extraction/precipitation step gives good recovery of most compounds of interest but also extracts significant amounts of fat.

SPE Cleanup

Take 1 mL of the supernatant (from step 1) for SPE cleanup using a Sep-Pak C_{18} Cartridge or plate (see SPE details in Figure 1).

This step removes fats and non-polar interferences.

Sep-Pak C_{18} Cartridge

1 cc, 100 mg

CONDITION:
1 mL 80:20 acetronile/water

Install Collection Tubes

PASS-THRU/COLLECT:
1 mL protein ppt sample

Install collection vessels

RINSE/COLLECT:
0.5 mL 80:20 acetronile/water

Add 0.25 mL ammonium formate in 50:50 ACN/methanol to buffer sample and protect acid labile analytes

RINSE/COLLECT:
0.5 mL 80:20 acetronile/water

Figure 1. SPE Cleanup Protocol

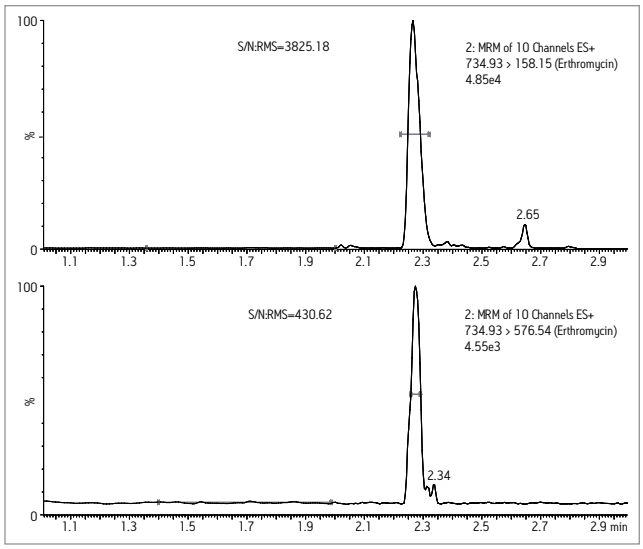


Figure 2. Typical LC/MS/MS Chromatogram Obtained from Pork Spiked with Erythromycin at 10 ng/g (Primary MRM Transition on Top).

Compound	Spike Level (ng/g)	Pork % Rec (%RSD) n=5	Chicken % Rec (%RSD) n=6	Salmon % Rec (%RSD) n=6
Carbadox	100.00	9 (36)	17 (14)	21 (13)
Chloramphenicol	100.00	57 (20)	51 (20)	89 (2)
Chlorotetracyline	100.00	42 (11)	49 (6)	54 (7)
Ciprofloxacin	100.00	130 (21)	61 (8)	88 (2)
Dexamethasone	100.00	70 (7)	61 (8)	91 (4)
Enrofloxacin	200.00	106 (4)	62 (9)	90 (2)
Erythromycin	10.00	36 (9)	33 (4)	43 (8)
Lincomycin	50.00	64 (17)	59 (10)	83 (8)
Oxacillin	100.00	51 (4)	48 (6)	55 (4)
Oxytetracycline	100.00	51 (8)	50 (10)	60 (5)
Penicillin	50.00	46 (7)	45 (8)	54 (9)
Phenybutazone	100.00	16 (16)	44 (10)	38 (8)
Ractopamine	300.00	74 (7)	62 (11)	88 (3)
Salbutamol	100.00	71 (14)	66 (12)	78 (7)
Sulfamerazine	100.00	63 (5)	59 (7)	82 (3)
Sulfamethazine	100.00	67 (5)	60 (8)	84 (3)
Sulfanilamide	100.00	74 (21)	65 (21)	74 (11)
Tetracycline	100.00	58 (10)	53 (8)	69 (2)
Tylosin	20.00	47 (11)	36 (12)	63 (14)

Table 2. Recovery data obtained from three types of spiked tissue samples

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC CSH C_{18}, 1.7 µm, 2.1 x 100 mm	186005297
Sep-Pak C_{18} Cartridge, 1 cc, 10 mg	WAT023590
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720004144EN

INTRODUCTION

Optimized sample preparation and analysis protocols were developed for tandem LC/MS/MS determination of a wide variety of veterinary drug residues in milk samples. Samples are initially precipitated and extracted with an equal volume of acetonitrile. After the resulting extract is treated with acidified acetonitrile to precipitate remaining proteins, a simple SPE cleanup is performed using a Sep-Pak® C_{18} Cartridge. After evaporation and reconstitution, the sample is analyzed using tandem LC/MS. Representative compounds were chosen from major classes of veterinary drugs including tetracyclines, fluoroquinolones, sulfonamides, macrolides, beta-lactams, NSAIDS, steroids, and beta-andrenergids.

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY UPLC CSH™ C_{18}, 1.7 µm, 100 mm x 2.1 mm (i.d.)
Mobile phase A:	0.1% formic in water
Mobile phase B:	0.1% formic acid acetonitrile
Injection volume:	7 µL
Injection mode:	Partial loop injection
Column temp.:	30 °C
Weak needle wash:	10:90 acetonitrile:water (600 µL)
Strong needle wash:	50:30:20 water:acetonitrile:IPA (200 µL)
Seal wash:	10:90 acetonitrile:water

Gradient:

Time (min)	Flow (mL/min)	Profile %A	Profile %B	Curve
Initial	0.4	85	15	6
2.5	0.4	60	40	6
3.9	0.4	5	95	6
4.9	0.4	5	95	6
5.0	0.4	85	15	6
7.0	0.4	85	15	6

MS CONDITIONS

MS System:	ACQUITY TQD
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas flow:	1000 L/Hr
Cone gas flow:	30 L/Hr
Collision gas flow:	0.15 mL/min
Data management:	MassLynx® v4.1

SAMPLE PREPARATION PROTOCOL

Initial Extraction/Precipitation

TRANSFER:
2 mL sample into a 15 mL centrifuge tube

ADD:
2 mL acetonitrile (ACN) and vortex for 30 seconds

CENTRIFUGE:
8000 x g for 4 minutes[1]

[1] This step gives good extraction of most compounds of interest but also extracts significant amounts of protein and some fats that may interfere with the LC/MS analysis.

Residual Protein Precipitation

TRANSFER:
2 mL of supernatant (from step 1)
to a second centrifuge tube

ADD:
3 mL of acidified acetonitrile (0.2 % formic acid)

CENTRIFUGE:
30 seconds[2]

[2] This step effectively precipitates the residual protein.

SPE Cleanup

Take 1 mL of the supernatant (from step 2)
for SPE cleanup using a Sep-Pak C_{18} Cartridge
(see SPE details in Figure 1).[3]

[3] This step removes fats and non-polar interferences.

CONDITION:
1 mL 80:20 acetronitrile/water

← install collection tubes

PASS-THRU/COLLECT:
1 mL protein ppt sample

RINSE/COLLECT:
0.5 mL 80:20 acetronitrile/water

← add 0.25 mL 200 mM ammonium formate in 50:50 ACN/methanol to buffer sample- and protect acid labile analytes

EVAPORATE/RECONSTITUTE:
0.2 mL 25:75 acetonitrile/buffer
(25 mM ammonium formate buffer @ pH 4.5)

Figure 1. SPE cleanup protocol.

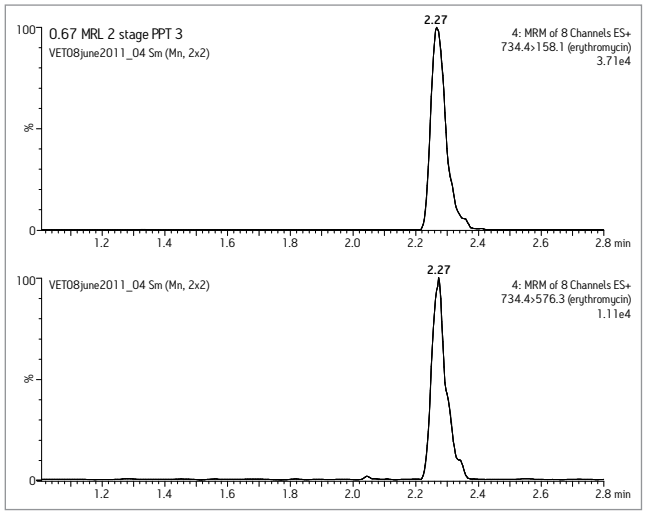


Figure 2. Typical LC/MS/MS Chromatogram obtained from milk spiked with erythromycin at 6.7 ng/g (primary MRM transition on top).

Compound	Spike Level (ng/g)	%REC (%RSD) (n=3)	%Suppression*
Carbadox	67.0	27 (27)	-43
Ciprofloxacin	67.0	67 (20)	32
Chloramphenicol	67.0	94 (16)	10
Chlortetracyline	67.0	22 (20)	7
Dexamethasone	67.0	87 (6)	-8
Enrofloxacin	134.0	76 (11)	26
Erythromycin	6.7	59 (10)	5
Lincomycin	33.0	102 (9)	25
Oxacillin	67.0	79 (12)	-9
Oxytetracycline	67.0	24 (16)	-9
Penicillin-G	33.0	73 (8)	-8
Phenylbutazone	67.0	67 (18)	20
Ractopamine	200.0	65 (14)	0
Salbutamol	67.0	80.4 (3)	96
Sulfamerazine	67.0	71 (4)	-16
Sulfamethazine	67.0	71 (6)	-74
Sulfanilamide	67.0	110 (30)	60
Tetracycline	67.0	31 (18)	-21

Table 2. Recovery and matrix effects.
** Negative number signifies matrix enhancement*

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC CSH C_{18}, 1.7 µm, 2.1 x 100 mm	186005297
Sep-Pak C_{18} Cartridge, 1 cc, 10 mg	WAT023590
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720004089EN

INTRODUCTION

Nitrofurans are a class of antibiotic drugs, used to treat bacterial infections, which may induce carcinogenic residues in animal tissues. Due to public health concerns, nitrofurans have been banned for use in food producing animals in many countries.

PRETREATMENT

1. Dilute 2 g honey sample with 5 mL of 0.12 M hydrochloric acid.

SPE PROCEDURE

SPE Step I: Oasis® HLB, 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 2 mL methanol
B. 2 mL water

↓

PASS-THROUGH:
2 g prepared honey in 5 mL of 0.1 N hydrochloric acid

↓

WASH:
2 mL water

↓

Combine the solutions

1. Collect quantitatively the eluent from the pass-through and wash steps into a 15 mL capped sample tube.
2. Add 300 µL of 50 mM 2-nitrobenzaldehyde in dimethyl-sulfoxide. Hydrolyse and derivatize for 18 hours at 37 °C.
3. Cool the sample to room temperature and adjust to pH 7 by adding 6 mL of 0.1 M dipotassium hydrogen phosphate.
4. Put sample through SPE Step II.

SPE Step II: Oasis HLB, 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 1 mL methanol
B. 1 mL water

↓

LOAD:
Prepared honey sample pH 7

↓

WASH:
A. 2 mL water
B. 2 mL 30% methanol in water

↓

DRY:
20 minutes

↓

ELUTE:
3 mL formic acid methyl-t-butyl/methanol/formic acid (79:9:2, v/v/v)

↓

Evaporate and reconstitute in 200 µL mobile phase

LC CONDITIONS

System:	Alliance® HPLC 2695
Column:	XTerra® MS C_{18}, 3.5 µm, 2.1 x 100 mm
Flow rate:	0.2 mL/min
Mobile phase:	Isocratic 70% 20 mM ammonium formate pH 4.0, 30% acetonitrile
Injection volume:	20 µL
Column temp.:	30 °C

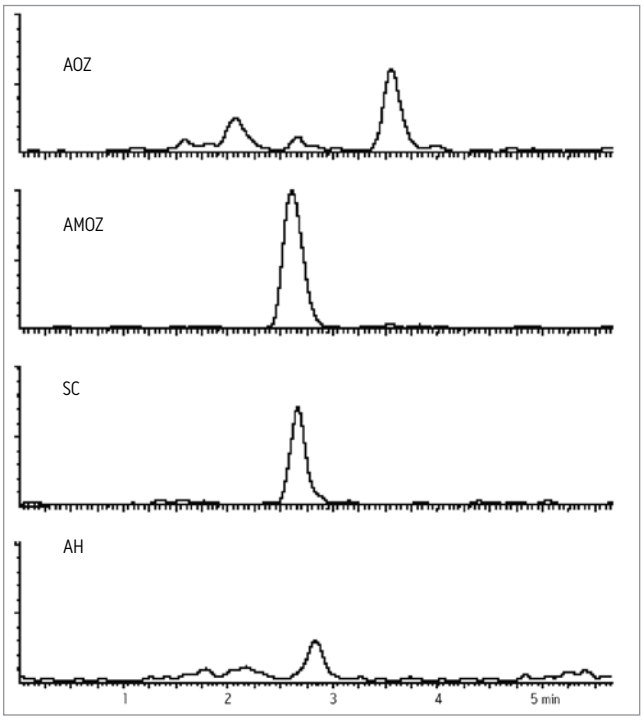
MS CONDITIONS

MS System:	Waters Quattro micro™ API
Ionization mode:	Positive electrospray (ESI+) Multiple reaction monitoring

Analyte	MRM Transition
AOZ	236→134
AMOZ	335→291
Semicarbizide (SC)	209→192
1-Aminohydantion (AH)	178→249

MRM method parameters.

RESULTS



Spiked honey (400 ng/kg) metabolites as 2-nitrobenzaldehyde derivatives.

Analytes	RSD (%)	
	Raw Wild Honey	Buckwheat Honey
Semicarbizide	9.8	9.7
AOZ	13.9	9.6
AMOZ	3.8	2.9
AH	14	3.8

Relative standard deviation obtained from two different lots of honey spiked at 500 ng/kg (ppt). Metabolite recovery was greater than 85% post-derivatization for each analyte. The samples used for spiking tested negative before the study.

ORDERING INFORMATION

Description	Part Number
Oasis HLB, 3 cc/60 mg, 30 µm, 100/box	WAT094226
XTerra MS C_{18}, 3.5 µm, 2.1 x 100 mm	186000404
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720001034EN

INTRODUCTION

The United States Food and Drug Administration (US FDA) banned Nitrofuran drugs are banned in food producing animals because they pose a public health risk. The rule went into effect as a result of evidence that the drugs may induce carcinogenic residues in animal tissues.

PRETREATMENT

1. Homogenize 10 g of sample in 100 mL of 0.12 M hydrochloric acid.
2. Take 1 mL aliquot and treat with 400 µL of 50 mM 2-nitrobenzaldehyde in dimethylsulfoxide.
3. Hydrolyze/derivatize the sample for 16 hours at 37 °C.
4. Adjust the sample to pH 7.4 with potassium hydrogen phosphate.
5. Centrifuge sample for 5 minutes at 8000 rpm.

SPE PROCEDURE

Oasis® HLB 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 1 mL methanol
B. 1 mL water

LOAD:
Approximatively 100 mL of sample

WASH:
A. 2 mL water
B. 2 mL 30% methanol in water

DRY:
20 minutes

ELUTE:
3 mL methyl-t-butyl/methanol/formic acid (89:9:2, v/v/v)

Evaporate and reconstitute in 200 µL mobile phase

LC CONDITIONS

System:	Alliance® HPLC 2695
Column:	XTerra® MS C_{18}, 3.5 µm, 2.1 x 100 mm
Flow rate:	0.2 mL/min
Mobile phase:	Isocratic 70% 20 mM ammonium formate pH 4, 30% acetonitrile
Injection volume:	20 µL
Column temp.:	30 °C

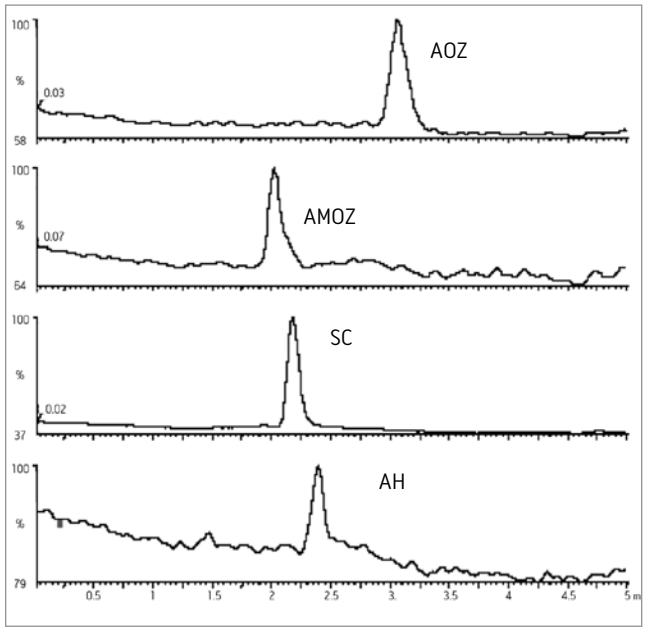
MS CONDITIONS

MS System:	Waters Quattro micro™ API
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

Analyte	MRM Transition
AOZ	236→134
AMOZ	335→291
SC	209→192
AH	249→178

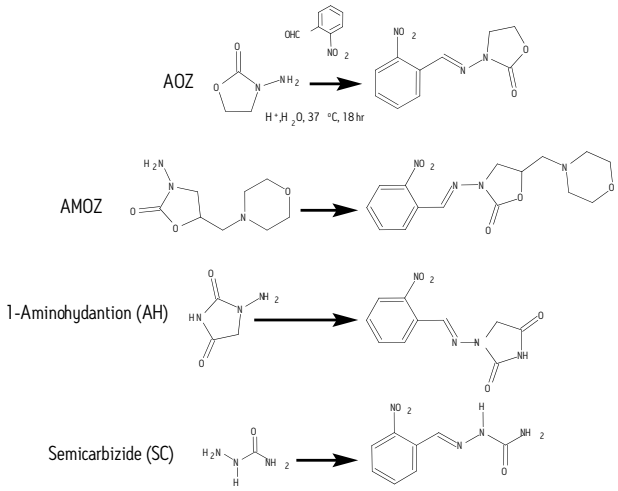
MRM method parameters.

RESULTS



Spiked chicken muscle (1 ng/g) metabolites as 2-nitrobenzaldehyde derivatives.

STRUCTURES



Chemical structures of 2-nitrobenzaldehyde derivatives.

ORDERING INFORMATION

Description	Part Number
Oasis HLB, 3 cc/60 mg, 30 µm, 100/box	WAT094226
XTerra MS C_{18}, 3.5 µm, 2.1 x 100 mm	186000404
Qsert™ Vials, LCGC Certified Combination Packs	186001126C

INTRODUCTION

World organizations are concerned about the overuse of antibiotics and antibacterials levels in foods due to possible bacteria resistances and health concerns.

PRETREATMENT

1. Homogenize 3 g of sample with 3 mL of 5% sodium tungstate, 3 mL of 0.17 M sulfuric acid and 30 mL of water.
2. Centrifuge 3100 rpm for 10 minutes.
3. Filter with glass fiber filter.

SPE PROCEDURE

Sep-Pak® Plus Short C_{18}, 360 mg

LOAD
WASH: 3 mL water
ELUTE: 4 mL 20% acetonitrile water
Concentrate to 2.5 mL and adjust the volume to 6 mL water

LC CONDITIONS

System: ACQUITY UPLC®

Column: ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm

Flow rate: 600 µL/min

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient:

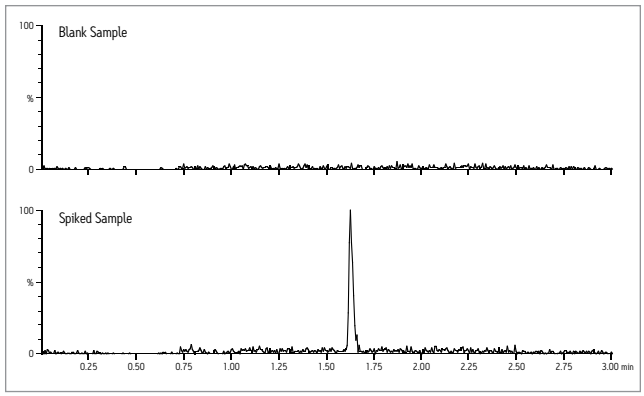
Time (min)	A%	B%
0.00	90	10
5.00	10	90
5.50	90	10
6.00	90	10

MS CONDITIONS

MS System: Waters Quattro Premier™ XE

Ionization mode: Positive electrospray (ESI+)
Multiple reaction monitoring

MRM transitions: 1: 335160
2: 335176



5 ppb penicillin spiked in pork muscle.

Compound Name: penicillin MRM 335→176	RT	Area
Pork blank 1	1.83	4.50
Pork blank 2	1.83	3.23
5 ppb spiked pork 1	1.83	23.30
5 ppb spiked pork 2	1.82	18.54
5 ppb spiked pork 3	1.83	21.82
5 ppb spiked pork 4	1.83	15.45
5 ppb spiked pork 5	1.82	21.50
RSD (%)	15.56	-
Recovery (%)	58.26	-

Compound Name: penicillin MRM 335→160	RT	Area
Pork blank 1	1.82	5.81
Pork blank 2	1.83	5.02
5 ppb spiked pork 1	1.83	42.97
5 ppb spiked pork 2	1.83	26.59
5 ppb spiked pork 3	1.83	21.89
5 ppb spiked pork 4	1.82	25.22
5 ppb spiked pork 5	1.83	39.03
RSD (%)	29.75	-
Recovery (%)	78.30	-

Recovery results for 5 ppb penicillin spiked in pork muscle.

ORDERING INFORMATION

Description	Part Number
Sep-Pak Plus Short C_{18}	WAT020515
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm	186002350
LCMS Certified Vials	600000751CV

INTRODUCTION

World organizations are concerned about the overuse of antibiotics and antibacterials. This method can be used to monitor antibacterials in milk.

PRETREATMENT

Milk (1 mL) is first extracted with 3 mL acetonitrile (1 minute shake). The sample is centrifuged and the supernatant is collected. The acetonitrile extract (extract 1) is evaporated to just under 1 mL using a gentle nitrogen stream and a water bath at 45 °C (this step extracts the penicillins and partially extracts the sulfonamides).

The milk solids pellet is then extracted with 3 mL of pH 5 succinate/EDTA buffer. The sample is centrifuged and the supernatant is collected (extract 2, this step extracts the tetracyclines and any remaining sulfonamides).

Extract 2 is combined with the evaporated extract 1 and the volume is made up to 10 mL with water. The combined extracts are then processed using an Oasis® HLB Cartridge.

SPE PROCEDURE

Oasis HLB 1cc/30mg

CONDITION/EQUILIBRATE:
1 mL MeOH, 1 mL water

LOAD SAMPLE:
From pretreatment

WASH:
0.5 mL 5% methanol/water

ELUTE:
60:40 methanol/water
60 mM ammonium acetate

LC CONDITIONS

Column: ACQUITY UPLC® BEH Shield RP18, 1.7 µm, 2.1 x 100 mm

Flow rate: 4 mL/min

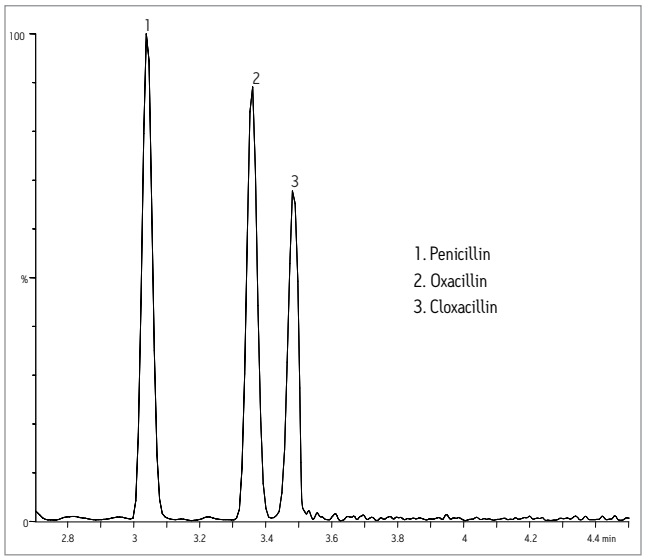
Mobile phase A: 0.1% Formic acid in water

Mobile phase B: Acetonitrile

Gradient:

Time (min)	A%	B%
Initial	85	15
2.00	60	40
2.50	40	60
3.00	10	90
4.50	10	90
4.60	85	15
5.50	85	15

RESULTS



100 ppb spiked in milk.

Compound	MRM Transition	Recovery
Oxytetracydine	461→426	78
Tetracycline	445→410	69
Chlortetracycline	479→444	76
Sulfadiazine	251→92	83
Sulfathiazole	256→92	87
Sulfapyridine	250→92	66
Sulfamerazine	265→92	95
Sulfamethazine	279→92	90
Sulfamethoxypyridazine	281→92	70
Sulfachloropyridazine	285→92	85
Sulfamethoxazole	254→92	97
Sulfadimethoxine	311→92	83
Penicillin G	335→160	93
Oxacillin	402→160	83
Cloxacillin	436→160	96

MRM method parameters and recovery data.

ORDERING INFORMATION

Description	Part Number
Oasis HLB, 1 cc/30 mg, 100/box	WAT094225
ACQUITY UPLC BEH SHIELD RP18, 1.7 µm, 2.1 x 100 mm	186002854
LCMS Certified Vials	600000751CV

INTRODUCTION

World health organizations are concerned about the antibiotics and antibacterials levels in food. This method can be used to monitor Spiramycin, a macrolide antibiotic, in pork.

PRETREATMENT

1. Weigh 5 g of sample into 25 mL of water. Homogenize.
2. Centrifuge at 3000 rpm for 10 minutes.
3. Collect supernatant.
4. Re-extract pellet with 15 mL 1.2% metaphosphoric acid/ methanol (50:50, v/v).
5. Vortex.
6. Centrifuge at 3000 rpm for 10 minutes. Collect supernatant.
7. Combine both supernatants.
8. Filter sample using glass fiber filter.

SPE PROCEDURE

Oasis® MCX, 6 cc/150 mg

CONDITION:
A. 3 mL phosphate buffer/methanol (1:9, v/v)
B. 5 mL water

LOAD:
Sample (approximately 40 mL)

WASH:
A. 3 mL 0.1 M dipotassium hydrogen phosphate (pH 3)
B. 15 mL 0.1 M potassium phosphate/methanol (1:9, v/v)

EVAPORATE:
Approximately to 1 mL and bring up to 2 mL with 20% methanol

SPE Buffer:

Dissolve 8.7 g dipotassium hydrogen phosphate in 1 L of deionized water, adjust to pH 3 with phosphoric acid.

LC CONDITIONS

System	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Flow rate:	400 µL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile

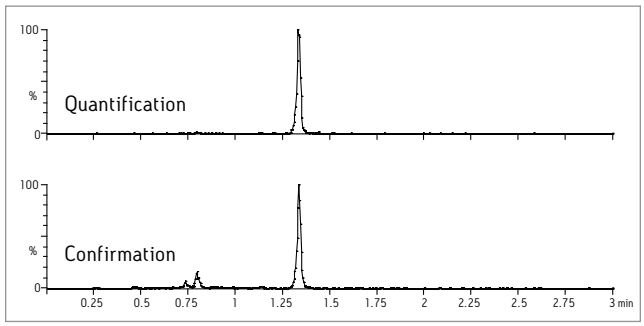
Gradient:

Time (min)	A%	B%
0.00	90	10
2.00	60	40
2.10	90	10
3.00	90	10

MS CONDITIONS

MS System:	Waters Quattro Premier™ XE
MRM transitions:	1: 422.50→101.00 2: 422.50→174.10
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

RESULTS



100 ppb spiramycin spiked sample in pork muscle.

Compound Name: Spiramycin 174.1	RT	Area
100 ppb spiked 1	1.34	902.79
100 ppb spiked 2	1.34	911.36
100 ppb spiked 3	1.34	1041.24
100 ppb spiked 4	1.34	1030.33
100 ppb spiked 5	1.34	1101.27
RSD (%)	-	7.97
Recovery (%)	-	73.26

Compound Name: Spiramycin 101	RT	Area
100 ppb spiked 1	1.34	1063.65
100 ppb spiked 2	1.34	1267.22
100 ppb spiked 3	1.34	1368.84
100 ppb spiked 4	1.34	1227.10
100 ppb spiked 5	1.34	1379.40
RSD (%)	-	5.96
Recovery (%)	-	74.82

Recovery data for 100 ppb spiramycin spiked sample in pork muscle.

ORDERING INFORMATION

Description	Part Number
Oasis MCX, 6 cc/150 mg, 30/box	186000256
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm	186002350
LCMS Certified Vials	600000751CV

INTRODUCTION

The analysis of steroids in products such as milk and meat is often very challenging due to the complexity of the samples. Sample preparation using QuEChERS allows for fast throughput and high sensitivity in food analysis. Although QuEChERS is commonly used for multi-residue pesticide analysis in fruits and vegetables, it is also applicable in the analysis of steroids in livestock products. In this application note, milk and ground beef are prepared and analyzed for steroid hormones at the ppb level, using QuEChERS methodology and UPLC®/MS/MS.

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY® BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Part number:	186002352
Injection volume:	3 µL
Column temp.:	40 ° C
Mobile phase A:	Water
Mobile phase B:	Methanol
Flow rate:	0.40 mL/min
Gradient:	30% B initial, linear gradient to 97% B in 5 minutes, hold until 8 minutes, back to 30% B at 8.1 minutes. Hold and re-equilibrate until 10 minutes.
Sample vials:	Maximum Recovery Vial
Part number:	600000670 CV

MS CONDITIONS

System:	Xevo® TQ-S
Ionization mode:	Electrospray positive (ESI+).

SAMPLE PREPARATION

Initial Extraction (QuEChERS)

Place 10 mL whole milk (pasteurized) or 10 g ground beef (85% lean) into a 50 mL centrifuge tube. Add 10 mL acetonitrile and shake the tube vigorously for 1 minute. Add the contents of DisQuE™ pouch salts for European Committee for Standardization (CEN) QuEChERS (P/N: 186006813) and shake vigorously for 1 minute. Centrifuge for 3 minutes @ 4000 rpm and take a 1 mL aliquot of the supernatant (top layer) for d-SPE cleanup.

d-SPE Cleanup

Transfer the 1 mL aliquot of supernatant to a 2 mL d-SPE cleanup tube that contains 150 mg of magnesium sulfate, 50 mg PSA sorbent and 50 mg C_{18} sorbent (P/N: 186004830) and shake vigorously for 1 minute. Centrifuge for 3 minutes at 4000 rpm and take a 0.5 mL aliquot as a sample for UPLC/MS/MS analysis.

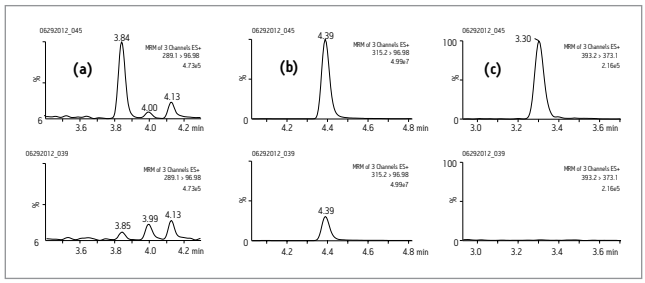


Figure 1. UPLC/MS/MS chromatograms of steroids obtained from ground beef samples; the top trace is the low level spiked sample, the bottom trace is a ground beef blank. (a) testosterone (b) progesterone (c) dexamethasone.

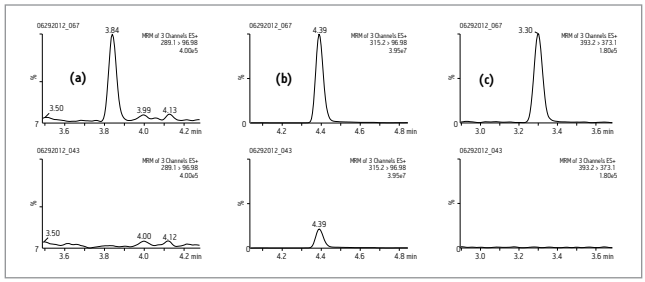


Figure 2. UPLC/MS/MS chromatograms of steroids obtained from milk samples; the top trace is the low level spiked sample, the bottom trace is a milk blank. (a) testosterone (b) progesterone (c) dexamethasone.

	Concentration Range (ppb)		Average % Recovery (%RSD) n=5			
			Ground Beef		Whole Milk	
Conc. Level	Low Level	High Level	Low Level	High Level	Low Level	High Level
Testosterone	0.5	5	87(2.5)	89(2.2)	89(5.4)	95(1.1)
Progesterone	50	100	91(1.9)	92(3.7)	94(1.4)	95(0.8)
Dexamethasone	10	50	86(4.3)	91(3.2)	90(3.9)	91(4.4)

Table 2. Recoveries of steroid hormones from ground beef and whole milk samples.

ORDERING INFORMATION

Description	Part Number
ACQUITY BEH C_{18}, 1.7 µm, 2.1 x 100 mm Column	186002352
CEN QuEChERS DisQuE Pouch	186006813
DisQuE 2 mL d-SPE Cleanup Tube (150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18})	186004830
DisQuE 50 mL Centrifuge Tube	186004837
Maximum Recovery Vial	600000670CV

Ref: Waters Application Note 720004441EN

INTRODUCTION

Bee keeping and honey production is a world-wide industry. Trace levels of the Streptomycin, an aminoglycoside, has been detected in honey. Streptomycin is not permitted in food products.

PRETREATMENT

1. Dissolve 20 g of honey in approximately 75 mL water.
2. Bring to a total volume of 100 mL with water.
3. Filter through a fluted filter to remove suspended solids.

SPE PROCEDURE

Sep-Pak® Vac 6 cc Accell™ Plus CM Cartridge

CONDITION:
2 x 5 mL 2% acetic acid

RINSE:
2 x 5 mL water*

LOAD:
50 mL of honey solution at approximately 2 drops per second

RINSE:
2 x 5 mL water

ELUTE:
2 x 5 mL (80:20, 2% acetic acid/acetonitrile)

Adjust to 10 mL with water

** Do not allow the cartridge to dry out through the procedure*

LC CONDITIONS

System:	Alliance® 2795 HPLC
Column:	Atlantis® HILIC Silica, 3 µm, 2.1 x 50 mm
Guard Column:	Atlantis HILIC Silica, 3 µm, 2.1 x 10 mm
Flow rate:	0.3 mL/min
Mobile phase A:	200 mM ammonium formate in 100 mM formic acid
Mobile phase B:	100 mM Formic Acid in acetonitrile
Column temp.:	30 °C
Injection volume:	20 µL

Gradient:

Time (min)	A%	B%
0.00	10	90
6.00	60	40
10.00	60	40
10.10	10	90
16.00	10	90

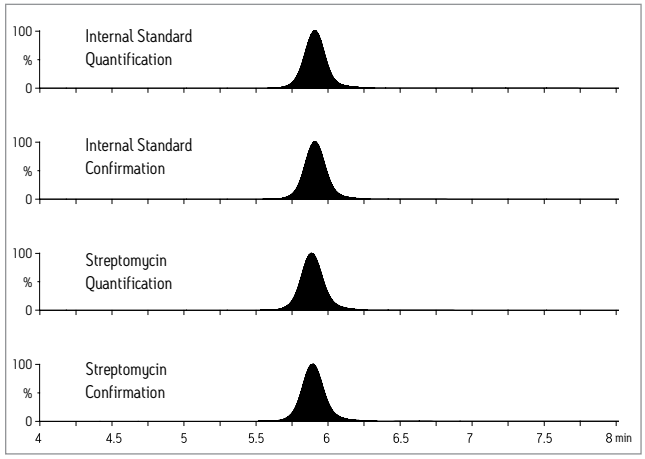
MS CONDITIONS

MS System: Waters Quattro micro™
Ionization mode: Positive electrospray (ESI+)
Multiple reaction monitoring

Analyte	MRM Transition
Streptomycin	582→263
	582→176
Internal Standard	584→263
	584→246

MRM method parameters.

RESULTS



MRM for streptomycin and the internal standard.

Spiked Concentration µg/kg	Mean	Std. Dev.	RSD (%)
2	1.99	0.09	4.5
20	20.17	0.076	3.7
100	100.91	3.75	3.7

Method accuracy and precision over three days.

ORDERING INFORMATION

Description	Part Number
Sep-Pak Vac, 6 cc Accell Plus CM Cartridge	WAT054545
Atlantis HILIC Silica, 3 µm, 2.1 x 10 mm	186002005
Atlantis HILIC Silica, 3 µm, 2.1 x 50 mm	186002011
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720000981EN

INTRODUCTION

Sulfonamides are widely used for therapeutic and prophylactic purposes in animals. When sulfonamides are retained in food stuff, this may result in allergic or toxic reactions in sensitive consumers. This method can be used to monitor the presence of sulfonamides in milk.

SPE PROCEDURE

Oasis® MCX, 3 cc/60 mg

CONDITION:
A. 2 mL methanol
B. 2 ml water

LOAD:
5 mL whole homogenized milk

WASH 1:
2 mL 5% methanol in water

WASH 2:
1 mL 0.5 M aqueous hydrochloric acid

WASH 3:
2 mL 20% methanol in water

WASH 4:
1 mL methanol

ELUTE:
2.5 mL 90:10 methanol/water (200 mM ammonium bicarbonate pH 8.5)

Reconstitute in 0.5 mL mobile phase

LC CONDITIONS

System: ACQUITY UPLC® System

Column: ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm

Flow rate: 0.2 mL/min

Mobile phase A: 0.05% formic acid/water
Mobile phase B: 0.05% formic acid/methanol

Gradient:

Time (min)	A%	B%
0.00	90	10
3.25	80	20
3.26	90	10

Detector: ACQUITY UPLC PDA Detector

MS CONDITIONS

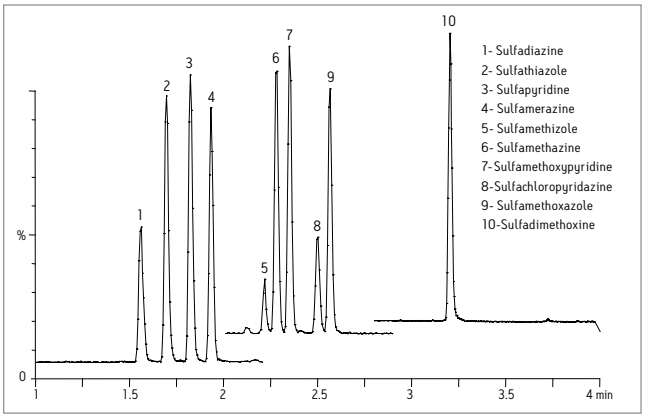
MS System: Waters Quattro Premier™ XE

Ionization mode: Positive electrospray (ESI^+)
Multiple reaction monitoring

Compounds	Precusor (m/z)
Sulfapyridine	249.80→91.90
	249.80→155.80
Sulfamethoxazole	253.70→91.90
	253.70→155.80
Sulfadiazine	250.80→91.90
	250.80→155.90
Sulfathiazole	255.70→91.90
	255.70→155.90
Sulfamerazine	264.80→92.00
	264.80→155.80
Sulfamethizole	270.90→91.70
	270.90→155.80
Sulfamethazine	278.80→91.90
	278.80→155.80
Sulfamethoxy-pyridazine	280.80→91.90
	280.80→155.80
Sulfachloropyridazine	284.70→92.00
	284.70→155.90
Sulfadimethoxine	310.80→91.90
	310.80→155.90

MRM method parameters.

RESULTS



Whole milk (5 ng/mL) typical LC/MS/MS analysis (MRM).

Compound	Recovery (%)	RSD (%)
1-Sulfadiazine	79	9.2
2-Sulfathiazole	75	6.5
3-Sulfapyridine	68	10.2
4-Sulfamerazine	77	9.1
5-Sulfamethizole	73	10.1
6-Sulfamethazine	67	7.0
7-Sulfamethoxypyridine	79	10.0
8-Sulfachloropyridazine	60	7.0
9-Sulfamethoxazole	71	9.1
10-Sulfadimethoxine	80	9.2

Recovery data for whole milk (5 ng/mL).

ORDERING INFORMATION

Description	Part Number
Oasis MCX, 3 cc/60 mg, 100/box	186000254
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352
LCMS Certified Vials	600000751CV

INTRODUCTION

Tetracyclines and sulfonamides are widely used for therapeutic and prophylactic purposes in animal diseases. When these classes of veterinary drugs are retained in food stuff, this may result in allergic or toxic reactions in sensitive consumers.

PRETREATMENT

1. Mix 1.5 mL milk with 6 mL pH 4 Mclivaine buffer.
2. Centrifuge.
3. Take supernatant/adjust to pH 10 with 0.75 mL 1 M NaOH.

SPE PROCEDURE

Oasis® MAX, 1 cc/30 mg

CONDITION/EQUILIBRATE:
2 mL MeOH, 2 mL water

LOAD SAMPLE:
From pretreatment

WASH 1:
0.5 mL 5% NH_2OH/water

WASH 2:
0.5 mL methanol

ELUTE:
0.5 mL 45.55 acetonitrile/75 mM oxalic acid.
Dilute 1:3 with water for LC

LC CONDITIONS

Column:	ACQUITY UPLC® BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Flow rate:	4 mL/min
Mobile phase A:	0.1% Formic acid/water
Mobile phase A:	Acetonitrile

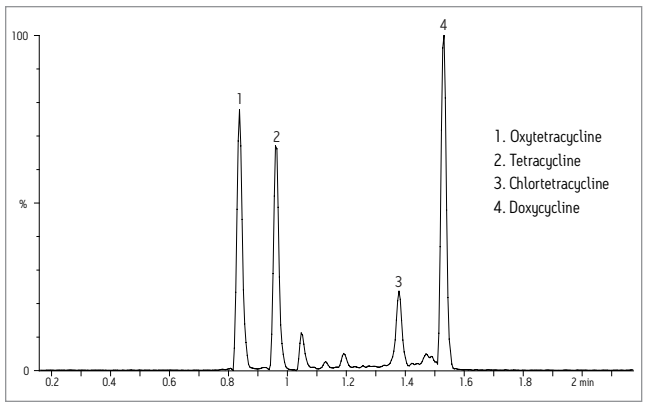
Gradient:

Time (min)	A%	B%
Initial	85	15
2.50	50	50
3.50	30	70
3.60	85	15
4.00	85	15

MS CONDITIONS

MS System:	Waters Quattro Premier™ XE
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

RESULTS



100 ppb spiked sample.

ORDERING INFORMATION

Description	Part Number
Oasis MAX, 1 cc/30 mg, 100/box	186000366
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm	186002350
LCMS Certified Vials	600000751CV

INTRODUCTION

Tetracyclines (TCs), an antibiotic, is not permitted in apiculture. This method can monitors the presence of TC's in honey.

PRETREATMENT

1. Add 30 mL EDTA-McIlvaines buffer to 6 g sample, mix thoroughly for 1 minute.
2. Centrifuge at 3000 rpm for 5 minutes and collect supernatant for SPE.

SPE PROCEDURE

Cartridge I: Oasis® HLB, 6 cc/500 mg

CONDITION/EQUILIBRATE:
A. 5 mL methanol
B. 10 mL water

LOAD:
Honey sample (3 mL/min)

WASH:
5 mL methanol/water (5:95, v/v)

DRY:
By vacuum for 20 minutes

ELUTE:
15 mL ethyl acetate

Solution I

Cartridge II: Sep-Pak® Accell™ CM, 3 cc/500 mg

CONDITION/EQUILIBRATE:
5 mL ethyl acetate

LOAD:
Solution I (3 mL/min)

WASH:
5 mL methanol

DRY:
By cartridge by vacuum for 5 minutes

ELUTE:
4 mL mobile phase

SOLUTIONS

McIlvaines Buffer

1. Thoroughly mix 1000 mL 0.1 M citric acid with 625 mL 0.1 ML disodium hydrogen phosphate dihydrate.
2. Adjust with sodium hydroxide or hydrochloric acid to pH 4 ± 0.05, if necessary.

EDTA-McIlvaines Buffer

1. Add 60.5 g disodium EDTA to 1625 mL McIlvaines Buffer and mix thoroughly.

LC CONDITIONS

Column:	SunFire® C_8, 3.5 µm, 2.1 x 150 mm
Mobile phase:	Acetonitrile:methanol: 0.4% fomic acid (18:4:78)
Flow rate:	0.2 mL/min
Injection volume:	20 µL
Column temp.:	25 °C

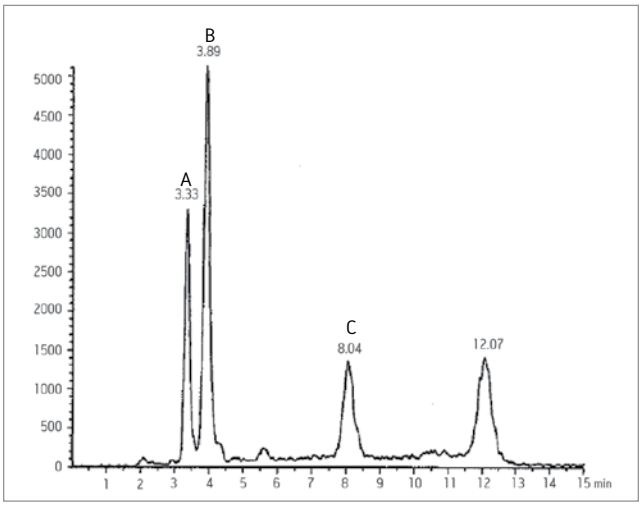
MS CONDITIONS

MS System:	Waters Quadrupole MS
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

Analyte	MRM for Quantification	MRM for Quantification
Oxytetracycline	461→426	461→426
	461→443	
	461→381	
Tetracycline	445→410	445→410
	445→154	
	445→428	
Chlortetracycline	479→444	479→444
	479→154	
	479→462	
Doxycycline	445→428	445→428
	445→410	
	445→154	

MRM method parameters.

RESULTS



A. Oxytetracycline, B. Tetracycline, C. Chlortetracycline and D. Doxycycline standards.

Analyte	Concentration (mg/kg)	Average Recovery
A. Oxytetracycline	0.002	88.0
	0.010	95.3
	0.100	95.8
	0.050	93.6
B. Tetracycline	0.002	81.9
	0.010	82.6
	0.050	84.5
	0.100	89.3
C. Chlortetracycline	0.002	87.2
	0.010	86.0
	0.050	86.6
	0.100	90.8
D. Doxycycline	0.002	85.2
	0.010	85.3
	0.050	86.8
	0.100	87.9

Recovery data for tetracyclines in honey.

ORDERING INFORMATION

Description	Part Number
Oasis HLB, 6 cc/500 mg, 60 µm 30/box	186000115
Sep-Pak Accell CM, 3 cc/500 mg, 50/box	WAT020855
SunFire C_8, 3.5 µm, 2.1 x 150 mm	186002712
LCMS Certified Vials	600000751CV

Ref: China GB/T 18932.23 - 2003

PESTICIDES AND CONTAMINANTS

The presence of contaminants in food, such as pesticides, herbicides, illegally-added dyes, mycotoxins, and melamine, are a concern to regulatory bodies, public health agencies, and the public at large. Methods in this section cover:

- Sample pretreatment
- Sample preparation
- Instrumentation methods and results

These methods meet, or exceed, the level of detection and quantitation required by government agencies.

This section of the notebook also includes applications of multi-residue pesticides analysis by QuEChERS and other official methods. There are QuEChERS* application briefs on a variety of different commodities to show examples requiring:

- Different pretreatment requirements, i.e. soaking of dry commodities before the extraction procedure
- Alternative d-SPE sorbent selection, i.e. for removing fats

** For further details on this method, see Waters QuEChERS White Paper on our website (literature number 720003643en).*

INTRODUCTION

Acrylamide, a chemical contaminant, is produced during the cooking of french fries, potato chips, and other processed foods. Acrylamide is considered to be a possible cancer causing agent.

PRETREATMENT

1. 1 g crushed potato product was weighed into a centrifuge tube.
2. 15 mL of 2 M sodium chloride and 10 µL of internal standard (acrylamide-D_3) solution was added to the tube. Shake contents vigorously for 30 minutes.
3. Centrifuge at 10000 x g for 12 minutes.
4. Take a 1.5 mL aliquot of the supernatant from the centrifuge tube for SPE extraction and cleanup.

SPE PROCEDURE

Cartridge I: Oasis® HLB, 6 cc/200 mg

CONDITION:
A. 2 mL methanol
B. 2 mL 2 M sodium chloride

LOAD:
1.5 mL potato extract

WASH:
0.8 mL water

ELUTE:
3 mL 1% formic acid in methanol

Cartridge II: Oasis MCX, 3 cc/60 mg

CONDITION:
2 mL methanol

A. Pass eluent from Part A.
B. Rinse vial in 0.5 mL methanol, combine with passed eluent
C. Collect in total

EVAPORATE AND RECONSTITUTE:
0.4 mL water

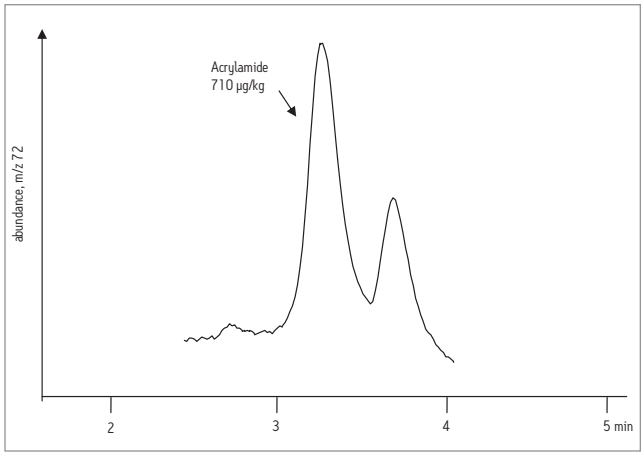
LC CONDITIONS

System:	Alliance® HPLC 2695
Column:	Atlantis® dC_{18}, 5 µm, 2.1 x 150 mm
Flow rate:	0.2 mL/min
Mobile phase:	0.1% formic acid in water
Injection volume:	20 µL
Column temp.:	30 °C

MS CONDITIONS

MS System:	Waters ZMD Mass Detector	
Ionization mode:	Positive Electrospray (ESI+) Selected-Ion Recording (SIR)	
Compound:	Mass	Cone Voltage (V)
Acrylamide	72	20
	55	40
Acrylamide-D_3	75	20
	58	40

RESULTS



LC/MS determination of acrylamide in potato chips.

Fortification Level (µg/kg)	Amount Found (µg/kg)	RSD (%)*
100	96	12
200	211	8.7
500	488	5.8
1000	1010	8.0
2000	2000	6.5

**Five replicate samples analyzed per level.*

ORDERING INFORMATION

Description	Part Number
Atlantis dC_{18}, 5 µm, 2.1 x 150 mm	186001301
Oasis HLB, 6 cc/200 mg	WAT106202
Oasis MCX, 3 cc/60 mg	186000254
Qsert™ Vials, LCGC Certified Combination Packs	186001126C

Ref: Waters Application Note, 720000688EN

INTRODUCTION

Contamination of foodstuffs with mycotoxins is one of the most concerning problems in food and feed safety. Aflatoxins, produced by the genus Aspergillus, are one of the most widely occurring mycotoxins. The main aflatoxins are B1, B2, G1, and G2. M1 and M2 are metabolites that appear when dairy animals eat grain contaminated with B1 and B2 aflatoxins.

LC CONDITIONS

System:	ACQUITY UPLC H-Class
Run time:	4.0 minutes
Column:	ACQUITY BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Column temp:	30 °C
Mobile phase A:	Water
Mobile phase B:	Methanol
Mobile phase C:	Acetonitrile
Flow rate:	0.4 mL/min
Injection volume:	20 µL (using optional 50 µL loop)

Gradient:

Time (min)	Flow Rate (mL/min)	%A	%B	%C
Initial	0.4	64	18	18

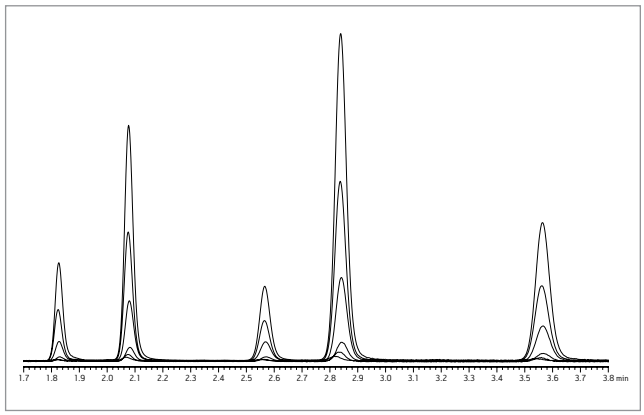
FLUORESCENCE CONDITIONS

Excitation:	365 nm
Emission:	429 nm (M1, B2, B1)
Emission:	456 nm (G2, G1)

AFLATEST SAMPLE PREPARATION PROCEDURE

- Using a blender, blend 25 g sample, 5 g sodium chloride, and 100 mL of a 80:20 methanol: water (HPLC grade) mix at high speed for 1 minute.
- Filter this mixture through fluted Whatman filter paper (filtrate 1).
- Mix 10 mL of filtrate 1 and 40 mL water.
- Filter through glass microfiber filter paper (filtrate 2).
- Load 10 mL of filtrate 2 onto a VICAM® AflaTest® Affinity Column, part no. G1024.
- Wash with two 10 mL portions of HPLC-grade water.
- Elute with 1 mL HPLC grade methanol.
- Dilute 1:1 with 1% aqueous acetic acid and inject.
- The resulting solution, assuming 100% recovery, had a calculated concentration of 1000 ng/L total aflatoxins B, G, and 12.5 ng/L M1 (spiked cereal). A total of 20 µL was injected for UPLC®-FLR analysis.

RESULTS



Representative overlay of standard mixes 1 to 6.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352

Ref: Waters Application Note 720003644EN

INTRODUCTION

Aflatoxins are naturally occurring mycotoxins. Aflatoxins often cause disease even when ingested in minute amounts and are most commonly known for causing acute, or chronic liver disease, and liver cancer.

PRETREATMENT

1. Weigh 50 g ground sample with 5 g sodium chloride and place in blender jar.
2. Add 100 mL 80:20 methanol: water (v/v) to jar.
3. Blend at high speed for 1 minute.
4. Filter extract with fluted filter paper. Collect filtrate in a clean vessel.
5. Pipette or pour 65 mL filtered extract into a clean vessel.
6. Dilute extract with 60 mL of phosphate buffer saline. Mix well.
7. Filter extract through glass microfiber filter into a clean vessel.

SPE PROCEDURE

AflaTest® Affinity Column

Pass 4 mL of filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest affinity column at a rate of about 1–2 drops/seconds until air comes through column

↓

Pass 10 mL of 20:80 methanol:water through the column at a rate of about 2 drops/second

↓

Repeat previous step once more until air comes through column

↓

Place glass cuvette under AflaTest column and add 1 mL HPLC grade methanol into glass syringe barrel

↓

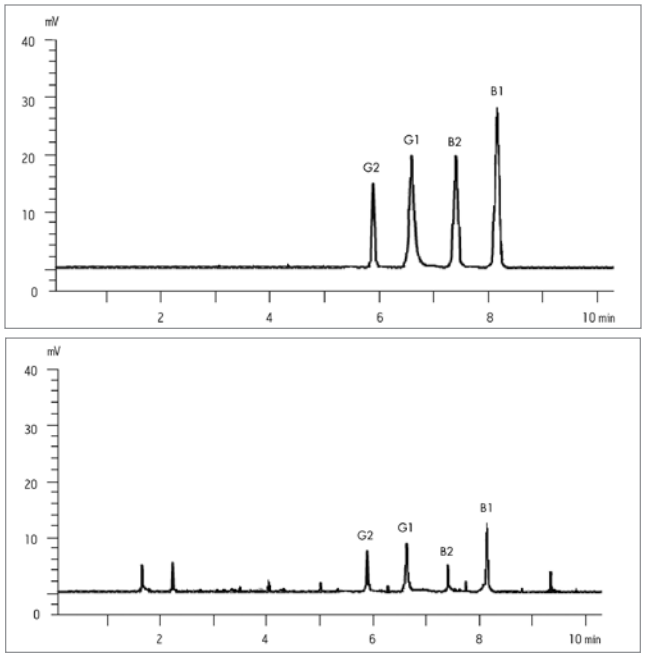
Elute AflaTest column at a rate of 1 drop/second by passing methanol through the column and collecting all the sample eluate (1 mL) in a glass cuvette

↓

Add 1 mL of purified water to eluate. Inject 20-100 µL onto HPLC

LC CONDITIONS

System:	Alliance® HPLC
Column:	XBridge® C_{18}, 5 µm, 4.6 x 250 mm
Flow rate:	1 mL/min
Mobile phase:	Acetonitrile/water/methanol (17:54:29, v/v/v)
Injection volume:	100 µL
Detector:	2475 Multi Wavelength Fluorescence
Detection:	Excitation Wavelength: 333 nm Emmission Wavelength: 460 nm



Aflatoxins in red pepper extract. Recovery: 76% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix).

Action Levels for Aflatoxins

United States (FDA) action levels (B1, B2, G2, G2, M1)

Food Stuff	Level	Regulation
All products, except milk, designated for humans	20 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Milk	0.5 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Corn for immature animals and dairy cattle	20 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Corn for breeding beef cattle, swine and mature poultry	100 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Corn for finishing swine	200 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Corn for finishing beef cattle	300 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
Cottonseed meal (as feed ingredient)	300 ng/g	Policy Guides 7120.26, 7106.10, 7126.33
All feedstuff other than corn	200 ng/g	Policy Guides 7120.26, 7106.10, 7126.33

Aflatoxin regulatory action limits.

ORDERING INFORMATION

Description	Part Number
VICAM AflaOchra HPLC™ Columns, 25/box	G1017
VICAM Glass Cuvette	34000
XBridge C_{18}, 5 µm, 4.6 x 250 mm	186003117
LCMS Certified Combination Packs	600000751CV

INTRODUCTION

Bisphenol A (BPA) is an additive primarily used in the production of polycarbonate plastics and epoxy resins. These synthetic materials are widely used in food packaging to protect the safety and integrity of foods and beverages. Polycarbonates are used to produce many food and beverage containers, such as baby bottles, tableware, and other food containers. BPA is an endocrine disruptor, which can mimic the body's own hormones and may lead to negative health effects. This application note presents the results for three bisphenols (A, B, and E) in baby food and powder milk formula.

SAMPLE PREPARATION CONDITIONS

DisQuE™

Sorbent pouch:	DisQuE Pouch (CEN Method), 4.0 g $MgSO_4$, 1.0 g NaCl, 1.5 g Na Citrate
dSPE:	15-mL tube, part #186004834 900 mg $MgSO_4$, 150 mg PSA, 150 mg C_{18}

SPE

Cartridge:	Oasis® HLB 30 mm, 60 mg/3 cc
Condition:	2 mL methanol
Equilibrate:	2 mL water
Load:	70 mL diluted extract
Flow rate:	< 5 mL/min
Wash:	2 mL 40% MeOH in Water
Elute:	1 mL 100% MeOH

LC conditions

System	ACQUITY UPLC®
Runtime:	5.0 minutes
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Column temp.:	40 °C
Mobile phase A:	0.5% NH_4OH in water
Mobile phase B:	0.5% NH_4OH in methanol
Elution:	3 minutes linear gradient from 5% (B) to 95% (B)
Flow rate:	0.5 mL/min
Injection volume:	50 µL

MS CONDITIONS

MS system:	Xevo® TQD
Ionization mode:	ESI negative
Capillary voltage:	3.5 kV
Cone voltage:	30.0 V
Source temp.:	140 °C
Desolvation temp.:	350 °C
Desolvation gas:	550 L/hr
Cone gas:	50 L/hr

Precipitation (10 g sample, 10 mL CH_3CN)
Centrifuge and collect supernatant

Add contents from DisQuE Pouch. Shake.
Centrifuge and collect 10 mL supernatant

Transfer supernatant to dSPE tube. Shake.
Centrifuge and collect supernatant

Dilute supernatant with 70 mL H_2O
Load on Oasis HLB (3 cc)

Wash – 2 mL 40 % MeOH
Elute – 1 mL 100 % MeOH

Dilute eluate with 1 mL H_2O
Inject 50 µL for LC/MS/MS

Figure 2. Final sample preparation protocol.

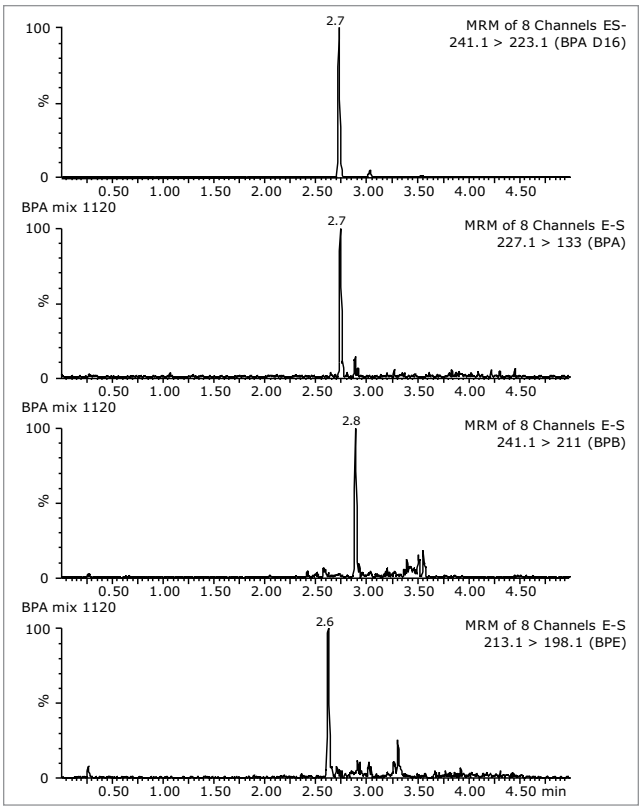


Figure 5. MRM chromatograms from an extract of a 1 ppb spike in powdered infant formula.

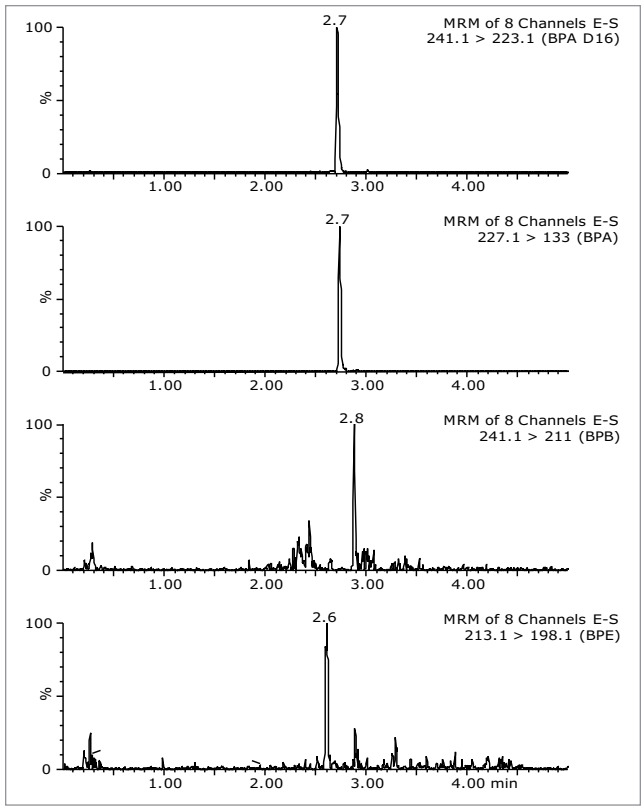


Figure 6. MRM chromatograms from an extract of a 1 ppb spike in baby food.

Bisphenols	Powdered Formula	Baby Food
BPA	102% (3.2%)	110% (7.8%)
BPB	95% (5.5%)	112% (6.7%)
BPE	81% (4.6%)	99% (6.1%)

Table 2. Calculated recoveries and RSDs (n = 3) for BPA, BPB and BPE spiked into powdered infant formula and pureed green beans.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC BEH C_{18} Column, 1.7 µm, 2.1 x 50 mm	186002350
DisQuE 50 mL Centrifuge Tube	186004837
DisQuE Pouch (CEN Method)	186006813
DisQuE 15 mL dSPE Cleanup Tube (900 mg $MgSO_4$, 150 mg PSA, 150 mg C_{18})	186004834
Oasis HLB, 3 cc/60 mg, 30 µm Cartridge	WAT094226
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720004192EN

INTRODUCTION

This application note presents a rapid QuEChERS-based method for the determination of BPA in infant formula at low ng/g (ppb) levels. The method employs a DisQuE™ Pouch product for QuEChERS-based sample preparation. The resulting extract is first cleaned-up using dispersive SPE (d-SPE), and is further cleaned-up and concentrated using an Oasis® HLB Cartridge. The QuEChERS extraction and subsequent SPE cleanup protocols provide a faster analysis with less toxic reagents, and similar detection limits when compared to other methods.

LC CONDITIONS

System:	ACQUITY UPLC® H-Class
Column:	ACQUITY® BEH C_{18}, 1.7 µm, 3.0 x 100 mm
Injection volume:	30 µL
Column temp.:	40 °C
Mobile phase A:	0.1% NH_4OH in water
Mobile phase B:	0.1% NH4OH in MeOH
Gradient:	50% B initial (hold for 0.2 minutes), linear gradient to 95% B for 3.0 minutes, hold for 5.0 minutes, back to 50% B for 5.1 minutes. Hold and re-equili brate for 7.0 minutes.
Flow rate:	0.6 mL/min
Vial:	Total Recovery

MS CONDITIONS

MS System: ACQUITY TQD

The mass spectrometer was operated in electrospray negative (ESI-) mode. The MRM transitions, cone voltages, and collision cell energies used for BPA analysis were as follows, with primary transition used for quantification appearing bold:

MRM Transition (*m/z*)	Cone (V)	Collision (eV)
227.3 > 133.0	40	23
227.3 > 212.1	40	17

SAMPLE DESCRIPTION

Initial Extraction (QuEChERS)

Place 10 g liquid infant formula into a 50-mL centrifuge tube. Add 10 mL acetonitrile (ACN), and shake the tube vigorously for 1 minute. Add contents of DisQuE Pouch for CEN QuEChERS (P/N 186006813), and shake vigorously for 1 minute. Centrifuge for 3 minutes at 4000 rpm, and take a 5-mL aliquot of the supernatant (top layer) for dSPE cleanup.

dSPE Cleanup

Transfer the 5-mL aliquot of supernatant to a 15-mL dSPE cleanup tube that contains 900 mg of magnesium sulfate, 150 mg PSA sorbent, and 150 mg of C_{18} sorbent (P/N 186004834), and shake vigorously for 1 minute. Centrifuge for 3 minutes at 4000 rpm, and take a 1.5-mL aliquot of the supernatant for Oasis HLB SPE enrichment and cleanup.

Oasis HLB SPE Enrichment and Cleanup

Transfer the 1.5-mL aliquot from d-SPE to a suitable container, dilute to 10.0 mL with reagent water, and mix. Condition an Oasis HLB Cartridge 10 mg, 1 cc with 0.5 mL methanol, followed by 0.5 mL of water. Load the diluted extract at a flow rate of 1 to 3 mL/minute. After sample loading is complete, wash the cartridge with 0.3 mL 40:60 methanol/water. Install collection vessel, and elute with 150 µL methanol. Dilute the eluted sample with 150 µL water prior to UPLC analysis. Transfer diluted sample to the Waters® Total Recovery Vial.

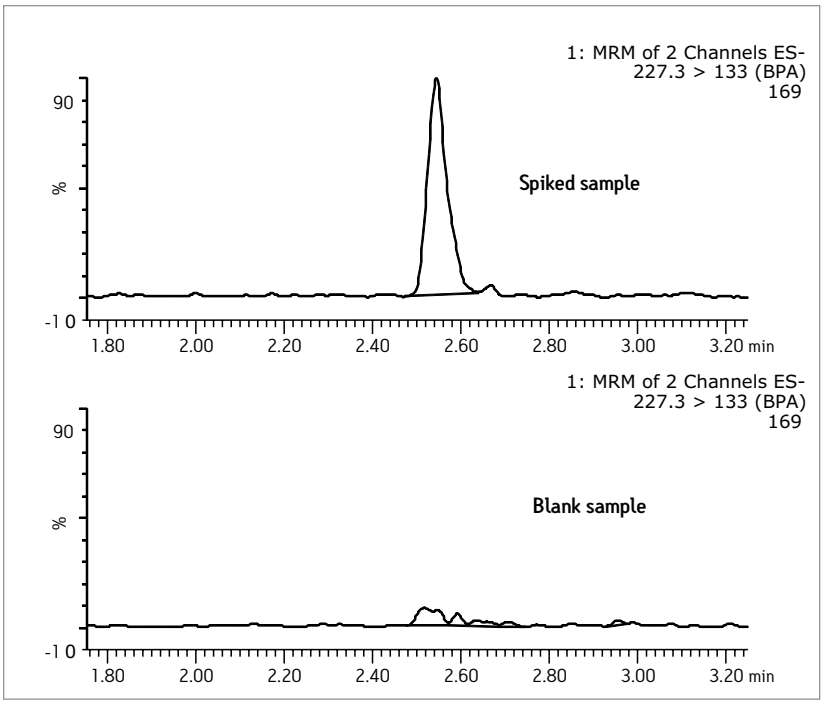


Figure 1. UPLC®/MS/MS chromatograms obtained from infant formula samples. The top trace is a 2 ng/g spiked sample, and the bottom trace is an infant formula blank.

ORDERING INFORMATION

Description	Part Number
ACQUITY BEH C_{18} Column, 1.7 µm, 3.0 x 100 mm	186005301
CEN QuEChERS DisQuE Pouch	186006813
DisQuE 15 mL d-SPE Cleanup Tube (900 mg $MgSO_4$, 150 mg PSA, 150 mg C_{18})	186004834
Total Recovery Vial	186005663CV

Ref: Waters Application Note 720004455EN

INTRODUCTION

Carbamates have been identified as a health risk. They affect the nervous system by reducing the ability of cholinesterase, an enzyme, to function properly in regulating the neurotransmitter acetylcholine.

PRETREATMENT

1. Add 50 mL of acetonitrile to 25 g of sample. Homogenize for 2 minutes and filter.
2. Collect 40-50 mL of filtrate into a flask containing 5–7 g sodium chloride.
3. Shake vigorously for 1 minute. Leave to stand at room temperature.
4. Take 10 mL aliquot from the acetonitrile layer and evaporate sample to dryness (80 °C under nitrogen or air).
5. Reconstitute with 2 mL methanol/dichloromethane (1:99, v/v).

SPE PROCEDURE

Sep-Pak® Aminopropyl, 6 cc/500 mg

CONDITION/EQUILIBRATE:
4 mL methanol/dichloromethane (1:99, v/v)

PASS-THROUGH:
2 mL sample

Collect the solution

ELUTE:
2 mL methanol/dichloromethane (1:99, v/v) x 2

Combine the solutions

Evaporate to dryness under nitrogen at 50 °C

Reconstitute in 2.5 mL methanol

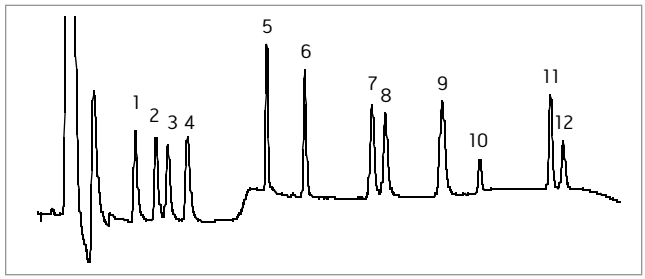
LC CONDITIONS

System:	Alliance® HPLC 2695
Column:	Carbamate Analysis Column, 3.9 x 150 mm
Flow rate:	1.5 mL/min
Mobile phase A:	Water
Mobile phase B:	Methanol
Mobile phase C:	Acetonitrile

Gradient:	Time (min)	A%	B%	C%
	0.00	88	12	0
	5.30	88	12	0
	5.40	68	16	16
	14.00	68	16	16
	16.10	50	25	25
	20.00	50	25	25
	22.00	88	12	0
	30.00	88	12	0

Sample:	10 ng of each analyte on column
Injection volume:	400 µL
Post column addition:	OPA*/NaOH @ 0.5 mL/min
Detector:	2475 Multi Wavelength Fluorescence Detector
Excitation wavelength:	339 nm
Emission wavelength:	445 nm

**OPA: Orthophthaldehyde*



Aldicarb standards.

Peak	Analyte	400 µL
1	Aldicarb Sulfoxide	3.77
2	Aldicarb Sulfone	4.66
3	Oxamyl	5.17
4	Methomyl	6.03
5	3-Hydroxycarbofuran	9.83
6	Aldicarb	11.46
7	Propoxur	14.35
8	Carbofuran	14.94
9	Carbaryl	17.37
10	1-Naphthol	18.99
11	Methiocarb	22.02
12	BDMC	22.56

Expected retention times for aldicarb standards.

ORDERING INFORMATION

Description	Part Number
Sep-Pak Aminopropyl, 6 cc/500 mg, 30/box	WAT054560
Carbamate Analysis Column, 3.9 x 150 mm	WAT035577
LC Certified Vials	186000272C

Ref: Ministry of Agriculture, China
(NY/T 761.1 – 2004 and NY/T761.3 – 2004)

INTRODUCTION

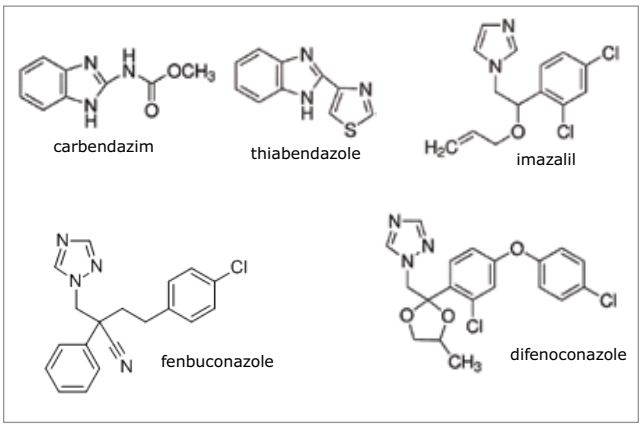
An examination of sample preparation strategies for determination of conazole fungicides, including carbendazim, in orange juice was undertaken. Sample preparation procedures utilizing QuEChERS (DisQuE) were proven to be suitable for five conazole fungicides at 10ppb levels when using UPLC/MS/MS. Solid-phase extraction (SPE) was demonstrated to reduce interferences and concentrate samples leading to detection of carbendazim at low ppb levels using HPLC/UV.

UPLC CONDITIONS FOR MASS SPECTROMETRY

System:	ACQUITY UPLC® H-Class
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Injection volume:	10 µL
Column temp.:	40 °C
Mobile phase A:	0.1% NH_4OH in water
Mobile phase B:	0.1% NH_4OH in MeOH
Flow rate:	0.40 mL/min
Gradient:	10% B initial, linear gradient to 90% B in 4 minutes, hold for 5 minutes, back to 10% B for 5.1 minutes. Hold and re-equilibrate for 7 minutes.

HPLC CONDITIONS WITH XP COLUMN FOR UV

System:	ACQUITY UPLC H-Class
Detection:	Photodiode Array (PDA)
Column:	XBridge® C_{18} ***XP*** Column, 2.5 µm, 4.6 x 100 mm
Injection volume:	50 µL
Column temp.:	40 °C
Mobile phase A:	20 mM Potassium phosphate in water (pH 6.8)
Mobile phase B:	Acetonitrile
Flow rate:	1.3 mL/min
Gradient:	25% B initial and hold for 3.4 minutes, then linear gradient to 65% B for 9.7 minutes, hold for 11.0 minutes, then linear gradient to 95% B for 11.7 minutes, hold for 13.0 minutes, then linear back to 25% B for 13.2 minutes. Hold and re-equilibrate for 18.3 minutes.



Structures of conazole fungicides.

EXPERIMENTAL

Sample description

The fungicides studied included the following: carbendazim, thiabendazole, imazalil, fenbuconazole, and difenoconazole. Structures for these analytes are shown in Figure 1. These compounds are bases that can be retained on Oasis® MCX mixed-mode cation-exchange sorbent for SPE.

Sample preparation

Initial extraction using QuEChERS Place 15-mL orange juice sample into a 50-mL centrifuge tube. Add 15 mL of 1% acetic acid in acetonitrile (ACN), and shake the tube for 1 minute. Add contents of DisQuE™ Pouch for AOAC QuEChERS method, and shake vigorously for 1 minute. Then centrifuge for 5 minutes at 3000 rpm. For UPLC/MS/MS analysis without SPE, dilute 0.5 mL of supernatant to 1.0 mL with water. The protocol for the initial QuEChERS extraction is shown in Figure 2.

SPE cleanup

SPE with the Oasis MCX Cartridge provides cleanup for basic compounds, such as conazole fungicides. For LC/UV analysis (or if cleanup is desired for UPLC/MS/MS analysis), take a 2-mL aliquot of supernatant from QuEChERS extract, add 6 mL 0.01 M aqueous HCl, and mix well. Proceed to the SPE cleanup protocol using a 3-cc Oasis MCX Cartridge, shown in Figure 3. The QuEChERS extract is diluted with aqueous acid to enhance mixed-mode SPE retention, while the aqueous dilution enhances reversed phase retention.

QuEChERS Extraction DisQuE™ Pouch

HOMOGENIZE:
Take 15 g sample
Add 15 mL 1% Acetic Acid in ACN

LIQUID EXTRACTION:
Shake for 1 minute

ADD POUCH CONTENTS
LIQUID/LIQUID PARTITION:
Shake for 1 minute Centrifuge

COLLECT:
Remove aliquot of supernatant for
LC/MS* or for clean-up for LC/UV**

* 0.5 mL diluted 1:1 with water
** 2.0 mL diluted to 8 mL with 0.01N HCl for SPE

Figure 2. QuEChERS extraction using DisQuE product for AOAC method.

Oasis MCX Protocol

CONDITION/EQUILIBRATE:
2 mL methanol/2 mL water

LOAD SAMPLE:
8 mL Diluted DisQuE extract

WASH 1:
1 mL 2% Formic Acid in Methanol

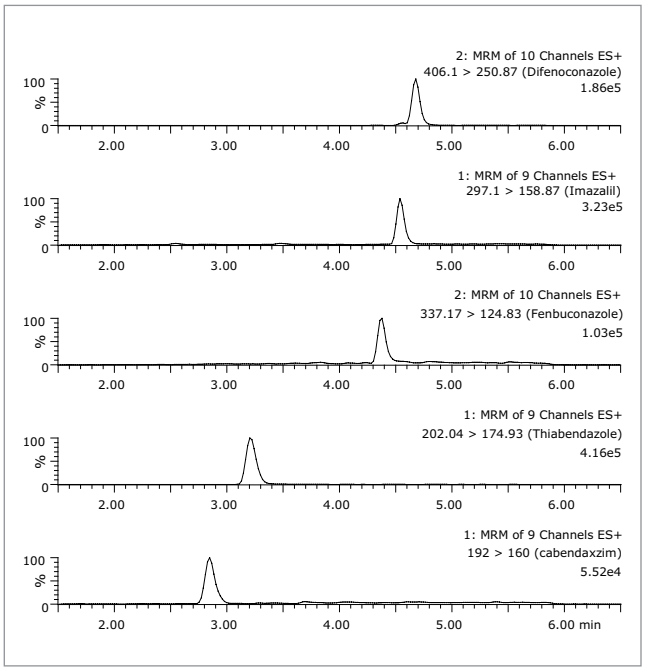
WASH 2:
1 mL Methanol

ELUTE:
2 mL 5% Ammonia in Methanol

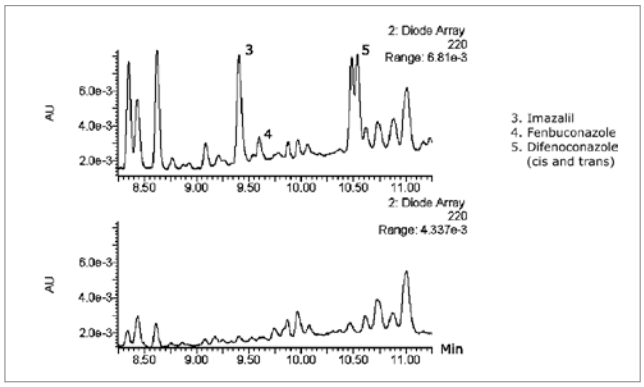
Figure 3. Oasis MCX SPE protocol for this study using a 3-cc cartridge.

Compound Conazole Fungicides	Spike Level (ppb)	% Recovery (%RSD)	% Suppression
Carbendazim	10	98.7 (1.3)	16.1
Thiabendazole	100	94.9 (2.3)	28.9
Imazalil	100	97.3 (0.9)	4.0
Difenoconazole	100	96.4 (0.6)	9.7
Fenbuconazole	100	97.6 (1.6)	8.6
Carbendazim	1	94.5 (4.2)	11.2
Thiabendazole	10	97.8 (4.1)	34.0
Imazalil	10	104 (3.7)	15.2
Difenoconazole	10	92.6 (6.5)	12.1
Fenbuconazole	10	95.2 (4.9)	5.0

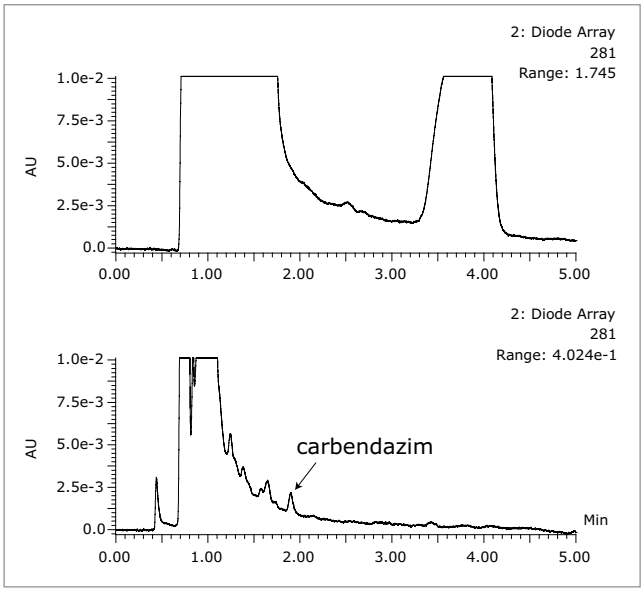
Summary of recovery data for conazole fungicides (n = 4) using the DisQuE Pouch for AOAC QuEChERS. Ion suppression is significantly reduced (<2%), if Oasis MCX cleanup is employed (data not shown).



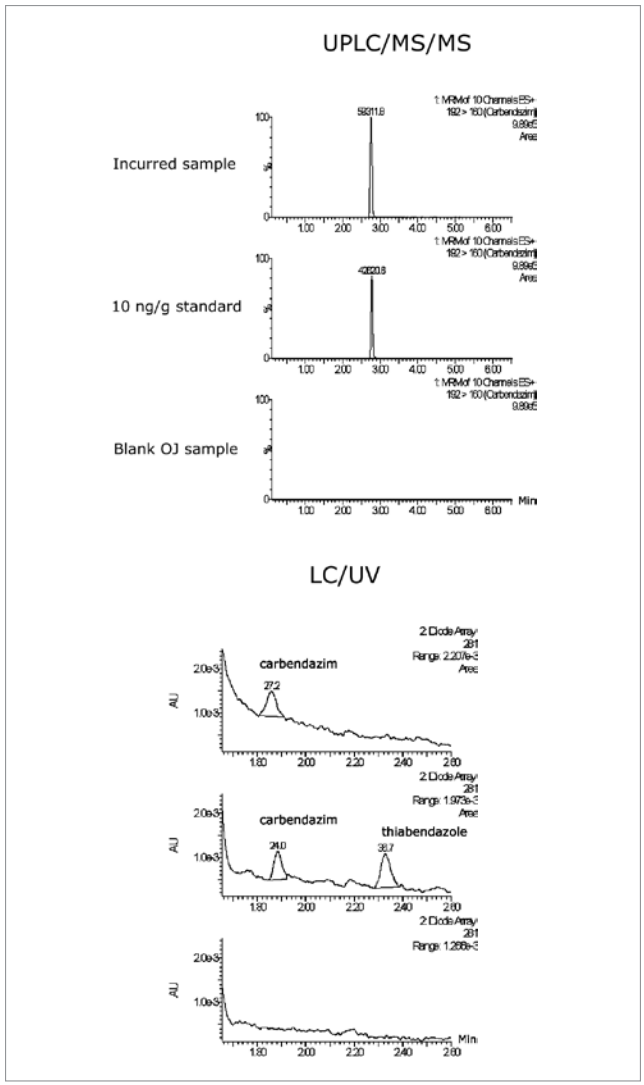
UPLC/MS/MS chromatogram obtained from DisQuE Pouch extract of orange juice spiked at 10 ng/g (ppb) of each fungicide (except 1 ppb carbendazim).



*LC/UV (220 nm) chromatograms obtained on an **XP** Column from a DisQuE Pouch extract of orange juice spiked at 100 ng/g (ppb) after Oasis MCX cleanup.*



LC/UV chromatograms (281 nm) obtained from a DisQuE Pouch extract of an orange juice sample with incurred carbendazim. The top chromatogram was obtained with no SPE cleanup, while the bottom chromatogram was obtained after cleanup with the Oasis MCX Cartridge.



Comparison of UPLC/MS/MS and LC/UV for commercial orange juice with incurred carbendazim.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352
XBridge C_{18} ***XP***, 2.5 µm, 4.6 x 100 mm	186006039
DisQuE Pouch for AOAC QuEChERS method	186006812
Oasis MCX 3-cc Cartridge	186000253

Ref: Waters Application Note 720004457EN

INTRODUCTION

Paraquat and diquat are quaternary ammonium herbicides used in great quantities worldwide for defoliation and weed control. Because these compounds are potentially toxic to wildlife and to humans, groundwater and surface water samples are monitored to assure that residue levels meet safety standards. A prior publication discussed HPLC/MS determination of quats after SPE with Oasis® WCX. The new UPLC® method requires half the analysis time compared with the HPLC method. Also, the SPE has been improved; the earlier protocol utilized trifluoroacetic acid, a toxic perfluorinated compound and persistant organic pollutant. In the new SPE protocol, formic acid is used as an acidic modifier for the SPE eluent.

LC CONDITIONS

System:	ACQUITY UPLC® H-Class
Column:	ACQUITY® BEH HILIC
Mobile phase (Isocratic):	40:60 A/B
Mobile phase A:	150 mM ammonium formate buffer (pH 3.7)
Mobile phase B:	Acetonitrile
Injection volume:	10 µL
Column temp.	30 °C
Weak needle wash:	50:50 methanol/water (600 µL)
Strong needle wash:	50:50 methanol/water (600 µL)
Seal wash:	10:90 acetonitrile/water
Flow rate:	0.50 mL/min

MS CONDITIONS

MS System:	TQD
Ionization mode:	Positive Electrospray
Source temp.:	150 °C
Desolvation temp.:	350 °C
Desolvation gas flow:	800 L/Hr
Cone gas flow:	30 L/Hr
Collision gas flow:	0.20 mL/Min
Data management:	MassLynx® v4.1

Sample Preparation

Note: Polypropylene containers should be used for sample collection and for all sample preparation steps. Polypropylene autosampler vials are recommended for UPLC analysis.

Sample Pretreatment

Transfer a 10 mL sample to an appropriate polypropylene container (15 mL centrifuge tubes were used for this study). For chlorinated samples add 10 mg of sodium thiosulfate and mix well. For all samples, adjust pH by addition of 25 µL of 400 mM pH 7 phosphate buffer.

SPE Enrichment and Cleanup

Perform SPE enrichment and cleanup using Oasis WCX Cartridges (see SPE details in Figure 2). To allow convenient loading of the 10 mL sample, attach a 30 cc polypropylene reservoir to each cartridge.

Oasis WCX, 3 cc 60mg, 30 µm
(part number 186002495)

CONDITION/EQUILIBRATE:
3 mL methanol
3 mL 25 mM pH 7 phosphate buffer

LOAD:
10 mL sample

WASH 1:
700 µL 25 mM ammonium formate
pH 8.0

WASH 2:
700 µL methanol

ELUTE:
2 x 1.0 mL 40:40:20
acetonitrile/isopropanol/formic acid

EVAPORATE/RECONSTITUTE:
500 µL 40:40:20
acetonitrile/IPA/formic acid

Figure 2. Oasis WCX Cartridge Protocol for Diquat/Paraquat Analysis

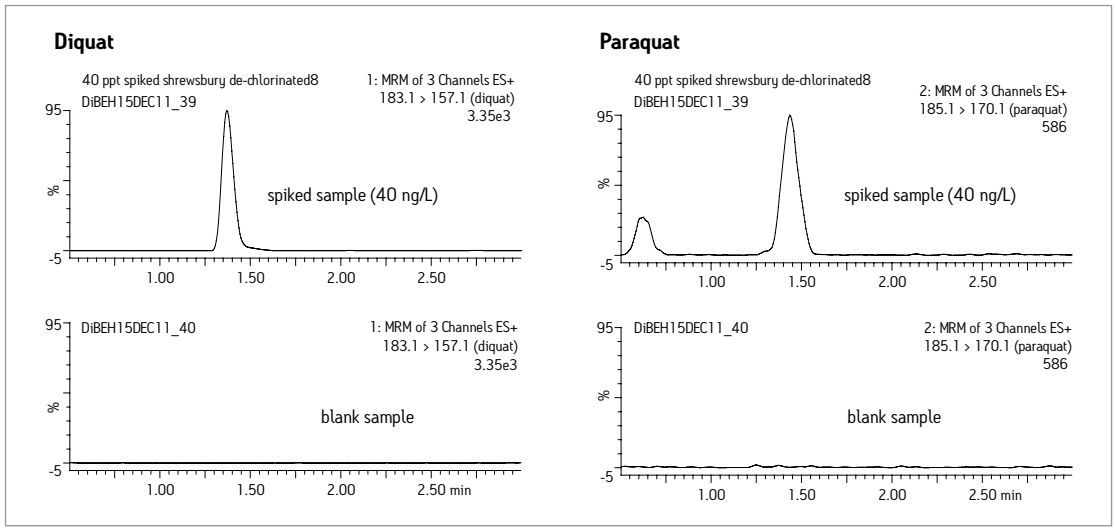


Figure 3. Typical LC/MS/MS Chromatograms.

Diquat/Paraquat Recovery Data (n=7) for Three Types of Water Spiked at 40 ng/L

Sample Type	Recovery (%RSD) Diquat	Recovery (% RSD) Paraquat
Groundwater	82 (8)	89 (16)
Tapwater	84 (5)	88 (8)
River water	83 (4)	89 (19)

Diquat

Correlation Coefficient: r = 0.996129, r^2 = 0.992272
Calibration Curve: 0.000420002 * x + -0.00197468
Response Type: Internal Std (Ref 3), Area* (*IS* Conc./*IS* Area)
Curve Type: Linear, Origin: Include, Weighting: 1/x, Axis trans: None

Paraquat

Correlation Coefficient: r = 0.995100, r^2 = 0.990223
Calibration Curve: 0.000575997 * x + -0.000150743
Response Type: Internal Std (Ref 4), Area* (*IS* Conc./*IS* Area)
Curve Type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

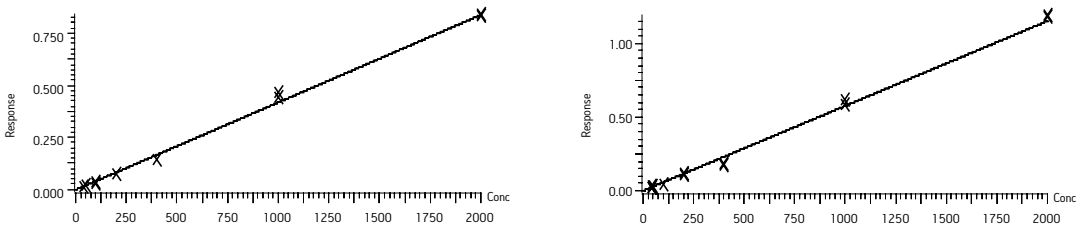


Figure 4. Typical LC/MS/MS Calibration Curves.

ORDERING INFORMATION

Description	Part Number
Oasis WCX, 3 cc/60 mg, 30 µm	186002495
ACQUITY BEH HILIC, 1.7 µm, 2.1 x 100 mm	186003461
Autosampler Vials	186002642
30 cc Polypropylene Reservoir	WAT011390

Ref: Waters Application Note 720004220EN

INTRODUCTION

Malachite Green (MG) is an effective and inexpensive fungicide used in aquaculture. During metabolism MG reduces to Leucomalachite Green (LMG), which has been shown to accumulate in fatty fish tissues. Both MG and LMG have demonstrated putative carcinogenic activity, and thus, have been banned for use in aquaculture by both the United States Food and Drug Administration (US FDA) and European Union (EU).

PRETREATMENT

1. Weigh 1 g sample into a 30 mL centrifuge tube.
2. Add 50 µL TMPD* solution (1 mg/mL).
3. Add standard solutions (MG, LMG, 0.1 µg/mL) and internal standard, leave to stand for 10 minutes.
4. Add 10 mL McIlvaines Buffer (pH 2.6) /methanol (50:50, v/v) solution; homogenize for 45 seconds.
5. Centrifuge at 5000 rpm for 20 minutes, transfer supernatant into centrifuge tube.
6. Repeat steps 4 and 5, combine the two portions of supernatant. A 20 mL aliquot will be used for SPE.

**TMPD= N, N, N', M'- Tetramethyl-1,4-phenylenediamine dihydrochloride*

SPE PROCEDURE

Oasis® MCX 6cc/150 mg

CONDITION/EQUILIBRATE:
A. 5 mL methanol
B. 5 mL water
C. 5 mL McIlvaines Buffer (pH 2.6)

LOAD:
20 mL of sample

WASH:
A. 5 mL 0.1 N hydrochloric acid
B. 2 x 5 mL water
C. 4 mL 50% methanol/water
D. 6 mL hexane, vacuum dry

ELUTE:
10 mL
50% ethyl acetate: 45% methanol: 5% ammonium hydroxide (v/v/v)

Dry eluant at 50 °C under nitrogen

Reconstitute with 50% acetonitrile (100 µL)

SOLUTIONS

McIlvaines Buffer (pH 2.6):

1. 0.1 M citric acid monohydrate (A) - Dissolve citric acid monohydrate (10.5 g) in water (500 mL).
2. 0.2 M disodium hydrogen phosphate dihydrate (B) - Dissolve disodium hydrogen phosphate dihydrate (14.2 g) in water (500 mL).
3. Mix A (445.5 mL) and B (54.5 mL).

McIlvaines Buffer (pH 2.6): methanol (50:50 v/v):

Mix McIlvaines Buffer (pH 2.6) (250 mL) with methanol (250 mL).

LC CONDITIONS

System:	Alliance® HPLC 2695
Column:	SunFire® C_{18}, 5 µm, 4.6 x 150 mm Self-packed PbO_2 oxidising column: 4.6 x 20 mm, (PbO_2 : diatomaceous earth = 3:1); Both ends are fitted with 2 µm stainless steel frits. This column is connected between the analytical columns and the detector.
Flow rate:	2 mL/min
Mobile phase A:	125 mM ammonium acetate, adjust to pH 4.5 with formic acid
Mobile phase B:	acetonitrile
Gradient:	Time (min) A% B% 0.00 45 55 7.00 10 90 10.00 10 90
Injection volume:	50 µL
Detector:	2487 Dual Wavelength UV Detector
UV wavelength:	619 nm

ORDERING INFORMATION

Description	Part Number
Oasis MCX, 6 cc/150 mg, 30 µm, 30/box	186000256
SunFire C_{18}, 5 µm, 4.6 x 150 mm	18600255
Qsert™ Vials	186001126

Ref: Jointly developed by Waters China applications team and Beijing CIQ

INTRODUCTION

Malachite Green (MG) is an effective and inexpensive fungicide used in aquaculture. During metabolism MG reduces to Leucomalachite Green (LMG), which has been shown to accumlate in fatty fish tissues. Both MG and LMG have demonstrated putative carcinogenic activity, and thus, have been banned for use in aquaculture by both the United States Food and Drug Administration (US FDA) and European Union (EU).

PRETREATMENT

1. Weigh 1 g sample into a 30 mL centrifuge tube.
2. Add 50 µL TMPD* solution (1 mg/mL).
3. Add standard solutions MG, LMG, 0.1 µg/mL and internal standard, leave to stand for 10 minutes.
4. Add 10 mL McIlvaines Buffer (pH 2.6) /methanol (50:50 v/v) solution; homogenize for 45 seconds.
5. Centrifuge at 5000 rpm for 20 minutes, transfer supernatant into centrifuge tube.
6. Repeat steps 4 and 5, combine the two portions of supernatant. A 20 mL aliquot will be used for SPE.

**TMPD= N, N, N', M'- Tetramethyl-1,4-phenylenediamine dihydrochloride*

SPE PROCEDURE

Oasis® MCX, 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 2 mL methanol
B. 2 mL water
C. 2 mL McIlvaines Buffer (pH 2.6)

LOAD:
20 mL of sample

WASH:
A. 2 mL 0.1N HCl
B. 2 x 2.5 mL water
C. 2 mL 50% methanol/water
D. 3 mL hexane, vacuum dry

ELUTE:
5 mL
50% ethyl acetate:45% methanol:5% ammonium hydroxide (v/v/v)

Dry eluant at 50 °C under nitrogen

Reconstitute with 50% acetonitrile (100 µL)

SOLUTIONS

McIlvaines Buffer (pH 2.6):

1. 0.1 M citric acid monohydrate (A) - Dissolve citric acid monohydrate (10.5 g) in water (500 mL).
2. 0.2 M disodium hydrogen phosphate dihydrate (B) - Dissolve disodium hydrogen phosphate dihydrate (14.2 g) in water (500 mL).
3. Mix A (445.5 mL) and B (54.5 mL).

McIlvaines Buffer (pH 2.6): methanol (50:50 v/v):

Mix McIlvaines Buffer (pH 2.6) (250 mL) with methanol (250 mL).

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Flow rate:	0.4 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile

Gradient:

Time (min)	A%	B%
0.00	40	60
3.00	5	95
5.00	40	60

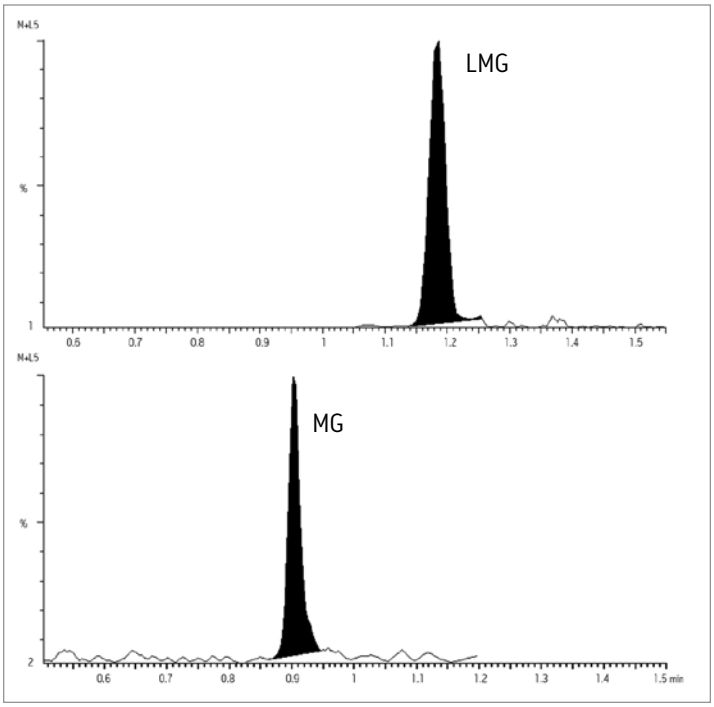
MS CONDITIONS

MS System:	Waters Quattro Premier™
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

Analyte	MRM Transition
LMG	331.2→239.1
	331.2→316.2
MG	329.2→208.1
	329.2→313.1

MRM method parameters.

RESULTS



The LOD of LMG and MG are 0.02 ppb and 0.01 ppb, respectively. The recoveries of LMG and MG in fish is between 50-80%.

ORDERING INFORMATION

Description	Part Number
Oasis MCX, 3 cc/60 mg, 30 µm, 100/box	186000254
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm	186002350
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm, 3/pk	176000863
Qsert™ Vials	186001126

INTRODUCTION

Responding to recent worldwide concern related to melamine in food, the United States Food and Drug Administration (US FDA) issued an interim method for the determination of residual melamine and cyanuric acid in foods using LC/MS/MS.

PRETREATMENT

1. Weigh 5 g of liquid infant formula, or 1 g of dry infant formula, and add 4 mL of water.
2. Add 500 ng (500 µL of 1 µg/mL stock) of isotopically-labeled melamine.
3. Add 2500 ng (250 µL of 10 µg/mL stock) of isotopically-labeled cyanuric acid.
4. Add 20 mL of 50:50 acetonitrile:water.
5. Shake 10-20 minutes.
6. Centrifuge for 10 minutes at 3400 rpm.

SPE PROCEDURE

Melamine SPE Cleanup
Oasis® MCX, 6 cc/150 mg

CONDITION:
A. 5 mL 0.1 M NaOH in acetonitrile
B. 5 mL 0.1M HCl in acetonitrile

EQUILIBRATE:
A. 5 mL acetonitrile
B. 5 mL 4% formic acid in water

LOAD:
A. 3 mL of 4% formic acid in water
B. Add 2 mL of sample supernatant

WASH:
A. 5 mL acetonitrile
B. 5 mL 0.2% diethylamine in acetonitrile

ELUTE:
4 mL 2% diethylamine in acetonitrile

Filter eluent into vials using 0.2 µm PTFE syringe filters and syringes

Cyanuric Acid SPE Cleanup
Oasis MAX Cartridges, 6 cc/150 mg

CONDITION:
A. 5 mL 0.1 M HCl in acetonitrile
B. 5 mL 0.1 M NaOH in acetonitrile

EQUILIBRATE:
A. 5 mL acetonitrile
B. 5 mL 5% NH_4OH in water

LOAD:
A. 3 mL 5% NH_4OH in water
B. Add 2 mL of sample supernatant

WASH:
5 mL acetonitrile

ELUTE:
2 mL 4% formic acid in acetonitrile

Filter eluent into vial using 0.2 µm PTFE syringe filters and syringes

DILUTE:
Cyanuric acid calibration standards accordingly

LC CONDITIONS

System:	ACQUITY UPLC®				
Column:	Atlantis® HILIC Silica, 3 µm, 2.1 x 150 mm				
Mobile phase A:	10 mM Ammonium acetate in 50/50 Acetonitrile/H_2O				
Mobile phase B:	10 mM Ammonium acetate in 95/5 Acetonitrile/H_2O				
Gradient:	Time (min)	Flow Rate (mL/min)	%A	%B	Curve
	Initial	0.5	0	100	-
	2.00	0.5	0	100	6
	3.50	0.5	60	40	6
	5.00	0.5	60	40	6
	5.20	0.8	0	100	6
	11.00	0.8	0	100	6
	11.10	0.5	0	100	6
	14.00	0.5	0	100	6
Injection volume:	20 µL				

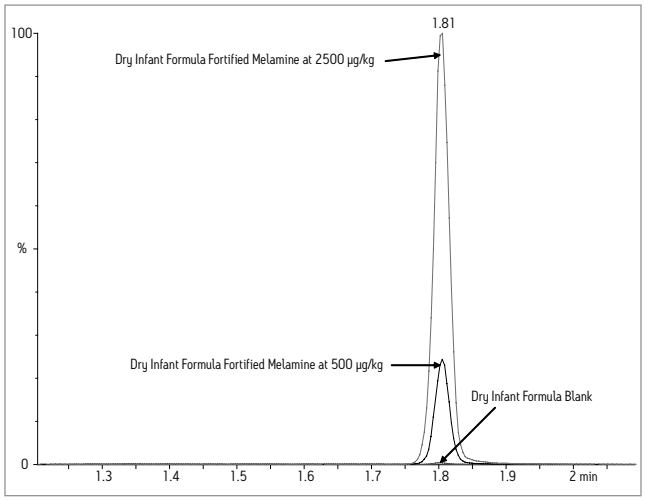
MS CONDITIONS

MS System: ACQUITY® TQD
Software: MassLynx® v.4.1
Ionization mode: ESI Positive (melamine and $^{13}C_3{}^{15}N_3$ melamine) ESI Negative (cyanuric acid and $^{13}C_3{}^{15}N_3$ cyanuric acid)

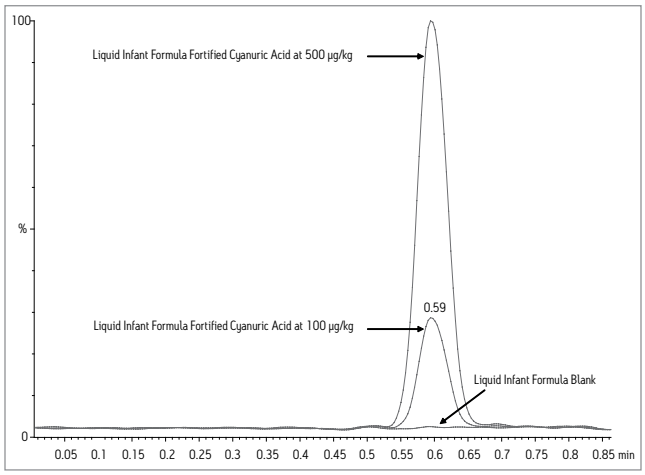
Compound	Ionization	MRM Transitions	Cone Voltage(V)	Collision Energy (eV)
Melamine	Positive	127→85	40	17
		127→68	40	25
$^{13}C_3{}^{15}N_3$ Melamine	Positive	133→89	40	17
		133→45	40	26
Cyanuric Acid	Negative	128→42	30	13
		128→85	30	11
$^{13}C_3{}^{15}N_3$ Cyanuric Acid	Negative	134→44	30	13
		134→89	30	11

MRM Conditions for Melamine, $^{13}C_3{}^{15}N_3$ Melamine, Cyanuric Acid, and $^{13}C_3{}^{15}N_3$ Cyanuric Acid.

RESULTS



Melamine in Dry Infant Formula at 500 ppb and 2500 ppb using Atlantis HILIC Silica Column.



Cyanuric Acid in Liquid Infant Formula at 100 ppb and 500 ppb using Atlantis HILIC Silica Column.

Spiking Concentration	Type	Single Day Results Average Spike % Recovery ± % RSD (n)	Multi-Day Results Average Spike % Recovery ± % RSD (n)
500 µg/kg	Dry	113.3 ± 8.8 (n = 5)	109.2.0 ± 7.7 (n = 11)
2500 µg/kg	Dry	108.5 ± 5.5 (n = 5)	-
10 µg/kg	Liquid	110.2 ± 13.2 (n = 5)	104.7 ± 8.2 (n = 8)
100 µg/kg	Liquid	113.8 ± 12.7 (n = 5)	116.4 ± 10.6 (n = 8)

Melamine Spike % Recovery in Dry and Liquid Infant Formula using Atlantis HILIC Silica Column.

Spiking Concentration	Type	Single Day Results Average Spike % Recovery ± % RSD (n)	Multi-Day Results Average Spike % Recovery ± % RSD (n)
500 µg/kg	Dry	115.7 ± 1.8 (n = 5)	113.9 ± 2.7 (n = 8)
2500 µg/kg	Dry	104.7 ± 3.9 (n = 5)	104.7 ± 3.1 (n = 8)
100 µg/kg	Liquid	109.2 ± 2.9 (n = 5)	108.4 ± 3.1 (n = 8)
500 µg/kg	Liquid	103.9 ± 2.1 (n = 5)	103.4 ± 3.3 (n = 8)

MRM method parameters.

ORDERING INFORMATION

Description	Part Number
HPLC Melamine Analysis Package	176001773
Oasis MCX, 6 cc/150 mg, 100/box	186000255
Oasis MAX, 6 cc/150 mg, 100/box	186000370
Atlantis HILIC Silica, 3 µm, 2.1 x 150 mm	186002015
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720002862EN

INTRODUCTION

Responding to recent worldwide concern related to melamine in food, the United States Food and Drug Administration (US FDA) issued an interim method for the determination of residual melamine and cyanuric acid in foods using LC/MS/MS.

PRETREATMENT

1. Weigh 5 g of liquid infant formula, or 1 g of dry infant formula, and add 4 mL of water.
2. Add 500 ng (500 µL of 1 µg/mL stock) of isotopically-labelled melamine.
3. Add 2500 ng (250 µL of 10 µg/mL stock) of isotopically-labelled cyanuric acid.
4. Add 20 mL of 50:50 acetonitrile:water.
5. Shake 10-20 minutes.
6. Centrifuge for 10 minutes at 3400 rpm.

SPE PROCEDURE

Melamine SPE Cleanup
Oasis® MCX, 6 cc/150 mg

CONDITION:
A. 5 mL 0.1 M NaOH in acetonitrile
B. 5 mL 0.1 M HCl in acetonitrile

EQUILIBRATE:
A. 5 mL acetonitrile
B. 5 mL 4% formic acid in water

LOAD:
A. 3 mL of 4% formic acid in water
B. Add 2 mL of sample supernatant

WASH:
A. 5 mL acetonitrile
B. 5 mL 0.2% diethylamine in acetonitrile

ELUTE:
4 mL 2% diethylamine in acetonitrile

Filter eluent into vials using 0.2 µm PTFE syringe filters and syringes

Cyanuric Acid SPE Cleanup
Oasis MAX Cartridges, 6 cc/150 mg

CONDITION:
A. 5 mL 0.1 M HCl in acetonitrile
B. 5 mL 0.1 M NaOH in acetonitrile

EQUILIBRATE:
A. 5 mL acetonitrile
B. 5 mL 5% NH_4OH in water

LOAD:
3 mL 5% NH_4OH in water
Add 2 mL of sample supernatant

WASH:
5 mL acetonitrile

ELUTE:
2 mL 4% formic acid in acetonitrile

Filter eluent into vial using 0.2 µm PTFE syringe filters and syringes

DILUTE:
Cyanuric acid calibration standards accordingly

LC CONDITIONS

System: ACQUITY UPLC®
Column: ACQUITY UPLC BEH HILIC, 1.7 µm, 2.1 x 100 mm
Mobile phase A: 10 mM Ammonium acetate
Mobile phase B: 10 mM Ammonium acetate in 95/5 Acetonitrile/H_2O
Gradient:

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.6	0	100	-
0.80	0.6	0	100	6
2.30	0.6	22	78	6
2.80	0.6	22	78	6
2.90	0.6	0	100	6
4.00	0.6	0	100	6

Injection volume: 10 µL

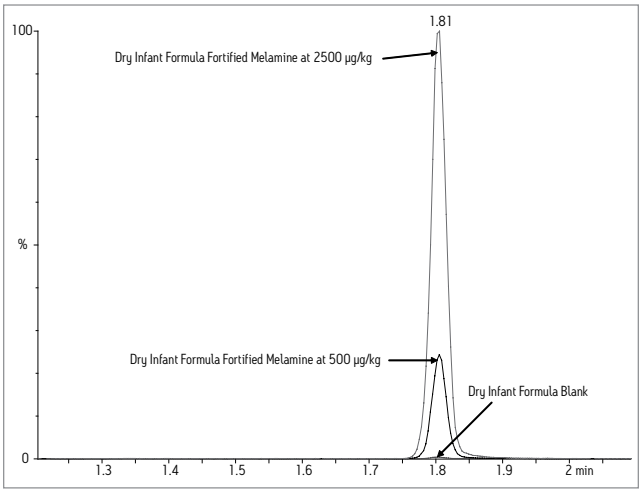
MS CONDITIONS

MS System: ACQUITY® TQD
Software: MassLynx® v.4.1
Ionization mode: ESI Positive (melamine and $^{13}C_3{}^{15}N_3$ melamine) ESI Negative (cyanuric acid and $^{13}C_3{}^{15}N_3$ cyanuric acid)

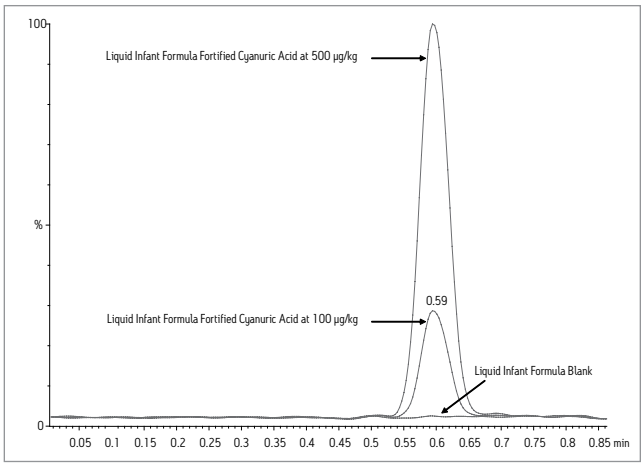
Compound	Ionization	MRM Transitions	Cone Voltage (V)	Collision Energy (eV)
Melamine	Positive	127→85	40	17
		127→68	40	25
$^{13}C_3{}^{15}N_3$ Melamine	Positive	133→89	40	17
		133→45	40	26
Cyanuric Acid	Negative	128→42	30	13
		128→85	30	11
$^{13}C_3{}^{15}N_3$ Cyanuric Acid	Negative	134→44	30	13
		134→89	30	11

MRM method parameters.

RESULTS



Melamine in Dry Infant Formula at 500 ppb and 2500 ppb using Atlantis HILIC Silica Column.



Cyanuric Acid in Liquid Infant Formula at 100 ppb and 500 ppb using Atlantis HILIC Silica Column.

Spiking Concentration	Type	Single Day Results Average Spike % Recovery ± % RSD (n)	Multi-Day Results Average Spike % Recovery ± % RSD (n)
500 µg/kg	Dry	115.0 ± 4.7 (n = 5)	110.7 ± 6.9 (n = 11)
2500 µg/kg	Dry	109.6 ± 3.1 (n = 5)	-
10 µg/kg	Liquid	103.9 ± 10.5 (n = 5)	104.7 ± 8.2 (n = 8)
100 µg/kg	Liquid	105.7 ± 3.2 (n = 5)	105.1 ± 4.5 (n = 8)

Melamine spike % recovery in dry and liquid infant formula using BEH HILIC column.

Spiking Concentration	Type	Single Day Results Average Spike % Recovery ± % RSD (n)	Multi-Day Results Average Spike % Recovery ± % RSD (n)
500 µg/kg	Dry	114.9 ± 3.9 (n = 5)	116.1 ± 4.8 (n = 8)
2500 µg/kg	Dry	109.6 ± 3.1 (n = 5)	104.9 ± 4.8 (n = 8)
100 µg/kg	Liquid	117.7 ± 4.0 (n = 5)	115.0 ± 5.0 (n = 8)
500 µg/kg	Liquid	103.8 ± 5.9 (n = 5)	103.1 ± 2.9 (n = 8)

Cyanuric Acid Spike % Recovery in Dry and Liquid Infant Formula using BEH HILIC Column.

ORDERING INFORMATION

Description	Part Number
UPLC® Melamine Analysis Package	176001791
Oasis MCX, 6 cc/150 mg, 30/box	186000255
Oasis MAX, 6 cc/150 mg, 30/box	186000370
ACQUITY UPLC BEH HILIC, 1.7 µm, 2.1 x 100 mm	186003461
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720002862EN

INTRODUCTION

Responding to recent worldwide concern related to melamine in food, the United States Food and Drug Administration (US FDA) issued an interim method for the determination of residual melamine and cyanuric acid in foods using LC/MS/MS.

SAMPLE EXTRACTION

1. Weigh 1 g infant formula.
2. Add 9 mL of sample extraction buffer into a 50 mL centrifuge tube.
3. Shake or vortex the solution for 1 minute.
4. Centrifuge for 15 minutes at 3500 rpm

Melamine SPE Cleanup
Oasis® MCX, 6 cc/150 mg

CONDITION:
A.5 mL 0.1 M NaOH in acetonitrile
B.5 mL 0.1M HCl in acetonitrile

CONDITION:
A. 5 mL Methanol
B. 5 mL 0.5N HCl

LOAD:
A. 3 mL of 0.5N HCl
B. 2 mL of sample supernatant

WASH:
A. 5 mL water
B. 2 mL acetonitrile

ELUTE:
4 mL 5% ammonium hydroxide in acetonitrile

Cyanuric Acid SPE Cleanup
Sep-Pak® Aminopropyl (NH_2), 6 cc/500 mg

CONDITION:
5 mL 5% diethylamine in acetonitrile

LOAD:
A. 2 mL 5% NH_4OH in water
B. 3 mL of sample supernatant

ELUTE:
5 mL 10:10:80 water:formic acid:acetonitrile

LC CONDITIONS

System: ACQUITY UPLC®
Column: ACQUITY UPLC BEH HILIC, 1.7 µm, 2.1 x 150 mm
Mobile phase A: 10 mM ammonium acetate at pH 3.9 in 50/50 water/acetonitrile
Mobile phase B: 10 mM ammonium acetate at pH 3.9 in 11.5/88.5 water/acetonitrile

Gradient:

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.45	0.0	100.0	-
5.00	0.45	0.0	100.0	6
6.00	0.45	0.0	100.0	11

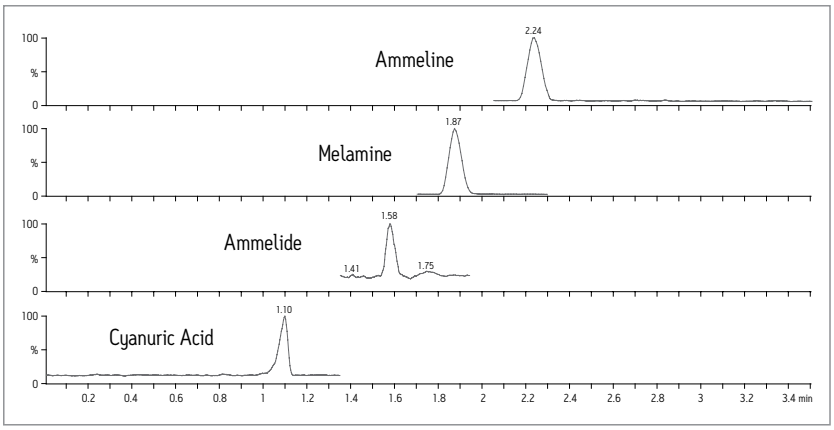
Injection volume: 10 µL

MS CONDITIONS

MS System: ACQUITY® TQD
Ionization mode: ESI Positive (melamine, ammelide and ammeline)
ESI Negative (cyanuric acid)

Compounds	MRM Transitions	Dwell (sec)	Cone Voltage (V)	Collision Energy (eV)
Melamine	127→85	0.07	30	18
	127→68	0.07	30	22
Ammelide	129→87	0.07	30	14
	129→43	0.07	30	18
Ammeline	128→86	0.08	30	15
	128→43	0.08	30	20
Cyanuric Acid	128→42	0.055	25	12
	128→85	0.055	25	6

MRM method parameters.



Melamine, cyanuric acid, ammelide, and ammeline of infant formula fortified at 1.0 µg/g.

Compound	Recovery Average Spike % Recovery ± % RSD (n)	Overall Recovery Average % Overall Recovery (n)
Cyanuric Acid	102.8 ± 12.8 (n = 5)	74.4 (n = 5)
Ammelide	97.4 ± 2.5 (n = 5)	60.2 (n = 5)
Melamine	106.8 ± 2.7 (n = 5)	58.5 (n = 5)
Ammeline	94.9 ± 3.5 (n = 5)	46.2 (n = 5)

Melamine, ammelide, and cyanuric acid spike % recovery in infant formula.

ORDERING INFORMATION

Description	Part Number
Oasis MCX, 6 cc/150 mg	186000256
Sep-Pak Aminopropyl (NH_2), 6 cc/500 mg	WAT054560
ACQUITY UPLC BEH HILIC, 1.7 µm, 2.1 x 150 mm	186003462
LCMS Certified Vials	600000751CV

Ref: Waters Application Note, 720000688EN

INTRODUCTION

Microcystin-LR is a potent mammalian toxin which is known to have been responsible for the deaths of domesticated animals, livestock loss, and the potential presence in potable water supplies.

PRETREATMENT

1. Filter water sample through 0.45 µm membrane filter.
2. Add 100 µL of enkephalin (concentration 10 µg/L) to 10 mL filtered water sample and mix thoroughly.

SPE PROCEDURE

Oasis® HLB, 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 3 mL methanol
B. 6 mL water

LOAD:
10 mL sample (1 mL/min)

WASH:
A. 3 mL water
B. 5 mL 20% methanol

Dry cartridge by vacuum for 1 minute

ELUTE:
5 mL methanol

Evaporate to dryness at 50 °C under nitrogen stream

Reconstitute residue with 1 mL 50% methanol

LC CONDITIONS

System: Alliance® HPLC 2695

Column: Symmetry300™ C_{18}, 3.5 µm, 4.6 x 75 mm

Flow rate: 0.2 mL/min

Mobile phase A: 0.2% formic acid in water

Mobile phase B: 0.2% formic acid in methanol

Gradient:

Time (min)	A%	B%
0.00	45	55
12.00	10	90
12.50	0	100
15.00	0	100
15.10	45	55
25.00	45	55

Injection volume: 10 µL

Column temp.: 30 °C

MS CONDITIONS

MS System: Waters Quattro Ultima Pt™

Ionization mode: Positive electrospray (ESI^+)
Multiple reaction monitoring

Analyte	MRM	MW	$[M+H]^+$	$[M+H]^{2+}$	Characteristic Ion Fragment
Enkephalin	556.1→278.0	555.6	556.1	N.D	278.0
					397.1
MCYST-LR	519.9→135.0	994.5	995.7	498.4	135.0
					861.5
MCYST-RR	498.4→135.0	1037.6	1038.4	519.9	135.0
					620.0
MCYST-LW	1025.8→891.7	1024.5	1025.8	N.D	897.1
					583.2
MCYST-LF	986.8→852.5	985.5	986.8	N.D	852.5
					544.0

MRM method parameters.

RESULTS

Analyte	Concentration (µg/L)	Average Recovery (%)	RSD (%)
MCYST-RR	0.10	100.0	6.45
	0.20	95.2	4.02
	0.40	90.0	4.35
MCYST-LR	0.02	105.0	5.40
	0.05	96.0	4.53
	0.08	93.8	4.22
MCYST-LW	0.40	103.8	5.30
	1.00	102.7	5.87
	1.60	93.8	5.67
MCYST-LF	0.20	103.0	7.03
	0.50	109.8	5.69
	0.80	102.3	4.57

Recovery data for spiked samples at various concentrations.

ORDERING INFORMATION

Description	Part Number
Oasis HLB, 3 cc/60 mg, 30 µm, 100/box	WAT094226
Symmetry300 C_{18}, 3.5 µm, 4.6 x 75 mm	186000189
Nylon Filter 0.45 µm	WAT200524
LCMS Certified Vials	600000751CV

Ref: Determination of Microcystins in Natural Water by Liquid Chromatography Tandem Mass Spectrometry, Chen Qi, Huang Baifen, Zhang Jing, Ren Yiping; Zhejiang Provincial Center for Disease Prevention and Control

INTRODUCTION

This application brief shows the step wise procedure of the Japan Ministry of Health, Labor and Welfare (JPMHLW) Official Method for multi-residue analysis of pesticides for grain and bean samples.

PRETREATMENT

1. Extract 10 g sample with 20 mL of water. Add 50 mL of acetonitrile.
2. Homogenize sample. Centrifuge and collect supernatant.
3. Repeat extraction with 20 mL of acetonitrile.
4. Collect acetonitrile layer and dilute to 100 mL with acetonitrile.
5. Sample 20 mL of diluted acetonitrile extract, add 10 g sodium chloride and 20 mL of 0.5 M of phosphate buffer. Shake for 10 minutes.
6. Collect acetonitrile layer for SPE treatment.

SPE PROCEDURE

Sep-Pak® Vac C_{18}, 1 g/6 cc

CONDITION:
10 mL of acetonitrile

Load the sample extract

ELUTE:
2 mL of acetonitrile

Collect eluate

Dry over anhydrous sodium sulfate

Filter

Evaporate to dryness

Dissolve the residue with 2 mL of toluene:acetonitrile (1:3, v/v)

Sep-Pak Vac Carbon Black/Aminopropyl, 6 cc/500 mg/500 mg

CONDITION:
10 mL of toluene:acetonitrile (1:3, v/v)

Load the sample extract

ELUTE:
20 mL of toluene:acetonitrile (1:3, v/v)

Collect eluate

Evaporate to dryness

Dissolve the residue with 1 mL of acetone:n-hexane (1:1, v/v)

ORDERING INFORMATION

Description	Part Number
Sep-Pak Vac C_{18}, 1 g/6 cc	WAT036905
Sep-Pak Vac Carbon Black/Aminopropyl, 6 cc/500 mg/500 mg	186003369
LCMS Certified Vials	600000751CV

INTRODUCTION

This application brief shows the Japan Ministry of Health, Labor and Welfare (JPMHLW) Method for multi-residue analysis of pesticides in vegetables and fruit. This sample preparation method calls for an extract from the commodity, followed by a SPE extract from a Sep-Pak Vac Carbon Black/Aminopropyl column.

SPE PROCEDURE

Sep-Pak® Vac Carbon Black/Aminopropyl, 6 cc/500 mg/500 mg

CONDITION:
Toluene/acetonitrile 10 mL (1:3 v/v). Do not allow to dry

LOAD:
Rinse vials with toluene/acetonitrile (1:3 v/v) solution and load 2 mL of extracted sample

ELUTE :
20 mL toluene/acetonitrile (1:3 v/v). Flow: 2 mL/min

LC CONDITIONS

System:	Alliance® HPLC 2695
Column:	XTerra® MS C_{18}, 3.5 µm, 2.1 x 150 mm
Flow rate:	0.2 mL/min
Mobile phase A:	water
Mobile phase B:	methanol
Mobile phase C:	100 mM ammonium acetate in water

Gradient:

Time (min)	A%	B%	C%
0.00	80	15	5
1.00	55	40	5
3.50	55	40	5
6.00	45	50	5
8.00	40	55	5
17.50	00	95	5
30.00	80	15	5
47.00	80	15	5

Injection volume:	5 µL
Column temp.:	40 °C

MS CONDITIONS

MS System:	Waters Quattro Premier™ XE
Ionization mode:	Positive electrospray (ESI+) Multiple reaction monitoring

Pesticides*	Spike Conc./µg/g	Recovery (%)
Abamectin	0.1	102.0
Anibfos	0.1	111.7
Azinphos-methyl	0.1	107.6
Benzofenap	0.1	139.5
Butafenacil	0.1	104.5
Chloridazon	0.1	106.0
Chromafenozide	0.1	108.2
Clomeprop	0.1	104.4
Cloquintocet-mexyl	0.1	108.7
Clothianidin	0.1	101.5
Cyazofamid	0.1	108.3
Cyflufenamid	0.1	110.1
Dimethirimol	0.1	101.0
Fenoxycarb	0.1	108.7
Ferimzone	0.1	112.6
Formetanate hydrochloride	0.1	86.7
Furathiocarb	0.1	100.5
Imidacloprid	0.1	111.8
Indoxacarb	0.1	121.2
Iprovalicarb	0.1	106.2
Isoxaflutole	0.1	99.5
Lactofen	0.1	106.8
Methoxyfenozide	0.1	103.3
Mibemectin A3	0.1	114.5
Mibemectin A4	0.1	101.2
Naproanilide	0.1	115.9
Oryzalin	0.1	103.8
Oxycarboxin	0.1	85.1
Oxydemeton-methyl	0.1	108.0
Phenmedipham	0.1	102.2
Pyrazolynate	0.1	72.7
Quizalofop-P-tefuryl	0.1	145.3
Simeconazole	0.1	106.0
Thiacloprid	0.1	109.2
Thiamethoxam	0.1	108.3
Tridemorph	0.1	94.6
Etriticonazole	0.1	113.3

**Five replicate samples analyzed per level.*

ORDERING INFORMATION

Description	Part Number
Sep-Pak Vac Carbon Black/Aminopropyl, 6 cc/500 mg/500 mg	186003369
XTerra MS C_{18}, 3.5 µm, 2.1 x 150 mm	186000506
LCGC Certified Vials	186000272C

INTRODUCTION

This multi-residue pesticide sample preparation shows the steps used to process fruit and vegetable samples, extract and concentrate the extract by an Oasis® HLB SPE method.

PRETREATMENT

1. Samples (lemon, raisin, tomato and avocado) were cut into small pieces.
2. A 20 g portion of homogenized sample was mixed with 60 mL 0.1% formic acid in methanol:water (80:20, v/v).
3. Extraction for 2 minutes with Ultra-Turrax at 8000 rpm.
4. Filtration and dilution with methanol:water 0.1% formic acid (80:20, v/v) to a final volume of 100 mL.
5. 2.5 mL aliquot diluted to 20 ml with 0.1% formic acid in water. Load 5 mL of the diluted extract onto the SPE Cartridge.

SPE PROCEDURE

Oasis HLB, 6cc/200mg

CONDITION:
A. 5 mL methanol
B. 5 mL methanol: MTBE* (10:90) 0.1% formic acid
C. 5 mL methanol 0.1% formic acid

EQUILIBRATE:
5 mL water 0.1% formic acid

LOAD:
5 mL diluted extract

DRY:
By passing air for through cartridge 1 hour

ELUTE:
5 mL methanol:MTBE (10:90, v/v) 0.1% formic acid, by means of gravity

SAMPLE POST-TREATMENT:
0.5 mL water added to the extract. Evaporate with nitrogen at 40 °C until 0.5 mL. Adjust final volume to 1 mL with methanol:water (10:90, v/v).

**MBTE: methyl-t-buthyl ether*

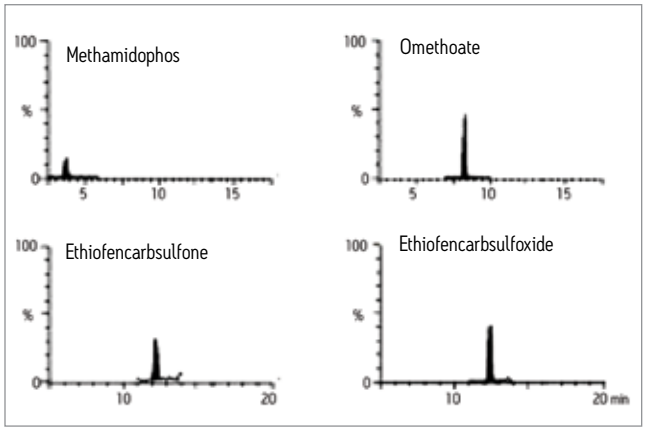
LC CONDITIONS

System:	Alliance® HPLC 2695		
Column:	Atlantis® dC_{18}, 5 µm, 2.1 x 100 mm		
Flow rate:	0.2 mL/min		
Mobile phase A:	0.01% formic acid in water		
Mobile phase B:	0.01% formic acid in methanol		
Gradient:	Time (min)	A%	B%
	0.00	95	5
	1.00	95	5
	8.50	50	50
	25.00	10	90
	28.00	10	90
	29.00	95	5
Injection volume:	20 µL		

MS CONDITIONS

MS System:	Waters Quattro micro™
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

RESULTS



LC/MS/MS data for 4 representative pesticides.

ORDERING INFORMATION

Description	Part Number
Oasis HLB, 6 cc/200 mg, 30/box	WAT106202
Atlantis dC_{18}, 5 µm, 2.1 x 100 mm	186001297
LCMS Certified Combination Packs	600000751CV

Ref: Journal of Chromatography A, 1109 (2006) 242-252

INTRODUCTION

Avocado is a fruit that contains fat; therefore, the recommended selection of the dispersive solid-phase extraction (SPE) tube should include C_{18} to remove the fats.

EXTRACTION PROCEDURE

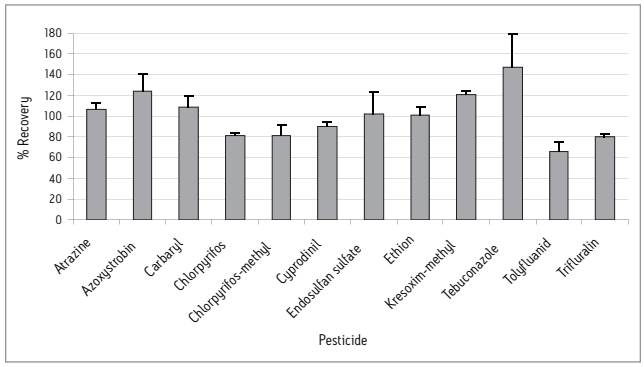
1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ Extraction Tube containing 1.5 g of sodium acetate and 6 g of magnesium sulfate.
2. Add 15 g of homogenized sample into the 50 mL tube.
3. Add any internal standards and standard mixture.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL dSPE clean-up tube containing 50 mg PSA, 150 mg $MgSO_4$, and 50 mg C_{18}.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 0.5 mL extract into test tube.
8. Add any post-extraction internal standards.
9. Add 0.25 mL toluene.
10. Evaporate at 50 °C with N_2 to < 0.1 mL.
11. Bring volume up to 0.2 mL with toluene.
12. Transfer to vial with insert for analysis.

GC CONDITIONS

GC System:	Agilent® 6890N
Column:	RTX-5MS, 30 x 0.25 mm, (0.25 µm film)
Carrier gas:	Helium
Flow rate:	1.0 mL/min
Temp. program:	Initial 100 °C, hold 1 minute, then 10 °C/min to 320 °C, hold for 7 minutes
Injection volume:	2 µL splitless

MS CONDITIONS

MS System:	Waters Quattro micro™ GC-MS
Ionization:	Electron Impact (70 eV)
Acquisition:	Single Ion Recording (SIR) Mode



Pesticides in Avocados by GC/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE 50 mL Tube-AOAC/Acetate	186004571
DisQuE 2 mL Tube-AOAC/C_{18}	186004830
LCGC Certified Vials	186000272C
Insert 300 µL with Poly Spring	WAT094170

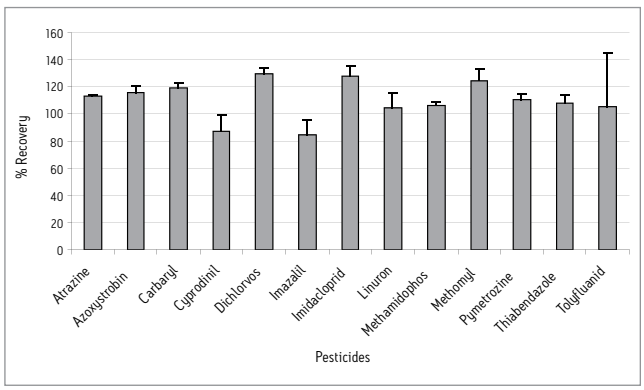
***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Avocado is a fruit that contains fat; therefore, the recommended selection of the dispersive solid-phase extraction (SPE) tube should include C_{18} to remove the fats.

EXTRACTION PROCEDURE

1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ Extraction Tube.
2. Add 15 g of homogenized sample into the 50 mL tube.
3. Add any internal standards and standard mixture.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL clean-up tube containing 50 mg PSA, 150 mg $MgSO_4$, and 50 mg C_{18}.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 100 µL of final extract into a 1.5 mL centrifuge tube.
8. Add any post-extraction internal standards.
9. Dilute as needed with an appropriate buffer or solvent.
10. Centrifuge > 16000 rcf for 5 minutes.
11. Transfer to autosampler vial.



Pesticides in Avocados by UPLC®/MS/MS

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Column temp.:	40 °C
Sample temp.:	4 °C
Flow rate:	0.3 mL/min
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Methanol + 0.1% formic acid

Gradient:

Time (min)	Flow Rate	A%	B%
0.00	0.3	75	25
0.25	0.3	75	25
7.75	0.3	5	100
8.50	0.3	0	100
8.51	0.5	75	25
10.50	0.5	75	25
11.0	0.3	75	25

Injection volume: 15 µL, Partial loop injection

MS CONDITIONS

MS System:	ACQUITY® TQ Detector
Ionization mode:	Positive electrospray (ESI^+)
Acquisition:	Multiple reaction monitoring (MRM)

ORDERING INFORMATION*

Description	Part Number
DisQuE 50 mL Tube-AOAC/Acetate	186004571
DisQuE 2 mL Tube-AOAC/C_{18}	186004830
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352
LCMS Certified Vials	600000749CV

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Avocado is a fruit that contains fat; therefore, the recommended selection of the dispersive SPE tube should include C_{18} to remove the fats.

EXTRACTION PROCEDURE

1. Add 15 g of homogenized sample to the 50 mL DisQuE™ extraction tube containing 1.5 g of sodium acetate and 6 g of magnesium sulphate.
2. Add 15 mL of 1% acetic acid in acetonitrile.
3. Add any pre-extraction internal standards.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 1 minute.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE extraction tube containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 250 µL of final extract into an autosampler vial.
8. Add any post-extraction internal standards.
9. Dilute as needed with an appropriate buffer or solvent.

GC CONDITIONS

GC System:	Agilent® 6890N
Column:	Restek® Rtx-5MS, 30 x 0.25 mm i.d., 0.25 µm df
Carrier gas:	Helium
Temp. program: to	Initial temp at 80 °C, hold for 1 minute, 10 °C/min 320 °C, hold for 5 minutes
Flow rate:	1.0 mL/min
Injection volume:	Split/splitless mode with 0.5 minute purge time and 1 µL injection

MS CONDITIONS

MS System:	Waters Quattro micro™ GC
Ionization mode:	Positive electrospray (70 eV)
	Selected-Ion Recording (SIR)

GC/MS (SIR)

Channel	Mass	Mass
Phenylphenol	170	141
Atrazine	200	173
Chlorpyrifos methyl	286	109
DDD	235	165
Ethion	231	153
Cylohalothrin	197	181

SIE method parameters.

	Chlorpyrifos Methyl	Phenylphenol	DDD	Ethion	Cyclohalothrin	Thiabendazole
%Recovery (± %RSD) in Grape	97.07 % (± 4.98%)	105.19% (± 10.31%)	109.42% (± 3.01%)	103.19% (± 2.10%)	102.27% (± 7.33%)	103.58% (± 3.99%)
%Recovery (± %RSD) in Avocado	107.45% (± 1.93%)	109.64% (± 4.45%)	96.85% (± 3.04%)	99.65% (± 9.03%)	95.62% (± 12.56%)	101.46% (± 3.86%)

GC separation of five basic/neutral pesticides. Compounds (1) phenylphenol, (2) atrazine (IS), (3) chloropyrifos methyl, (4) DDD, (5) ethion, (6) cyclohalothrin.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
LCGC Certified Vials	186000272C

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

Ref: Waters Application Note 720002755EN

INTRODUCTION

The application brief uses QuEChERS extraction procedure plus UPLC®/MS/MS to analyze pesticides in fruit- and meat-based baby food extracts. This method exceeds both current European and worldwide legislation requirements.

EXTRACTION PROCEDURE

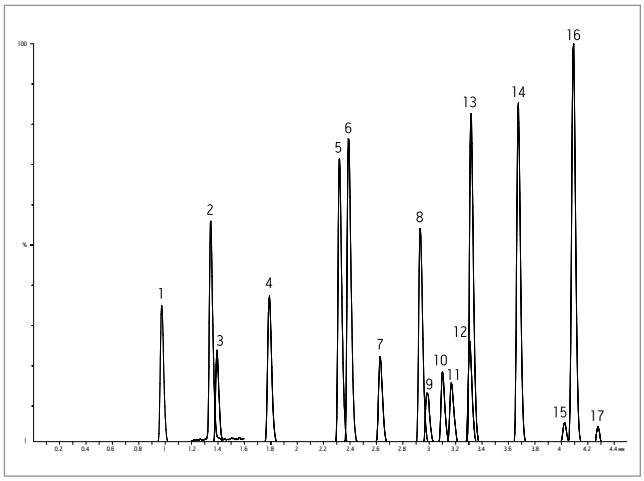
1. Add 15 g of homogenized sample to the 50 mL DisQuE™ extraction tube containing 1.5 g of sodium acetate and 6 g of magnesium sulphate.
2. Add 15 mL of 1% acetic acid in acetonitrile.
3. Add any pre-extraction internal standards.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 1 minute.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE extraction tube containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 250 µL of final extract into an autosampler vial.
8. Add any post-extraction internal standards.
9. Dilute as needed with an appropriate buffer or solvent.

LC CONDITIONS

System:	ACQUITY UPLC®		
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm		
Column temp.:	40 °C		
Sample temp.:	4 °C		
Flow rate:	0.7 mL/min		
Mobile phase A:	Water + 0.1% formic acid		
Mobile phase B:	Methanol + 0.1% formic acid		
Gradient:	Time (min)	A%	B%
	0.00	99	1
	5.00	1	99
	6.00	1	99
	6.10	99	1
	8.00	99	1
Total run time:	8 minutes		
Injection volume:	50 µL, full loop injection		

MS CONDITIONS

MS System:	Xevo® TQ MS
Ionization mode:	Positive electrospray (ESI^+)
	Multiple reaction monitoring



17 pesticide residues in one injection at 1 ng/mL in water.

Peak	Pesticide	RT	MRM Transitions	Dwell Time (s)	Cone Voltage (V)	Collision Energy (eV)
1	Omethoate	0.97	214→183 214→155	0.08	16	12 15
2	Oxydemeton-S-methyl	1.35	247→169 247→109	0.04	18	14 18
3	Demeton-S-methyl sulfone	1.39	263→169 263→121	0.04	20	16 16
4	Dimethoate	1.79	230→125 230→171	0.10	12	20 14
5	Fensulfothion-oxon	2.32	293→237 293→265	0.04	22	18 13
6	Fensulfothion-oxon-sulfone	2.39	309→253 309→175	0.04	19	15 25
7	Demeton-S-methyl	2.63	231→89 231→61	0.10	12	12 22
8	Disulfoton sulfoxide	2.93	291→185 291→97	0.04	15	13 32
9	Disulfoton sulfone	2.98	307→97 307→115	0.02	16	28 23
10	Fensulfothion	3.10	309→281 309→157	0.02	25	14 24
11	Fensulfothion sulfone	3.17	325→269 325→297	0.02	19	15 11
12	Terbufos sulfone	3.30	321→171 321→115	0.03	19	11 28
13	Terbufos sulfoxide	3.32	305→187 305→131	0.03	10	11 27
14	Ethoprophos	3.68	243→131 243→173	0.10	18	19 14
15	Disulfoton	4.03	275→89 275→61	0.08	14	10 32
16	Cadusafos	4.09	271→159 271→131	0.02	16	14 22
17	Terbufos	4.28	289→103 289→233	0.06	12	9 5

Xevo TQ MS MRM method parameters.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm	186002350
LCMS Certified Vials	600000751CV

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

Ref: Waters Application Note 720002812EN

INTRODUCTION

Flour is low water content commodity that requires the addition of water and soak time as a pretreatment step before extraction.

SAMPLE PRETREATMENT

1. Add 5 g of flour and 10 mL of water in a tube and soak for 10 minutes.

EXTRACTION PROCEDURE

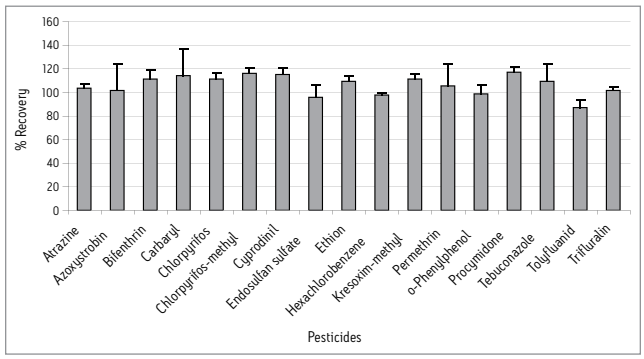
1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ extraction tube 1 containing 1.5 g of sodium acetate and 6 g of magnesium sulfate.
2. Add soaked flours sample into the 50 mL tube.
3. Add any internal standards and standard mixture.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE dSPE tube, containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 0.5 mL extract into a tube.
8. Add any post-extraction internal standards.
9. Add 0.25 mL toluene.
10. Evaporate at 50 °C with N_2 to < 0.1 mL.
11. Bring volume up to 0.2 mL with toluene.

GC CONDITIONS

GC System:	Agilent® 6890N
Column:	RTX-5MS, 30 x 0.25 mm, (0.25 µm film)
Carrier gas:	Helium
Flow rate:	1.0 mL/min
Temp. program:	Initial 100 °C, hold 1 minute, then 10 °C/min to 320 °C, hold for 7 minutes
Injection volume:	2 µL splitless

MS CONDITIONS

MS System:	Waters Quattro micro™ GC-MS
Ionization mode:	Electron Impact (70 eV)
Acquisition:	Single Ion Recording (SIR) Mode



Pesticides in Flour by GC/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
LCGC Certified Vials	186000272C
Insert 300 µL with Poly Spring	WAT094170

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Flour is low water content commodity that requires the addition of water and soak time as a pretreatment step before extraction.

SAMPLE PRETREATMENT

1. Add 5 g of flour and 10 mL of water in a tube and soak for 10 minutes.

EXTRACTION PROCEDURE

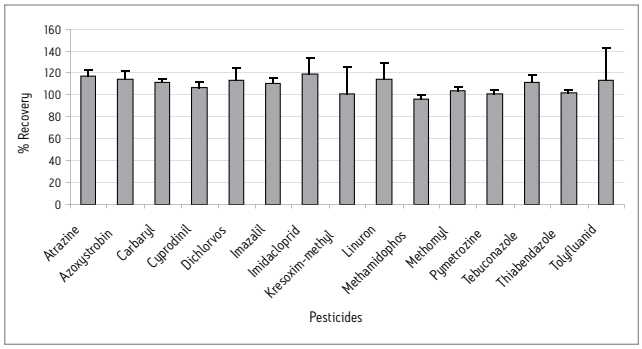
1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ extraction tube 1 containing 1.5 g of sodium acetate and 6 g of magnesium sulfate.
2. Add soaked flours sample into the 50 mL tube.
3. Add any internal standards and standard mixture.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE clean-up tube 2, containing 50 mg PSA and 150 mg of magnesium sulphate.

LC CONDITIONS

System:	ACQUITY UPLC®			
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm			
Column temp.:	40 °C			
Sample temp.:	4 °C			
Flow rate:	0.3 mL/min			
Mobile phase A:	Water + 0.1% formic acid			
Mobile phase B:	Methanol + 0.1% formic acid			
Gradient:	Time (min)	Flow Rate	A%	B%
	0.00	0.3	75	25
	0.25	0.3	75	25
	7.75	0.3	5	100
	8.50	0.3	0	100
	8.51	0.5	75	25
	10.50	0.5	75	25
	11.0	0.3	75	25
Injection volume:	15 µL, Partial loop injection			

MS CONDITIONS

MS System:	ACQUITY® TQ Detector
Ionization mode:	Positive electrospray (ESI^+)
Acquisition:	Multiple reaction monitoring (MRM)



Pesticides in Flour by UPLC/MS/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352
LCMS Certified Vials	600000749CV

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Grapes are a commodity containing an ample amount of water. This sample uses the standard Association of Analytical Communities (AOAC) extraction and dSPE tubes.

EXTRACTION PROCEDURE

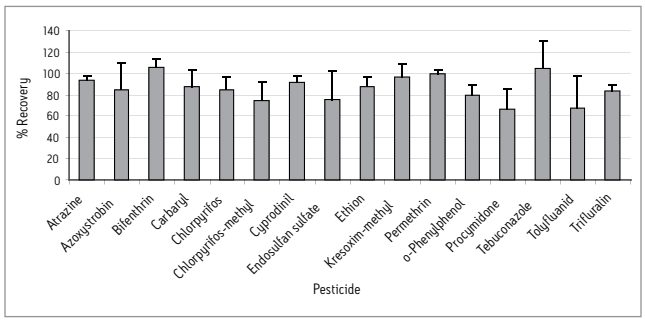
1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ extraction tube 1 containing 1.5 g of sodium acetate and 6 g of magnesium sulfate.
2. Add 15 g of homogenized sample into the 50 mL tube.
3. Add any internal standards and standard mixture.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE clean-up tube 2, containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 0.5 mL extract into a tube.
8. Add any post-extraction internal standards.
9. Add 0.25 mL toluene.
10. Evaporate at 50 °C with N_2 to < 0.1 mL.
11. Bring volume up to 0.2 mL with toluene.
12. Transfer to vial with insert for analysis.

GC CONDITIONS

GC System:	Agilent® 6890N
Column:	RTX-5MS, 30 x 0.25 mm, (0.25 µm film)
Carrier gas:	Helium
Flow rate:	1.0 mL/min
Temp. program:	Initial 100 °C, hold 1 minute, then 10 °C/min to 320 °C, hold for 7 minutes
Injection volume:	2 µL splitless

MS CONDITIONS

MS System:	Waters Quattro micro™ GC-MS
Ionization mode:	Electron Impact (70 eV)
Acquisition:	Single Ion Recording (SIR) Mode



Pesticides in Grapes by GC/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
LCGC Certified Vials	186000272C
Insert 300 µL with Poly Spring	WAT094170

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Grapes are a commodity containing an ample amount of water. This sample uses the standard Association of Analytical Communities (AOAC) extraction and clean-up tubes.

EXTRACTION PROCEDURE

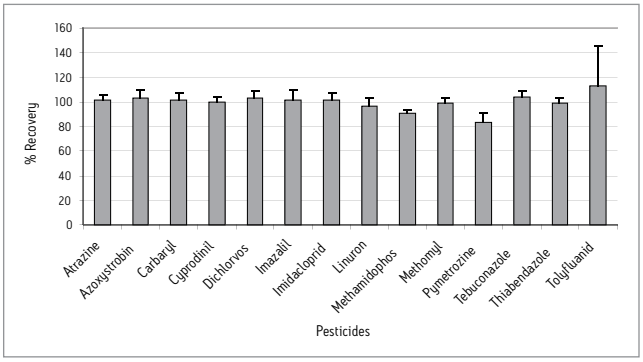
1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ extraction tube 1 containing 1.5 g of sodium acetate and 6 g of magnesium sulfate.
2. Add 15 g of homogenized sample into the 50 mL tube.
3. Add any internal standards and standard mixture.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE clean-up tube 2, containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 100 µL of final extract into an autosampler vial.
8. Add any post-extraction internal standards.
9. Dilute as needed with an appropriate buffer or solvent.

LC CONDITIONS

System:	ACQUITY UPLC®			
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm			
Column temp.:	40 °C			
Sample temp.:	4 °C			
Flow rate:	0.3 mL/min			
Mobile phase A:	Water + 0.1% formic acid			
Mobile phase B:	Methanol + 0.1% formic acid			
Gradient:	Time (min)	Flow Rate	A%	B%
	0.00	0.3	75	25
	0.25	0.3	75	25
	7.75	0.3	5	100
	8.50	0.3	0	100
	8.51	0.5	75	25
	10.50	0.5	75	25
	11.0	0.3	75	25
Injection volume:	15 µL, Partial loop injection			

MS CONDITIONS

MS System:	ACQUITY® TQ Detector
Ionization mode:	Positive electrospray (ESI^+)
Acquisition:	Multiple reaction monitoring (MRM)



Pesticides in Grapes by UPLC/MS/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352
LCMS Certified Vials	600000749CV

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Oranges are a commodity containing an ample amount of water. This sample uses the standard Association of Analytical Communities (AOAC) extraction and clean-up tubes.

EXTRACTION PROCEDURE

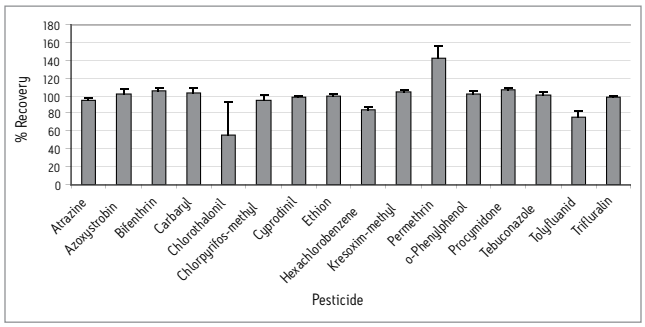
1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ extraction tube 1 containing 1.5 g of sodium acetate and 6 g of magnesium sulfate.
2. Add 15 g of homogenized sample into the 50 mL tube.
3. Add any internal standards and standard mixture.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE clean-up tube 2, containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 0.5 mL extract into a tube.
8. Add any post-extraction internal standards.
9. Add 0.25 mL toluene.
10. Evaporate at 50 °C with N_2 to < 0.1 mL.
11. Bring volume up to 0.2 mL with toluene.
12. Transfer to vial with insert for analysis.

GC CONDITIONS

GC System:	Agilent® 6890N
Column:	RTX-5MS, 30 x 0.25 mm, (0.25 µm film)
Carrier gas:	Helium
Flow rate:	1.0 mL/min
Temp. program:	Initial 100 °C, hold 1 minute, then 10 °C/min to 320 °C, hold for 7 minutes
Injection volume:	2 µL splitless

MS CONDITIONS

MS System:	Waters Quattro micro™ GC/MS
Ionization mode:	Electron Impact (70 eV)
Acquisition:	Single Ion Recording (SIR) Mode



Pesticides in Oranges by GC/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE 50 mL Tube-AOAC/Acetate	186004571
DisQuE 2 mL Tube-AOAC/C_{18}	186004830
LCGC Certified Vials	186000272C
Insert 300 µL with Poly Spring	WAT094170

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Oranges are a commodity containing an ample amount of water. This sample uses the standard Association of Analytical Communities (AOAC) extraction and clean-up tubes.

EXTRACTION PROCEDURE

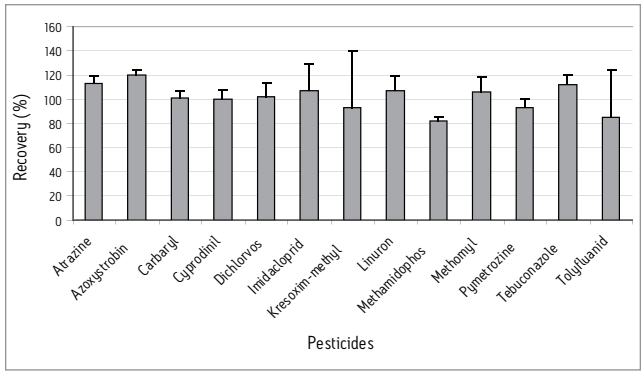
1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ extraction tube 1.
2. Add 15 g of homogenized orange with skin into the 50 mL tube.
3. Add any internal standards and standard mixture.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL clean-up tube containing 50 mg PSA, 150 mg $MgSO_4$, and 50 mg C_{18}.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 100 µL of final extract into a 1.5 mL centrifuge tube.
8. Add any post-extraction internal standards.
9. Dilute as needed with an appropriate buffer or solvent.
10. Centrifuge > 16000 rcf for 5 minutes.
11. Transfer to autosampler vial.

LC CONDITIONS

System:	ACQUITY UPLC®			
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm			
Column temp.:	40 °C			
Sample temp.:	4 °C			
Flow rate:	0.3 mL/min			
Mobile phase A:	Water + 0.1% formic acid			
Mobile phase B:	Methanol + 0.1% formic acid			
Gradient:	Time (min)	Flow Rate	A%	B%
	0.00	0.3	75	25
	0.25	0.3	75	25
	7.75	0.3	5	100
	8.50	0.3	0	100
	8.51	0.5	75	25
	10.50	0.5	75	25
	11.0	0.3	75	25
Injection volume:	15 µL, Partial loop injection			

MS CONDITIONS

MS System:	ACQUITY® TQ Detector
Ionization mode:	Positive electrospray (ESI^+)
Acquisition:	Multiple reaction monitoring (MRM)



Pesticides in Oranges by UPLC®/MS/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE 50 mL Tube-AOAC/Acetate	186004571
DisQuE 2 mL Tube-AOAC/C_{18}	186004830
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352
LCMS Certified Vials	600000749CV

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Rolled oats are a low water content commodity that requires the addition of water and soak time as a pretreatment step before extraction.

SAMPLE PRETREATMENT

1 Add 7.5 g of ground rolled oats and 15 mL of water in a tube and soak for 10 minutes.

EXTRACTION PROCEDURE

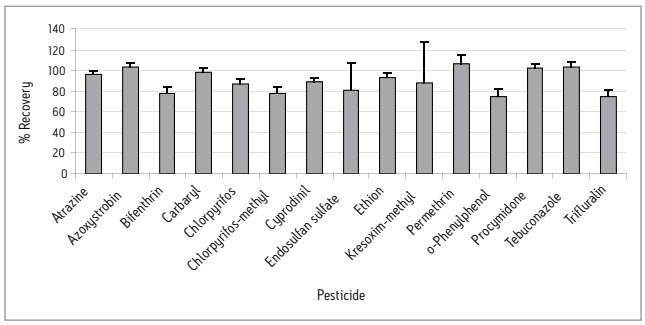
1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ extraction tube 1 containing 1.5 g of sodium acetate and 6 g of magnesium sulfate.
2. Add soaked oat sample into the 50 mL tube.
3. Add any internal standards and standard mixture.
4. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE clean-up tube 2, containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 0.5 mL extract into a tube.
8. Add any post-extraction internal standards.
9. Add 0.25 mL toluene.
10. Evaporate at 50 °C with N_2 to < 0.1 mL.
11. Bring volume up to 0.2 mL with toluene.
12. Transfer to vial with insert for analysis.

GC CONDITIONS

GC System:	Agilent® 6890N
Column:	RTX-5MS, 30 x 0.25 mm, (0.25 µm film)
Carrier gas:	Helium
Flow rate:	1.0 mL/min
Temp. program:	Initial 100 °C, hold 1 minute, then 10 °C/min to 320 °C, hold for 7 minutes
Injection volume:	2 µL splitless

MS CONDITIONS

MS System:	Waters Quattro micro™ GC/MS
Ionization mode:	Electron Impact (70 eV)
Acquisition:	Single Ion Recording (SIR) Mode



Pesticides in Rolled Oats by GC/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
LCGC Certified Vials	186000272C
Insert 300 µL with Poly Spring	WAT094170

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Rolled oats are a low water content commodity that requires the addition of water and soak time as a pretreatment step before extraction.

EXTRACTION PROCEDURE

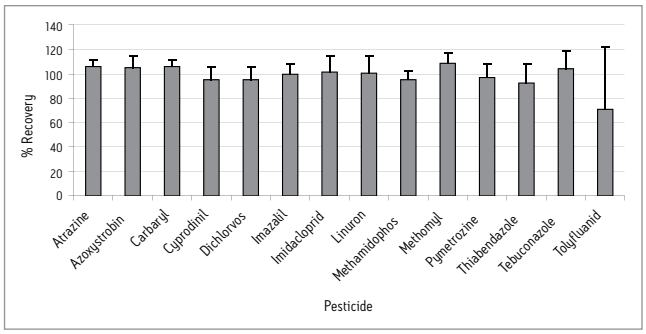
1. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ extraction tube (tube 1) containing 1.5 g of sodium acetate and 6 g of magnesium sulfate.
2. Diluted 7.5 g ground rolled oats with 15 mL water and soak for 10 minutes.
3. Add sample into the 50 mL tube.
4. Add any internal standards and standard mixture.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE dSPE clean-up tube (tube 2), containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Transfer 1 mL of the acetonitrile extract into the clean-up tube 2.
7. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
8. Transfer 100 µL of final extract into a 1.5 mL centrifuge tube.
9. Add any post-extraction internal standards.
10. Dilute as needed with an appropriate buffer or solvent.
11. Centrifuge > 16000 rcf for 5 minutes.
12. Transfer to autosampler vial.

LC CONDITIONS

System:	ACQUITY UPLC®			
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm			
Column temp.:	40 °C			
Sample temp.:	4 °C			
Flow rate:	0.3 mL/min			
Mobile phase A:	Water + 0.1% formic acid			
Mobile phase B:	Methanol + 0.1% formic acid			
Gradient:	Time (min)	Flow Rate	A%	B%
	0.00	0.3	75	25
	0.25	0.3	75	25
	7.75	0.3	5	100
	8.50	0.3	0	100
	8.51	0.5	75	25
	10.50	0.5	75	25
	11.0	0.3	75	25
Injection volume:	15 µL, Partial loop injection			

MS CONDITIONS

MS System:	ACQUITY® TQ Detector
Ionization mode:	Positive electrospray (ESI^+)
Acquisition:	Multiple reaction monitoring (MRM)



Pesticides in Rolled Oats by UPLC®/MS/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352
LCMS Certified Vials	600000749CV

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Teas are a low water content commodity that requires the addition of hot water and soak time as a pretreatment step before extraction.

SAMPLE PREPARATION PROCEDURE

1. Tare weigh an empty beaker.
2. Weigh out 100 g of tea leaves in the beaker.
3. Add in 600 g of hot water at 80-85 °C to the beaker. Brew the tea for 20 minutes.
4. Weigh the beaker with water and tea.
5. Calculate the weight of water loss due to evaporation.

   Add water to the beaker to make up for the loss of water.
6. Homogenize the sample until it reaches consistent texture.

EXTRACTION PROCEDURE

1. Transfer 15 g of homogenized sample into an empty 50 mL tube.
2. Add any internal standards and standard mixture.
3. Add 15 mL 1% acetic acid in acetonitrile into the 50 mL DisQuE™ extraction tube 1 containing 1.5 g of sodium acetate and 6 g of magnesium sulfate.
4. Transfer all the powder in the DisQuE extraction tube 1 into the 50 mL containing sample and solvent.
5. Shake vigorously for 1 minute and centrifuge > 1500 rcf for 5 minutes.
6. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE clean-up tube 2, containing 50 mg PSA and 150 mg of magnesium sulphate.
7. Shake for 30 seconds and centrifuge >1600 rcf for 5 minutes.
8. Transfer 100 µL of final extract into an autosampler vial.
9. Add any post-extraction internal standards.
10. Dilute as needed with an appropriate buffer or solvent.

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Column temp.:	40 °C
Sample temp.:	4 °C
Flow rate:	0.3 mL/min
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Methanol + 0.1% formic acid

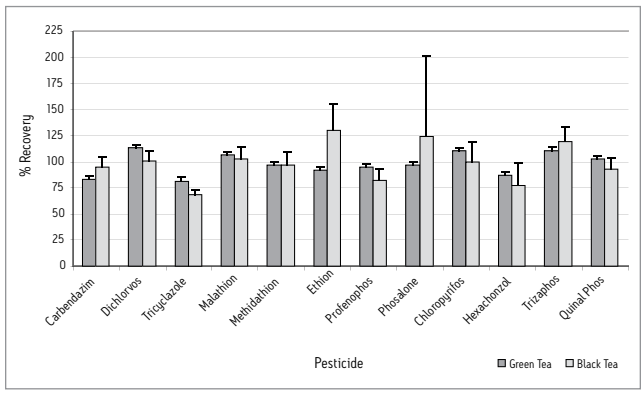
Gradient:

Time (min)	Flow Rate	A%	B%
0.00	0.3	75	25
0.25	0.3	75	25
7.75	0.3	5	100
8.50	0.3	0	100
8.51	0.5	75	25
10.50	0.5	75	25
11.0	0.3	75	25

Injection volume: 15 µL, Partial loop injection

MS CONDITIONS

MS System:	ACQUITY® TQ Detector
Ionization mode:	Positive electrospray (ESI^+)
Acquisition:	Multiple reaction monitoring (MRM)



Pesticides in Teas by UPLC®/MS/MS.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352
LCMS Certified Vials	600000749CV

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

INTRODUCTION

Most fruits and vegetables are commodities containing an ample amount of water. These samples use the standard Association of Analytical Communities (AOAC) extraction and clean-up tubes.

EXTRACTION PROCEDURE

1. Add 15 g of homogenized sample to the 50 mL DisQuE™ extraction tube containing 1.5 g of sodium acetate and 6 g of magnesium sulphate.
2. Add 15 mL of 1% acetic acid in acetonitrile.
3. Add any pre-extraction internal standards.
4. Shake vigorously for 1 minute and centrifuge >1500 rcf for 1 minute.
5. Transfer 1 mL of the acetonitrile extract into the 2 mL DisQuE extraction tube containing 50 mg PSA and 150 mg of magnesium sulphate.
6. Shake for 30 seconds and centrifuge >1500 rcf for 1 minute.
7. Transfer 250 µL of final extract into an autosampler vial.
8. Add any post-extraction internal standards.
9. Dilute as needed with an appropriate buffer or solvent.

LC CONDITIONS

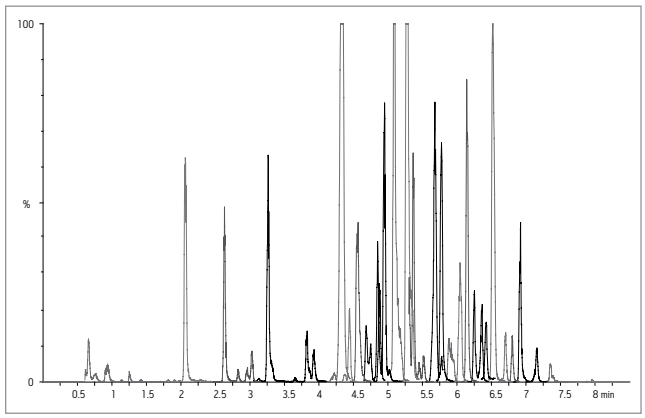
System: ACQUITY UPLC®
Column: ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Column temp.: 40 °C
Sample temp.: 4 °C
Flow rate: 0.450 mL/min
Mobile phase A: 98:2 water: 0.1% formic acid in methanol
Mobile phase B: Methanol + 0.1% formic acid
Gradient:

Time (min)	A%	B%
0.00	90	10
0.25	90	10
7.75	0	100
8.50	0	100
8.51	90	10

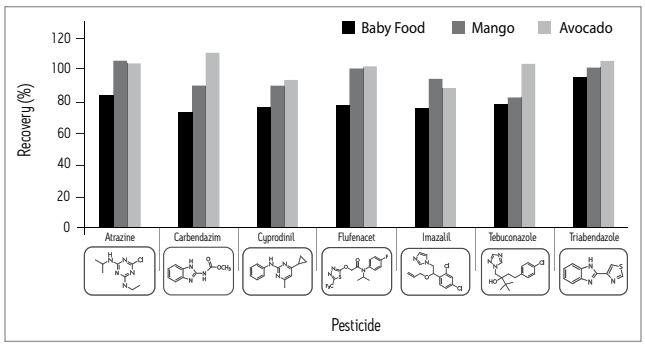
Total run time: 10 minutes
Injection volume: 20 µL, full loop injection

MS CONDITIONS

MS System: ACQUITY® TQ Detector
Ionization mode: Positive electrospray (ESI+)
Multiple reaction monitoring



402 pesticide residues in one 10 minute run in injection solvent.



Recovery data for three types of sample matrices.

ORDERING INFORMATION*

Description	Part Number
DisQuE Dispersive Sample Preparation Kit (100/pk)	176001676
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100	186002352
LCMS Certified Vials	600000751CV

***For all AOAC and CEN QuEChERS methods, refer to the DisQuE Dispersive Sample Preparation Brochure (lit code: 720003048EN)**

Ref: Waters Application Note 720002578EN

INTRODUCTION

The results of this study indicate that QuEChERS methodology is well-suited as a sample preparation step prior to GC/MS determination of pesticides in beef tissue. The QuEChERS extraction and subsequent dispersive SPE cleanup steps provide a faster analysis with less toxic reagents, and similar detection limits compared with other methods.

SAMPLE DESCRIPTION

Initial Extraction (QuEChERS)

Place 10 g of homogenized ground beef into a 50-mL centrifuge tube. Add 2 mL water and 10 mL acetonitrile (ACN), then shake the tube vigorously for 1 minute. Add contents of DisQuE™ Pouch salts for CEN QuEChERS, and shake vigorously for 1 minute. Centrifuge for 3 minutes at 4000 rpm, and take a 1-mL aliquot of the supernatant (top layer) for dSPE cleanup.

dSPE Cleanup

Transfer the 1-mL aliquot of supernatant to a 2-mL dSPE cleanup tube that contains 150 mg of magnesium sulfate, 50 mg PSA sorbent, and 50 mg C_{18} sorbent. Shake vigorously for 1 minute. Transfer a portion of the supernatant to the LCMS Certified Vial for GC/MS analysis.

GC CONDITIONS

GC System:	Agilent® 6890
Column:	Rxi®-5Sil MS, 30 meter x 0.25 mm (I.D.), 0.25 µm df
Injection volume:	1 µL
Carrier gas:	Helium
Flow rate:	1.0 mL/min (constant flow)
Temp. program:	80 °C initial (hold for 1 minute), 10 °C/min to 280 °C, and hold for 10 minutes
Sample vials:	LC/MS Certified (P/N 600000751CV)

MS CONDITIONS

MS System: Quattro micro GC™

The mass spectrometer was operated in positive electron impact (EI+) mode. Data was collected at 70 electron energy (eV) using selected ion monitoring (SIR). The ions monitored consisted of the following, with the principal quantification ion listed first.

Compound	SIR (m/z)	Retention Time (min)
Dimethoate	87.0, 93.0,125.0	11.8
Chlorpyrifos methyl	286.1, 288.1,125.0	13.1
Malathion	173.2, 127.1, 125.0	13.7
Tribufos	169.1, 202.2, 113.0	15.6
Coumaphos	362.3, 226.2, 210.1	19.6

Compound *OP Pesticides*	Spike level (ppb)	% Recovery	% RSD
Dimethoate	20	89.4	7.2
Chlorpyrifos methyl	20	74.6	6.8
Malathion	20	93.9	13.8
Tribufos	20	71.8	3.7
Coumaphos	20	88.0	6.5
Dimethoate	200	98.9	6.1
Chlorpyrifos methyl	200	88.0	7.5
Malathion	200	107.0	9.6
Tribufos	200	74.8	6.9
Coumaphos	200	95.1	7.3

Table 1. Recovery for organophosphorus pesticides in ground beef using the DisQuE Pouch for QuEChERS (n=5).

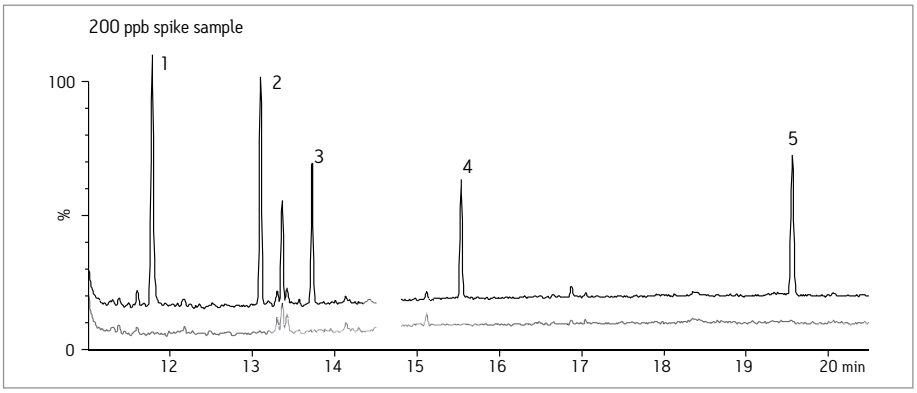


Figure 1. GC/MS chromatograms of unspiked ground beef samples (blue trace), and ground beef samples spiked with 200 ppb organophosphorus pesticides (black trace).

ORDERING INFORMATION*

Description	Part Number
CEN QuEChERS DisQuE Pouch	186006813
DisQuE 2 mL d-SPE Cleanup Tube (150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18})	186002352
DisQuE 50 mL Centrifuge Tube	186006814
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720004456EN

INTRODUCTION

Paraquat is one of the most widely used herbicides in the world. As with many herbicides it is dangerously poisonous to humans if swallowed.

PRETREATMENT

1. Adjust sample to pH 7 by adding 1 M ammonium phosphate buffer dropwise to 20 mL river water.

SPE PROCEDURE

Oasis® WCX, 3cc/60mg

CONDITION/EQUILIBRATE:
A. 1 mL methanol
B. 1 mL water

LOAD:
20 mL sample

WASH:
A. 1 mL 1 M pH 7 phosphate buffer
B. 1 mL water
C. 1 mL methanol

ELUTE:
1.5 mL acetonitrile/water/trifuoroacetic acid (84:14:12, v/v/v)

Reconstitute in 0.5 mL mobile phase

LC CONDITIONS

System:	Alliance® HPLC 2695
Column:	Atlantis® HILIC, 3.5 µm, 2.1 x 150 mm
Flow rate:	0.4 mL/min
Mobile phase:	40% acetonitrile 60% 250 mM ammonium formate (pH 3.7)
Injection volume:	20 µL
Column temp.:	30 °C

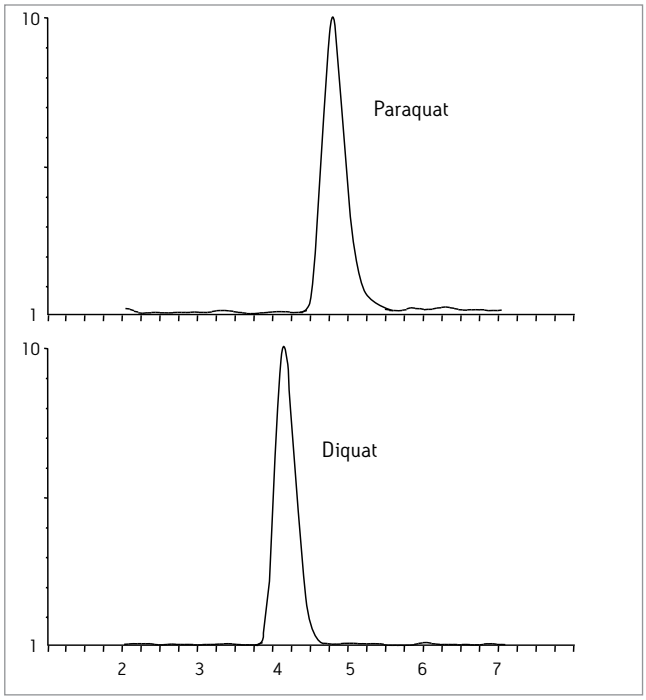
MS CONDITIONS

MS System:	Waters Quattro micro™
Ionization mode:	Positive electrospray (ESI+) Multiple reaction monitoring

Analyte	MRM Transition
Paraquat	171→77
	171→155
Diquat	183→157
	183→168

MRM method parameters.

RESULTS



LC/MS separation of paraquat and diquat at 0.5 µg/L.

	Paraquat	Diquat
Day 1	1.08 µg/L (8.1% RSD)	1.05 µg/L (2.9% RSD)
Day 4	1.10 µg/L (8.0% RSD)	1.09 µg/L (5.9% RSD)
Day 5	0.95 µg/L (7.1% RSD)	1.08 µg/L (4.4% RSD)

Intraday results obtained from spiked water samples (spike level 1.0 µg/L).

ORDERING INFORMATION

Description	Part Number
Oasis WCX, 3 cc/60 mg, 60 µm, 100/box	186002497
Atlantis HILIC, 3.5 µm, 2.1 x 150 mm	186002015
750 µL Polypropylene Vials	186002635

Ref: Waters Application Note WA40524

INTRODUCTION

Patulin is a mycotoxin that is produced by certain species of Penicillium, Aspergillus, and Byssochylamys molds that may grow on a variety of foods including fruit, grains, and cheese. Patulin is a safety concern in apple juice.

SPE PROCEDURE

Oasis® HLB, 3cc/60mg

CONDITION:
A. 3 mL methanol
B. 3 mL water

LOAD:
2.5 mL sample

WASH 1:
3 mL 1% sodium bicarbonate (1g/100mL)

WASH 2:
1 mL 0.1% acetic acid

DRY:
Using vacuum manifold

ELUTE:
2 x 1.5 mL 10% ethyl acetate in methyl t-butyl ether (MTBE)

RECONSTITUTE:
500 µL water

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH Shield RP18, 1.7 µm, 2.1 x 100 mm
Flow rate:	600 µL/min
Mobile phase A:	0.1% aqueous ammonium hydroxide
Mobile phase B:	0.1% ammonium hydroxide in acetonitrile

Gradient:

Time (min)	A%	B%
0.00	99	1
1.80	99	1
2.30	10	90
2.80	10	90
2.81	99	1

Injection volume:	20 µL, Full loop injection
Column temp.:	40 °C
Sample temp.:	4 °C
Detector:	ACQUITY UPLC PDA
Detection:	276 nm

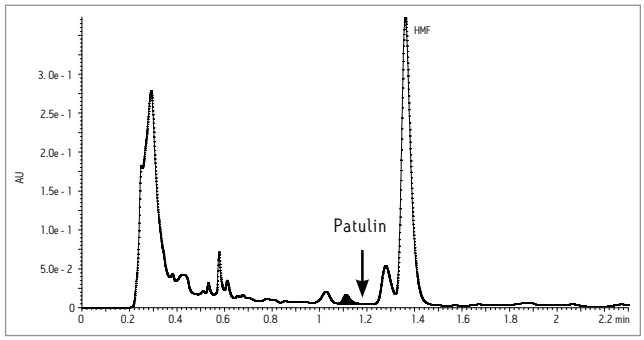
MS CONDITIONS

MS System:	ACQUITY® TQ Detector
Ionization mode:	Negative electrospray (ESI^-) Multiple reaction monitoring

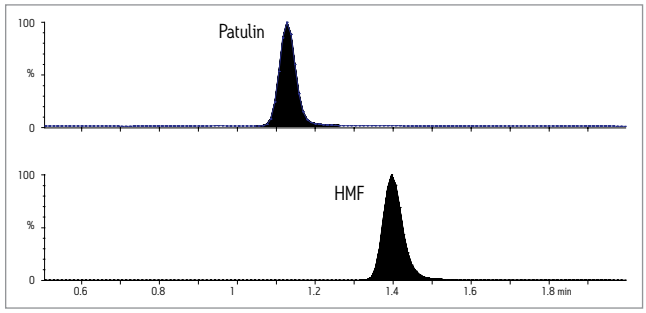
Analytes	MRM Transition
Patulin	153→109
	153→81
5-Hydroxymethylfurfural (HMF)	125→95

MRM method parameters.

RESULTS



Apple juice extract at 50 µg/kg containing patulin and 5-hydroxymethylfurfural (HMF) at 276 nm.



Apple juice extract at 50 µg/kg containing patulin and 5-hydroxymethylfurfural in negative electrospray mode.

Concentration	Average Recovery (%RSD)
5 µg/kg	86.1% (13.6)
50 µg/kg	95.4% (5.9)
500 µg/kg	89.9% (17.5)

Recovery data obtained from Oasis HLB extraction of patulin in apple juice. Four data points were measured at each level.

ORDERING INFORMATION

Description	Part Number
Oasis HLB, 3 cc/60 mg, 100/box	WAT094226
ACQUITY UPLC BEH Shield RP18, 1.7 µm, 2.1 x 100 mm	186002854
LCMS Certified Vials	600000749CV

Ref: Developed by Vural Gökmen, Food Engineering Department, Hacettepe University, Ankara, Turkey and John Martin, Waters Corporation

INTRODUCTION

The Pesticide Screening Application Solution with UNIFI® has been designed to confidently report the presence and absence of pesticide residues at Maximum Residue Limits (MRLs).

EXTRACTION PROCEDURE

1. Weigh 15 g (±0.05 g) of homogenised sample material into a 50 mL tube.
2. Add 15 mL of 1% glacial acetic acid acetonitrile.
3. Vigorously shake the extraction tubes for 1 minute.
4. Add the contents of the sorbent pouch, shake for 2 minutes venting periodically, and centrifuge at 3500 rpm for 1 minute.
5. Remove 5 mL of supernatant and place in 15 mL tube 2.
6. Shake for 1 minute, and centrifuge at 3500 rpm for 1 minute.
7. Remove 1 mL of supernatant from tube 2 and place under a gentle stream of nitrogen and heat (< 40 °C). Dry extract to consistent near dryness.
8. Reconstitute with 1 mL of 25% CH_3CN (or alternative desired diluent) and place in autosampler vial for LC-QTof analysis.

LC CONDITIONS

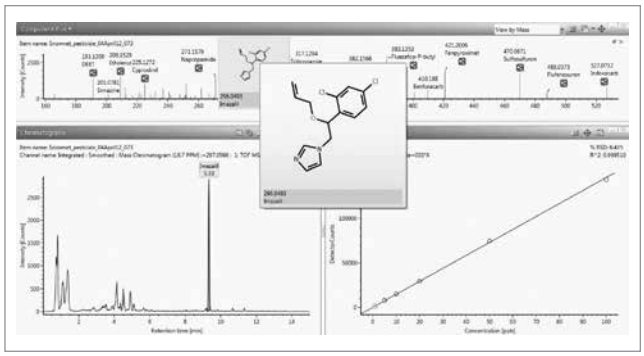
System:	ACQUITY UPLC® I-Class
Run time:	15 minutes
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm
Column temp.:	45 °C
Mobile phase A:	10 mM ammonium acetate dissolved in water
Mobile phase B:	10 mM ammonium acetate dissolved in methanol
Flow rate:	0.45 mL/min
Injection volume:	5 µL

Gradient:

Time (min)	Flow Rate (mL/min)	%A	%B
Initial	0.450	98	2
0.250	0.450	98	2
12.25	0.450	1	99
13.00	0.450	1	99
13.10	0.450	98	2
17.00	0.450	98	2

MS^E CONDITIONS

MS System:	Xevo® G2-S QTof
Ionization mode:	ESI positive



UNIFI component plot (upper data panel) shows the pesticide residues identified in a test sample and the calibration series for imazalil (1 to 100 ppb) and correlation coefficient R2=0.999.

Analyte	Assigned Value (mg/kg)	Found Value (mg/kg)	Z-Score	Z-Score Classification	Correlation Coefficient (R^2)	Mass Accuracy (ppm)
Carbendazim	1.25	0.97	-0.9	Acceptable	0.989	1.49
EPN*	0.42	0.70	2.7	Questionable	0.985	-1.75
Imazalil	1.30	1.36	0.2	Acceptable	0.999	0.73
Indoxacarb	0.79	0.94	0.7	Acceptable	0.993	-0.32
Methomyl	0.20	0.10	-1.9	Acceptable	0.998	-0.95
Oxamyl	0.13	0.14	0.3	Acceptable	0.992	1.69
Prochloraz	0.30	0.43	1.8	Acceptable	0.984	-0.93
Pyriproxyfen*	0.44	0.50	0.6	Acceptable	0.994	0.30
Spinosad	0.61	0.89	1.8	Acceptable	0.998	-0.68
Thiabendazole	0.81	0.85	0.2	Acceptable	0.990	2.27

The comparative performance of the pesticide screening solution for the LC amenable pesticides identified in an EU-RL proficiency test sample (mandarin FV-13 2011) assessed against the published median calculated results.
**Compounds normally analyzed using GC.*

ORDERING INFORMATION

Description	Part Number
DisQuE Pouch (CEN)	186006813
50 mL DisQuE Extraction Tube (1.5 g sodium acetate, 6 g $MgSO_4$)	186004571
15 mL DisQuE Clean-up Tube (900 mg $MgSO_4$, 150 mg PSA)	186004833
DisQuE 50 mL Centrifuge Tube	186006814
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100	186002352

Ref: Waters Application Note 720004491EN

INTRODUCTION

Perfluorinated compounds (PFCs) such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid are persistent organic pollutants (POPs). PFCs may be toxic and have bioaccumulative properties. There is growing interest in the development of analytical methods for PFCs in food, drinking water, tissue, plasma, and blood.

PRETREATMENT

Water samples

1. Adjust 100 mL of sample to pH 3 with formic acid prior to SPE.

Chicken liver samples

1. Homogenize 1 g of sample and extract with 10 mL of 10 mM potassium hydroxide in methanol. Shake for 16 hours.
2. Centrifuge the sample for 10 minutes at 8000 rpm.
3. Dilute 1 mL aliquot of supernatant to 20 mL with water and adjust the pH to 4-5 using 2% formic acid.

SPE PROCEDURE

Oasis® WAX, 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 2 mL methanol
B. 2 mL water

↓

LOAD:
100 mL water or 20 mL diluted tissue sample

↓

WASH:
A.1 mL 2% formic acid
B. 2 mL methanol

↓

ELUTE:
2 mL 1% ammonia in methanol

Note:

- The SPE eluate is collected in polypropylene test tubes, diluted with 2 mL of 2% aqueous formic acid and brought to 5 mL with water.
- Alternatively, the eluate may be evaporated and reconstituted in 1 mL mobile phase prior to analysis. Polypropylene labware should be used exclusively.

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Flow rate:	0.40 mL/min
Mobile phase A:	20 mM ammonium acetate in water/acetonitrile (90:10)
Mobile phase B:	Acetonitrile/methanol

Gradient:

Time (min)	A%	B%
0.00	85	15
8.00	5	95

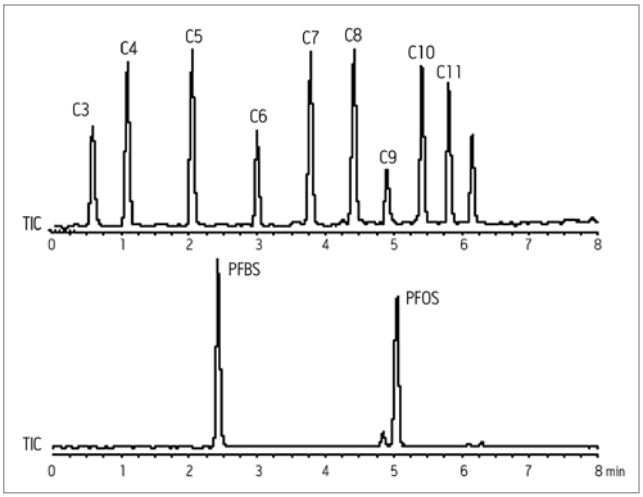
Injection volume:	10 µL (full loop injection mode)
Column temp.:	40 °C

MS CONDITIONS

MS System:	Waters Quattro Premier™ XE
Ionization mode:	Negative electrospray (ESI^-) Multiple reaction monitoring

PFC	MRM
PFBS (Perfluorobutane Sulfonate)	299→80
PFOS (Perfluorooctane Sulfonate)	499→80
C3	163→119
C4	213→169
C5	263→219
C6	313→269
C7	363→319
C8	413→369
C9	463→419
C10	513→469
C11	563→519

MRM method parameters.



UPLC®/MS/MS of 12 PFCs spiked at 10 µg/kg in chicken liver.

Recovery from Drinking Water (%)											
Spike Level µg/L	PFBS	PFOS	C3	C4	C5	C6	C7	C8	C9	C10	C11
0.10	122	109	108	119	97	184	107	83	121	101	101
0.30	110	117	95	132	105	110	119	126	137	118	94
0.70	102	98	91	107	93	118	100	78	103	126	119
1.0	113	94	128	106	98	130	100	88	100	110	117
4.0	104	86	101	99	99	102	102	92	115	99	84
10	104	100	98	101	100	87	89	82	103	99	101

Recovery data for PFCS from drinking water.

Recovery from Chicken Liver (%)											
Spike Level µg/kg	PFBS	PFOS	C3	C4	C5	C6	C7	C8	C9	C10	C11
2	LOQ	–	81	LOQ	–	108	132	165	100	97	–
5	98	LOQ	138	148	LOQ	97	100	133	89	73	LOQ
10	93	50	102	134	121	99	87	123	95	101	33
20	102	50	128	144	94	96	110	117	90	80	25
30	87	51	104	102	124	89	86	103	91	84	20
50	92	54	92	92	118	94	97	97	86	86	22

Recovery data for PFCs from chicken liver.

ORDERING INFORMATION

Description	Part Number
PFC Analysis Kit	176001744
PFC Column Kit	176001692
PFC QC Standard	186004597
Oasis WAX, 3 cc/60 mg, 60 µm, 100/box	186002492
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm	186002350
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm, 3/pk	176000863
750 µL Polypropylene Vial	186002635

Ref: Waters Application Note 720001817EN

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are toxic compounds commonly found in nature, and are constituents of coal and petroleum. The US EPA has classified seven PAHs as probable human carcinogens. The US FDA has set the limit of concern (LOC) for benzo[a]pyrene, one of the most widely occurring and potent PAHs, at 35 ppb in shellfish. This application note demonstrates that the QuEChERS extraction and cleanup technology can be applied to other types of analytes, and other types of sample matrices as an alternative to more cumbersome methods of sample preparation.

LC CONDITIONS

System:	ACQUITY UPLC® H-Class with Large Volume Flow Cell (LVFC)
Column:	PAH, 3 µm, 4.6 x 50 mm
Column temp.:	350 °C
Mobile phase A:	Water
Mobile phase B:	Methanol
Mobile phase C:	Acetonitrile
Flow rate:	2.0 mL/minute
Gradient:	30% A, 70% B initial, linear gradient to 70% B, 30% C at 2.25 minutes, to 100% C at 3.5 minutes, back to 30% A, 70% B at 3.6 minutes, and re-equilibrate.
Injection volume:	10 µL
Detection:	Fluorescence (FLR) using programmed wavelength changes
Vials:	LCGC Certified Vials

Fluorescence program:

Time (min)	Excitation (nm)	Emission (nm)
0.00	276	331
1.00	295	315
1.33	248	380
1.62	246	488
1.97	275	380
2.40	300	422
2.70	364	408
2.89	298	410
3.17	305	500

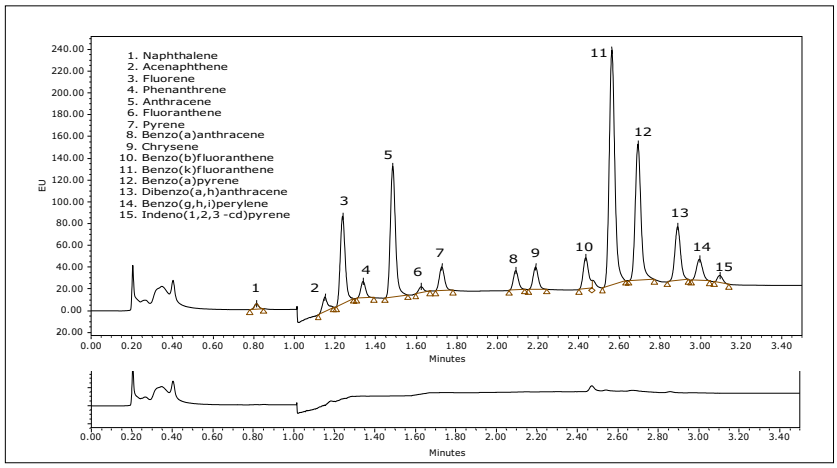


Figure 1. LC/FLR chromatogram obtained from a shrimp sample spiked with 20 ppb PAH compounds. The bottom chromatogram is an unspiked shrimp blank.

PAH Compound	% Recovery (%RSD)	
	20 ppb spike	200 ppb spike
Naphthalene	73 (21)	86 (3.5)
Acenaphthene	83 (9.1)	91 (0.8)
Fluorene	87 (5.4)	93 (0.9)
Phenanthrene	93 (4.0)	94 (1.5)
Anthracene	93 (4.1)	94 (1.4)
Fluoranthene	94 (3.9)	94 (1.9)
Pyrene	94 (4.2)	92 (2.1)
Benzoanthracene	94 (5.2)	90 (2.2)
Chrysene	95 (5.1)	97 (2.3)
Benzo(b)fluoranthene	88 (3.1)	88 (2.4)
Benzo(b) fluoranthene	98 (5.1)	87 (1.7)
Benzo(a) pyrene	88 (6.0)	83 (2.6)
Dibenzo(a,h)anthracene	99 (6.1)	84 (3.6)
Benzo(g,h,i)perylene	96 (6.3)	73 (2.8)
Indeno(1,2,3 -cd)pyrene	98 (8.6)	98 (14.0)

Table 1. Recovery from DisQuE™ extraction of 20 ppb and 200 ppb spiked shrimp samples.

ORDERING INFORMATION

Description	Part Number
Waters PAH Column, 3 µm, 4.6 x 50 mm	186001260
DisQuE 50 mL Centrifuge Tube	186006814
AOAC QuEChERS Pouch	186006812
LCGC Certified Vials	186000307C

Ref: Waters Application Note 720004454EN

INTRODUCTION

Propham is the active substance used as herbicides and potato sprout inhibitor. This analytical method can be used to monitor Propham residues in potatoes.

PRETREATMENT

1. Add 15 g of ground potatoes into a 50 mL centrifuge tube.
2. Add 15 mL 1% acetic acid in acetonitrile and shake.
3. Add 1.5 g anhydrous sodium acetate and 6 g anhydrous magnesium sulfate (equivalent to contents of DisQuE Pouch, AOAC method).
4. Centrifuge >1500 rcf for 1 minute.
5. Take out 7.5 mL extract and dilute to 10 mL with 2.5 mL toluene.

SPE PROCEDURE

Sep-Pak® Vac Carbon Black/Aminopropyl, 6 cc/500 mg/500 mg

CONDITION:
10 mL 25:75 toluene: actonitrile (v/v)

Add 200 mg anhydrous magnesium sulfate to top of cartridge to remove water

LOAD:
Extract (collect)

ELUTE:
10 mL 25:75 toluene: acetonitrile (collect)

Combine both collected fractions and adjust volume to exactly 20 mL by addition of toluene:acetonitrile (25:75, v/v)

Take 5 mL and evaporate to just below 1 mL and bring up to 1 mL with toluene. Inject onto GC/MS.

GC CONDITIONS

GC System:	Agilent® 6890
GC Column:	DB-5ms, 30m x 0.25mm (i.d.), 0.25 µm film. Direct connection of column to injection-port liner
Transfer line to MS:	300 °C
Source temp.:	200 °C
Injection volume:	1 µL splitless
Injection port temp.:	180 °C
Initial temp.:	80 °C
Time at initial temp.:	1 minute
	Then Program at 10 °C/ min to 200 °C
	Then at 25 °C minute to 300, hold 5 minutes

GC/MS CONDITIONS

GC/MS System:	Waters Quattro micro™ GC
Ionization mode:	Electron Impact (70 eV) Selected-Ion Recording (SIR)

HP6890 GC FLOW 1

Initial Flow: 1 mL/min

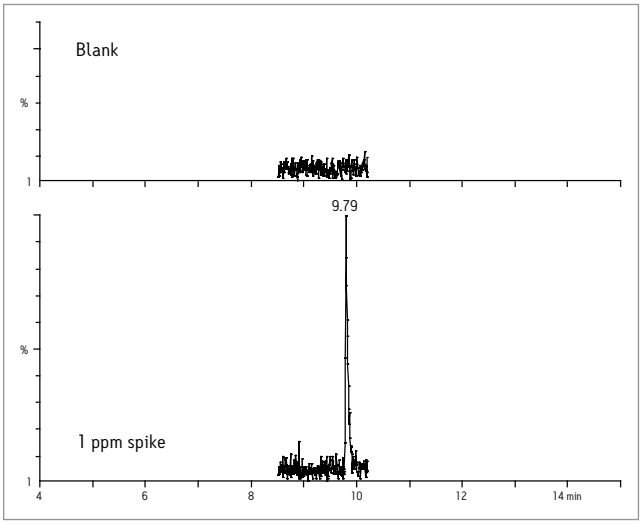
Time (min)	Rate Final (mL/min)	Flow (mL/min)
0.00	50	3
0.50	50	3
0.60	50	1

GC/MS (SIR)

Channel	Mass
1 (Quantification)	92.8
2 (Confirmation)	119
3 (Confirmation)	120

SIR method parameters.

RESULTS



1 µg/g spiked potato sample.

Compound Name: propham 92.8	RT	Area
1 ppm spiked 1	9.78	379.00
1 ppm spiked 2	9.82	382.00
1 ppm spiked 3	9.80	458.00
1 ppm spiked 4	9.79	399.00
1 ppm spiked 5	9.75	421.00
Mean	-	407.80
RSD (%)	-	8.01
Recovery (%)	-	95.73

Recovery data for 1 µg/g spiked potato sample.

ORDERING INFORMATION

Description	Part Number
Sep-Pak Vac Carbon Black/Aminopropyl, 6 cc/500 mg/500 mg	186003369
DisQuE Pouch (AOAC Method)	186006812
LCMS Certified Vials	600000751CV

INTRODUCTION

Propham is the active substance used as herbicides and potato sprout inhibitor. This analytical method can be used to monitor Propham residues in potatoes.

PRETREATMENT

1. Add 15 g of ground potatoes to 50 mL centrifuge tube.
2. Add 15 mL 1% acetic acid in acetonitrile and shake.
3. Add 1.5 g anhydrous sodium acetate and 6 g anhydrous magnesium sulfate (equivalent to contents of DisQuE Pouch, AOAC method).
4. Centrifuge >1500 rcf for 1 minute.

SPE PROCEDURE

Sep-Pak® Light NH_2

Transfer 5 mL to another tube and add 0.5 mg of anhydrous magnesium sulfate

↓

Vortex and allow powder to settle

↓

Pass through 2 mL of prepared sample extract

↓

Take out 200 µL dilute with 800 µL water

↓

Inject onto LC/MS (10 µL)

LC CONDITIONS

System: ACQUITY UPLC®

Column: ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient:

Time (min)	A%	B%
0.00	80	20
3.00	20	80
3.20	80	20
5.00	80	20

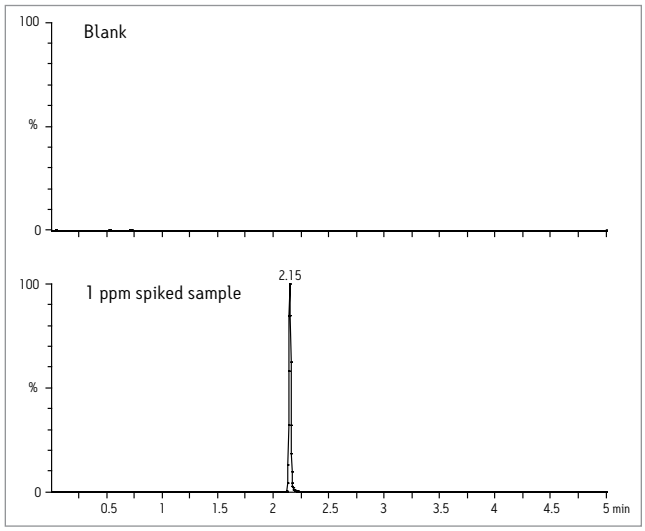
MS CONDITIONS

MS System: Waters Quattro micro™

Ionization mode: Positive electrospray (ESI^+)
Multiple reaction monitoring

MRM transitions: 1. 180.3→120.3
2. 180.3→138.3

RESULTS



Pass through 1 µg/g spiked potato sample.

Compound 1: propham 138.3	RT	Area
1 ppm spiked 1	2.15	5895.34
1 ppm spiked 2	2.15	6424.93
1 ppm spiked 3	2.15	6996.63
1 ppm spiked 4	2.15	7557.80
1 ppm spiked 5	2.15	7567.60
Mean	-	6888.46
RSD (%)	-	10.57
Recovery (%)	-	84.43

Compound 2: propham 120.3	RT	Area
1 ppm spiked 1	2.15	1849.85
1 ppm spiked 2	2.15	1950.71
1 ppm spiked 3	2.15	2091.4
1 ppm spiked 4	2.15	2276.4
1 ppm spiked 5	2.15	2306.56
Mean	-	2094.98
RSD (%)	-	9.5
Recovery (%)	-	81.35

Recovery data for of 1 µg/g spiked potato sample.

ORDERING INFORMATION

Description	Part Number
Sep-Pak Light NH_2	WAT023513
DisQuE Pouch (AOAC Method)	186006812
ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm	186002352

INTRODUCTION

Sudan dyes are red dyes that are used for coloring solvents, oils, waxes, petrol, shoe and floor polishes. Sudan dyes are not allowed to be added to food in by the United States Food and Drug Administration (US FDA), European Union (EU), and other countries.

PRETREATMENT

For Chilli Oil

1. Dilute 0.1 g chilli oil in 1 mL with hexane.

For Chilli Powder

1. Homogenize and extract 1 g chilli powder with 10 mL acetone.
2. Centrifuge.
3. A 1 mL aliquot is evaporated to complete dryness and the residue is taken up in 1 mL hexane.

SPE PROCEDURE

Sep-Pak® Alumina B, 3 cc/500 mg

CONDITION/EQUILIBRATE:
A. 2 mL methanol
B. 2 mL ethyl acetate
C. 3 mL hexane

LOAD:
1 mL of hexane pre-extract

WASH:
A. 3 mL hexane
B. 1 mL ethyl acetate

ELUTE:
4 mL ethyl acetate/methanol (90:10)

Evaporate and reconstitute in 200 µL methanol

LC CONDITIONS

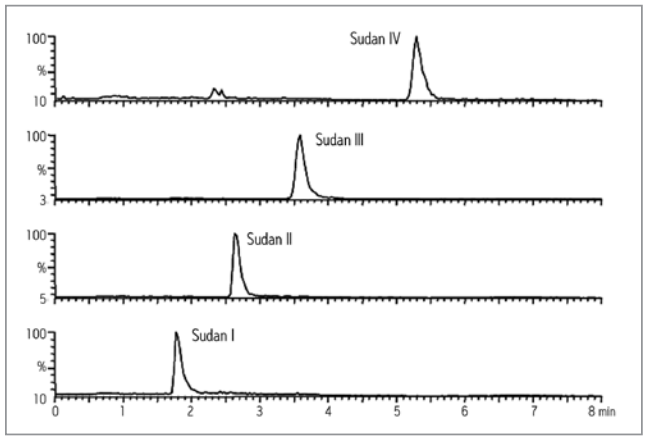
System:	Alliance® HPLC 2695		
Column:	Atlantis® dC_{18}, 3 µm, 2.1 x 100 mm		
Flow rate:	0.4 mL/min		
Mobile phase A:	0.1% formic acid in water		
Mobile phase B:	Acetonitrile		
Gradient:	Time (min)	A%	B%
	0.00	20	80
	10.00	5	95
Injection volume:	15 µL		
Column temp.:	30 °C		

MS CONDITIONS

MS System: Waters Quattro micro™ API
Ionization mode: Positive electrospray (ESI^+)
Multiple reaction monitoring

Analyte	MRM Transition
Sudan I	249→156
	249→93
	249→128
Sudan II	277→156
	277→121
	277→106
Sudan III	353→77
	353→120
	353→196
Sudan IV	381→91
	381→106
	381→224

RESULTS



LC/MS spiked chilli powder (n=6, 80 µg/kg).

Analyte	Recovery (%)	RSD (%)
Sudan I	99	11
Sudan II	91	11
Sudan III	93	6
Sudan IV	122	11

Recovery data for spiked chilli powder (n=6, 80 µg/kg).

ORDERING INFORMATION

Description	Part Number
Sep-Pak Alumina B, 3 cc/500 mg, 50/box	WAT020825
Atlantis dC_{18}, 3 µm, 2.1 x 100 mm	186001295
Qsert™ Vials, LCGC Certified Combination Packs	186001126C

Ref: Waters Applications 720001440EN

INTRODUCTION

Sudan dyes are red dyes that are used for coloring solvents, oils, waxes, petrol, shoe and floor polishes. Sudan dyes are not allowed to be added to food in by the United States Food and Drug Administration (US FDA), European Union (EU), and other countries.

PRETREATMENT

1. Homogenize and extract 1 g of chilli with 10 mL acetone.
2. Dilute 1 mL aliquot to 5 mL with aqueous sodium hydroxide (adjust to pH 11).

SPE PROCEDURE

Oasis® MAX, 3 cc/60 mg

CONDITION/EQUILIBRATE:
A. 2 mL ethyl acetate
B. 2 mL methanol
C. 1 mL 0.1 M sodium hydroxide
D. 2 mL water

LOAD:
5 mL of diluted acetone pre-extract

WASH:
A. 2 mL 70% methanol in water
B. 1 mL 1 M sodium hydroxide in water
C. 2 mL methanol
D. 1 mL ethyl acetate

ELUTE:
2 mL ethyl acetate/methanol/formic acid (89:9:2, v/v/v)

Evaporate and reconstitute in 200 µL acetonitrile/water (90:10, v/v)

LC CONDITIONS

System:	Alliance® HPLC 2695		
Column:	Atlantis® dC_{18}, 3 µm, 2.1 x 100 mm		
Flow rate:	0.4 mL/min		
Mobile phase A:	0.1% formic acid in water		
Mobile phase B:	Acetonitrile		
Gradient:	Time (min)	A%	B%
	0.00	20	80
	10.00	5	95
Injection volume:	15 µL		
Column temp.:	30 °C		

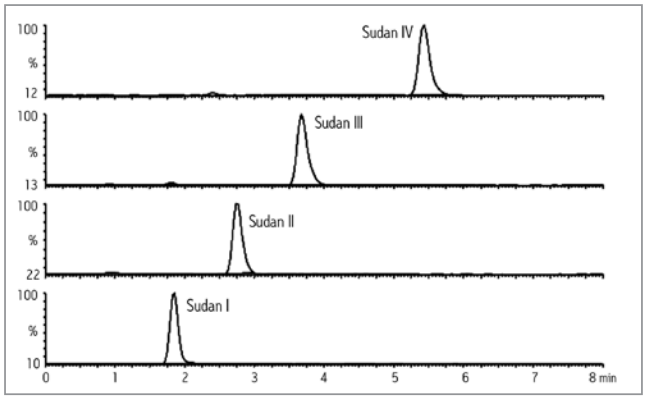
MS CONDITIONS

MS System:	Waters Quattro micro™ API
Ionization mode:	Positive electrospray (ESI^+) Multiple reaction monitoring

Analyte	MRM Transition
Sudan I	249→156
	249→93
	249→128
Sudan II	277→156
	277→121
	277→106
Sudan III	353→77
	353→120
	353→196
Sudan IV	381→91
	381→106
	38 →224

MRM method parameters.

RESULTS



LC/MS spiked chilli sauce (n=6, 80 µg/kg), Oasis MAX method.

Analyte	Recovery (%)	RSD (%)
Sudan I	83	9
Sudan II	83	1
Sudan III	77	3
Sudan IV	75	4

ORDERING INFORMATION

Description	Part Number
Oasis MAX, 3 cc/60 mg, 60 µm, 100/box	186000368
Atlantis dC_{18}, 3 µm, 2.1 x 100 mm	186001295
Qsert™ Vials, LCGC Certified Combination Packs	186001126C

Ref: Waters Application Note 720001440EN

INTRODUCTION

Tetracycline antibiotics are used in veterinary medicine for treatment of animals grown for production of meat or milk. Of this class of antibiotics, oxytetracycline is the most commonly used for milk-producing cattle. Ingestion of antibiotic residues in milk can result in increased antibiotic resistance as well as potential allergic reactions among the consuming population.

PRETREATMENT

Transfer 1.5 mL milk to a 15-mL centrifuge tube. Add 6 mL of EDTA/McIlvaine buffer, and vortex for 30 seconds. Centrifuge at 4000 rpm for 5 minutes. Collect the supernantant and adjust to pH 10 with 0.75 mL 1 M NaOH.

SPE PROCEDURE

Oasis MAX Cartridge (1 cc, 30 mg)

CONDITION/EQUILIBRATE:
2 mL methanol, 2 mL water

LOAD:
Supernatant from pretreatment

WASH 1:
0.5 mL 5% ammonium hydroxide in water

WASH 2:
0.5 mL methanol

ELUTE:
0.5 mL 45:55 acetonitrile.75 mM aqueous oxalic acid

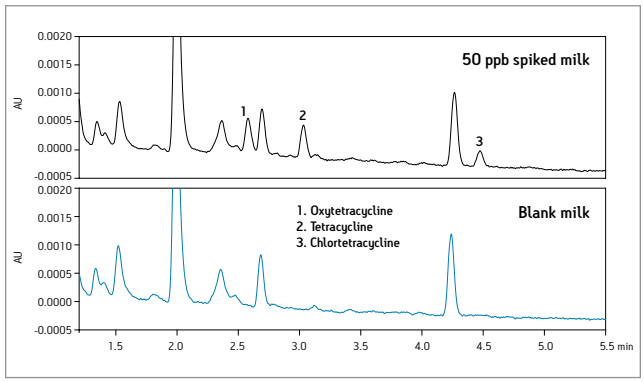
DILUTE:
1.5 mL with reagent-grade water

LC CONDITIONS

System:	Alliance® 2690/5 HPLC with 2998 PDA Detector
Column:	XBridge® BEH C_{18} ***XP***, 2.5 µm, 4.6 x 100 mm
Injection volume:	35 µL
Column temp.:	30 °C
Mobile phase A:	10 mL oxalic acid in water
Mobile phase B:	10 mM oxalic acid in acetonitrile
Flow rate:	1.20 mL/min
Gradient:	15% B initial, linear gradient to 50% B in 8.00 minutes, hold until 11.25 minutes, back to 15% B at 11.60 minutes. Hold and re-equilibrate until 12.85 minutes.
UV detection:	PDA (extracted 355 nm)

RESULTS

Method recovery was greater than 80% for all tetracyclines. A typical HPLC-UV chromatogram (355 nm) obtained from analysis of a sample spiked with 50 ppb (ng/g) of three tetracyclines.



*A typical HPLC/UV chromatogram (355 nm extracted wavelength, **XP** Column) obtained from analysis of a sample spiked with 50 ppb (ng/g) of three tetracyclines.*

ORDERING INFORMATION

Description	Part Number
XBridge BEH C_{18} ***XP***, 2.5 µm, 4.6 x 100 mm	186006039
Oasis MAX 1 cc, 30 mg Cartridge	186000366

Ref: Waters Application Note 720004582EN

FOOD TESTING AND QUALITY CONTROL (QC)

The methods in this section may be used for simple QC testing or to monitor for adulteration. The methods offer:

- Sample extraction
- Sample preparation
- Chromatographic conditions

INTRODUCTION

Amino acid analysis has been used in the food and feed industries to verify and characterize materials and processes. The total amino acid content, as well as the proportions of growth-limiting amino acids, is an essential characteristic of the nutritional value of feeds.

SAMPLES

Swine diet, poultry diet, whole soybean, and soybean meal samples were acid-hydrolyzed in an independent laboratory as part of a collaborative study. The samples were supplied at an estimated concentration of 1.0 mg/mL in 0.1 M HCl and sealed under argon in ampoules. Samples were stored at -80 °C until analysis. The standard was NIST 2389 Amino Acids in 0.1 mol/L HCl Reference Material, and it was diluted to 5, 100, and 250 pmol/µL.

SAMPLE DERIVATIZATION

The samples were diluted 1:16 with 0.1 M HCl prior to derivatization. The standard derivatization protocol was modified to include neutralization of excess acid with 0.1 M NaOH. Conditions for pre-column derivatization and analysis are described in detail in the Waters UPLC® Amino Acid Analysis Solution System Guide (P/N 71500129702). These derivatization conditions were modified to include additional base.

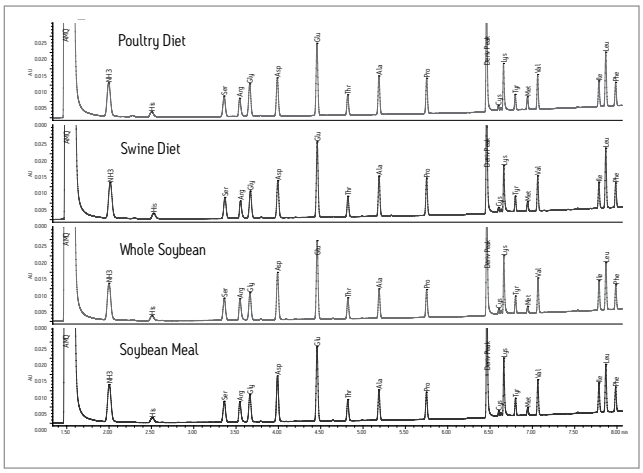
1. 60 µL AccQ•Tag™ Ultra Borate Buffer
2. 10 µL diluted sample
3. 10 µL 0.1 N NaOH
4. 20 µL reconstituted AccQ•Tag Ultra Reagent

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	AccQ•Tag Ultra, 1.7 µm, 2.1 x 100 mm
Column temp.:	55 °C
Sample temp.:	20 °C
Flow rate:	700 µL/min
Mobile phase A:	1:20 Dilution of AccQ•Tag Ultra Eluent A with MilliQ® water (prepared fresh daily)
Mobile phase B:	AccQ•Tag Ultra Eluent B
Gradient:	AccQ•Tag Ultra Hydrolysate Method (provided in the UPLC Amino Acid Analysis Solution)
Total run time:	9.5 minutes
Injection volume:	1 µL, partial loop with needle overfill
Detection:	UV (TUV), 260 nm

Amino Acid	% RSD
His	1.03
Ser	0.43
Arg	0.60
Gly	0.39
Asp	0.24
Glu	0.23
Thr	0.26
Ala	0.27
Pro	0.30
Cys	0.18
Lys	0.23
Tyr	0.17
Met	0.21
Val	0.22
Ile	0.23
Leu	0.24
Phe	0.23

Summary of retention times, in minutes, for the hydrolysate standard throughout the five days of analyses.



Animal feed hydrolysates, 6 ng on column, using the UPLC Amino Acid Analysis Solution.

Amino Acid	Poultry Diet	Swine Diet	Whole Soybean	Soybean Meal	Mean	Std. Dev.
His	2.513	2.504	2.526	2.534	2.521	0.016
Ser	3.363	3.358	3.370	3.373	3.367	0.008
Arg	3.546	3.544	3.553	3.555	3.551	0.006
Gly	3.665	3.661	3.671	3.673	3.668	0.006
Asp	3.990	t3.987	3.995	3.997	3.993	0.005
Glu	4.457	4.455	4.461	4.462	4.459	0.004
Thr	4.820	4.819	4.823	4.823	4.822	0.002
Ala	5.189	5.187	5.190	5.191	5.189	0.002
Pro	5.752	5.751	5.750	5.751	5.751	0.001
Cys	6.599	6.601	6.602	6.603	6.602	0.001
Lys	6.661	6.663	6.662	6.663	6.663	0.001
Tyr	6.799	6.800	6.801	6.801	6.801	0.001
Met	6.944	6.945	6.945	6.945	6.945	0.000
Val	7.064	7.065	7.064	7.066	7.065	0.001
Ile	7.787	7.788	7.787	7.787	7.787	0.001
Leu	7.868	7.869	7.868	7.869	7.869	0.001
Phe	7.985	7.985	7.985	7.986	7.985	0.001

Retention time summary, in minutes, for the different sample types from one day of analyses. Each reported value represents the mean value of fifteen injections.

ORDERING INFORMATION

Description	Part Number
Amino Acid Analysis Kit	176001235
AccQ•Tag Ultra Column, 1.7 µm, 2.1 x 100 mm	186003837
Amino Acid Standard, Hydrolysate, 10 x 1 mL ampules	WAT088122
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720002804EN

INTRODUCTION

Free amino acids can exist as nutrients, metabolic intermediates, or as waste products to a biological process and can be used to identify the genotype and origin of a plant. In a similar fashion, amino acids can be used to relate a profile to valuable characteristics in high value foods. By monitoring the free amino acids during and after a process, it is possible to identify and control physiology that affects the product quality.

As an example of this application, we have followed a yeast fermentation in the production of a beer. Characterization of raw materials is demonstrated with the free amino analysis of three different starting fermentation barley malts. Each of these malt varieties were then carried through a fermentation process, and the changes in free amino acid levels were observed at different stages of the fermentation.

SAMPLE HANDLING

Starting Barley Malts

- 2-row Pale Malt; 6-row Pale Malt; 2-row Pilsener Malt
- Samples were collected from early suspension of malts and stored at -80 °C prior to analysis.
- Each thawed sample was centrifuged for one minute at 16110 RCF x g, then the supernatant was diluted 1:10 with 0.1 M HCl.
- Derivatization volumes: 70 µL Borate buffer, 10 µL diluted sample, 20 µL AccQ•Tag™ Ultra reagent

Brewing Fermentation

- Samples: Beginning, 24 hour, day 4, and end of primary fermentation
- Samples were drawn from fermentor at intervals during primary fermentation and stored at -80 °C prior to analysis.
- Each thawed sample was centrifuged for one minute at 16110 RCF x g, then the supernatant was diluted 1:10 with 0.1 M HCl.
- Derivatization volumes: 70 µL Borate buffer, 10 µL diluted sample, 20 µL AccQ•Tag Ultra reagent

Commercial Pale Ales

- Two lots each of two brands of pale ale
- Samples were collected from 12 oz. bottles, and stored at -80 °C prior to analysis.
- Each thawed sample was centrifuged for one minute at 16110 RCF x g, then the supernatant was diluted 1:10 with 0.1 M HCl.
- Derivatization volumes: 50 µL Borate buffer, 10 µL diluted sample, 20 µL 0.1 M NaOH (to neutralize excess acid), 20 µL AccQ•Tag Ultra reagent

SAMPLE DERIVATIZATION

The derivatization reagent reacts with both primary and secondary amines at an optimal pH of 8.5. The batch-derivatized samples are stable at room temperature for up to one week when tightly capped. Conditions for pre-column derivatization and analysis are described in detail in the Waters UPLC Amino Acid Analysis Solution System Guide (P/N 71500129702).

LC CONDITIONS

System:	ACQUITY UPLC®
Column:	AccQ•Tag Ultra, 1.7 µm, 2.1 x 100 mm
Column temp:	60 °C
Sample temp:	20 °C
Flow rate:	700 µL/min
Mobile phase A:	1:10 Dilution of AccQ•Tag Ultra Eluent A with Milli-Q® water
Mobile phase B:	AccQ•Tag Ultra Eluent B
Weak needle wash:	95:5 Water:Acetonitirile
Strong needle wash:	5:95 Water:Acetonitrile
Gradient:	AccQ•Tag Ultra Cell Culture Method (provided in the UPLC Amino Acid Analysis Solution)
Total run time:	9.5 minutes
Injection volume:	1 µL, Partial loop with needle overfill
Detection:	UV (TUV), 260 nm

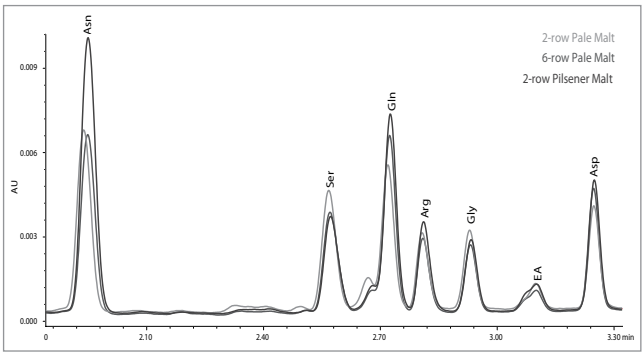


Figure 2: Amino acid differences between the different starting barley malts.

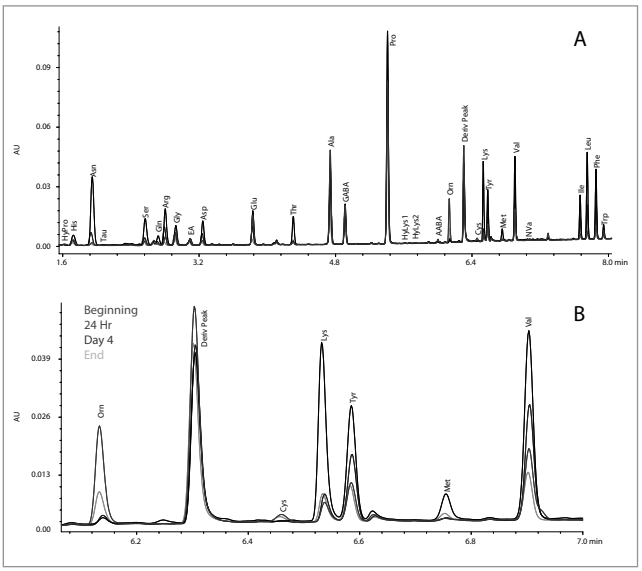


Figure 3: (A) Amino acid levels at various stages of a primary beer fermentation process, using the UPLC Amino Acid Analysis Solution. (B) Magnified region showing time dependent changes throughout the fermentation process.

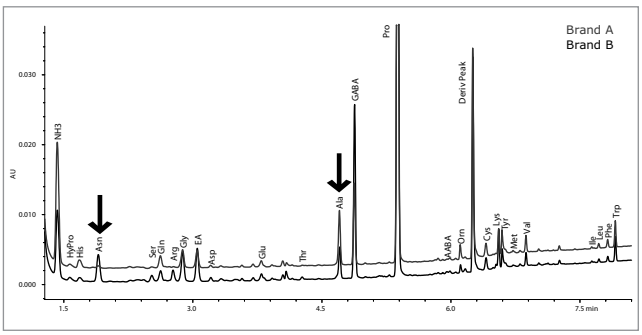


Figure 4: Different free amino acid profile of two commercially-produced pale ales. Arrows indicate significant differences between brands.

Table 1: Lot-to-lot variability for one brand of pale ale. The values (expressed as pmoles/µL sample) include triplicate injections of two derivatizations.

	Lot 1		Lot 2	
Amino Acid	Mean	Std. Dev.	Mean	Std. Dev.
His	81.25	0.85	91.40	1.35
Asn	18.83	0.69	19.18	0.46
Ser	12.45	0.91	11.55	0.62
Arg	5.94	0.46	4.49	0.26
Gly	156.58	3.02	169.21	1.43
EA	146.13	2.40	156.45	4.50
Asp	16.42	1.27	14.11	0.22
Glu	49.06	1.47	49.22	0.40
Thr	5.58	1.61	3.54	0.12
Ala	357.08	6.09	342.39	1.89
GABA	475.44	8.68	487.51	2.22
Pro	3479.25	42.23	4091.81	14.83
Orn	36.70	1.26	45.19	0.34
Cys	41.75	0.52	46.05	0.39
Tyr	100.27	1.99	99.40	0.39
Met	13.12	1.64	12.91	2.13
Val	101.21	1.73	73.73	0.76
Ile	12.87	0.39	9.49	0.34
Leu	26.03	0.41	19.58	0.31
Phe	41.36	0.55	34.08	0.29
Trp	104.12	1.88	107.50	1.17

ORDERING INFORMATION

Description	Part Number
Amino Acid Analysis Kit	176001235
AccQ•Tag Ultra Column, 1.7 µm, 2.1 x 100 mm	186003837
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720003357EN

INTRODUCTION

L-theanine is a free (non-protein) amino acid found almost exclusively in tea plants. It is the predominant amino acid in green tea leaves, giving tea its characteristic taste.

SAMPLE PREPARATION

Free amino acids were analyzed in tea leaves. Tea leaves were standard consumer single serving products. The tea samples were between 2.5-3.5 g. The tea leaves were extracted in 6 oz. of bottled water at an initial temperature of 72 °C for 2 hours, unless otherwise specified. After a set period of time, the supernatant of the mixture was transferred to a separate vial. Extracted tea samples in water were stored at -20 °C until analysis.

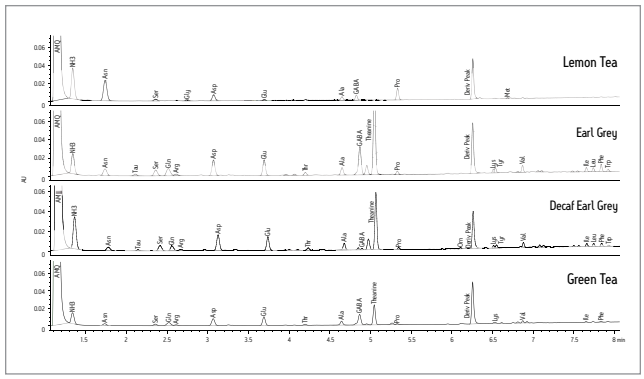
SAMPLE DERIVATIZATION

The extracted tea samples were derivatized neat following the standard AccQ•Tag™ Ultra derivatization protocol. Conditions for pre-column derivatization and analysis are described in detail in the Waters UPLC® Amino Acid Analysis Solution System Guide (P/N 71500129702). The derivatization conditions included:

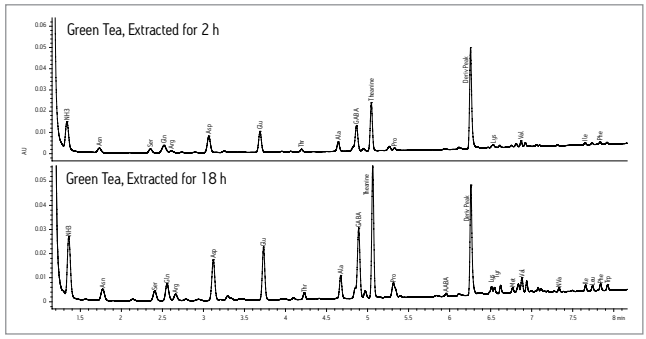
1. 70 µL of AccQ•Tag Ultra borate buffer
2. 10 µL of tea extract
3. 20 µL of derivatization reagent

LC CONDITIONS

System:	ACQUITY UPLC® with TUV
Column:	AccQ•Tag Ultra, 1.7 µm, 2.1 x 100 mm
Column temp.:	60 °C
Sample temp.:	20 °C
Flow rate:	700 µL/min
Mobile phase A:	1:10 dilution of AccQ•Tag Ultra Eluent A concentrate with MilliQ® water
Mobile phase B:	AccQ•Tag Ultra Eluent B
Gradient:	AccQ•Tag Ultra Cell Culture Method (provided in the UPLC Amino Acid Analysis Solution)
Total run time:	9.5 minutes
Injection volume:	1 µL, partial loop with needle overfill
Detection:	UV (TUV), 260 nm



Extracted teas using the UPLC Amino Acid Analysis Solution. Theanine was confirmed by MS detection. Amino acids levels vary with type of tea.



Two separate aliquots of a single type of green tea using the UPLC Amino Acid Analysis Solution. Each aliquot was extracted for 2 or 18 hours. Theanine (5.1 minutes) was confirmed by MS detection. All amino acid levels increase with duration of extraction.

ORDERING INFORMATION

Description	Part Number
AccQ•Tag Ultra Column, 1.7 µm, 2.1 x 100 mm	186003837
Amino Acid Standard, Hydrolysate, 10 x 1 mL ampules	WAT088122
LCMS Certified Vials	600000751CV

INTRODUCTION

In this application, UPLC®/MS is used for the rapid and direct analysis of simple and complex carbohydrates in a variety of sample matrices. The stability of the ACQUITY UPLC® BEH Amide column allows the use of high pH mobile phases that improve chromatographic resolution and enhance MS signal intensity without the need for derivatization, metal complexing agents, or post-column addition.

SAMPLE PREPARATION

The food sugar standard (fructose, glucose, sucrose, maltose, and lactose) was prepared at 10 µg/mL in 50/50 ACN/H_2O. Other standards were prepared in 50/50 ACN/H_2O at the specified concentrations. Beer samples were prepared by diluting 1:1 with 50/50 ACN/H_2O. Natural sweetener powder was dissolved in 50/50 ACN/H_2O. Maple syrup samples were prepared at a concentration of 0.5 to 1 mg/mL in 50/50 ACN/H_2O. All samples were filtered using a 0.45 µm PVDF syringe filter.

LC CONDITIONS

System:	ACQUITY UPLC with ACQUITY® SQD
Data system:	MassLynx® version 4.1
Columns:	ACQUITY UPLC BEH Amide, 1.7 µm, 2.1 x 50 mm, 1.7 µm, 2.1 x 100 mm, or 1.7 µm, 2.1 x 150 mm
Weak needle wash:	75/25 ACN/H_2O (500 µL)
Strong needle wash:	20/80 ACN/H_2O (800 µL)
Seal wash:	50/50 ACN/H_2O
Injection mode:	Partial loop with needle overfill (PLNO)

Mobile phases, flow rate, gradient conditions, column temperature, and Injection volume are all listed in figure captions.

MS CONDITIONS

Ionization mode:	ES-
Capillary:	2.8 kV
Cone:	25 V
Source temp.:	120 °C
Desolvation temp.:	350 °C
Desolvation gas:	500 L/hr
Cone gas:	50 L/hr
Dwell time:	40 or 80 ms
	SIR m/z: 179.2 (fructose, glucose) 341.3 (sucrose, maltose, lactose) 503.4, 665.5, 827.6, 989.7, 1151.8 (maltooligosaccharides n=1 to 5) 121.1 (erythritol), 803.8 (stevioside), 950.1 (rebaudioside C), 966.1 (rebaudioside A)

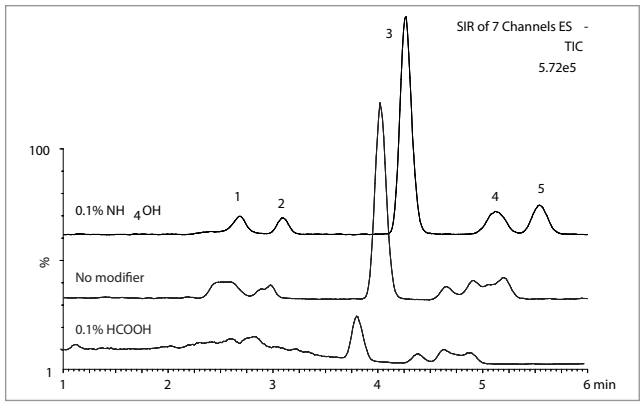


Figure 1. UPLC/MS analysis of carbohydrates in 75/25 ACN/H_2O containing formic acid, no modifier, and ammonium hydroxide. The flow rate is 0.13 mL/min, the column temperature is 35 °C, and the Injection volume is 0.7 µL. The concentration of each analyte is 10 µg/mL in 50/50 ACN/H_2O. The column dimensions are 2.1 x 50 mm, 1.7 µm. Peaks: (1) fructose, (2) glucose, (3) sucrose, (4) maltose, and (5) lactose.

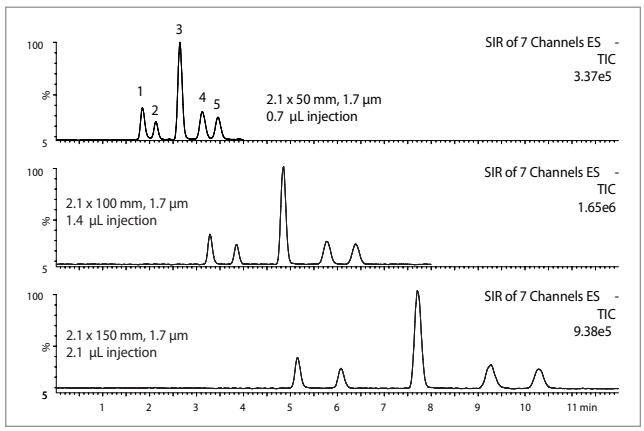


Figure 2. UPLC/MS analysis of carbohydrates using acetone as the organic solvent. The isocratic mobile phase was 77/23 acetone/H_2O with 0.05% NH_4OH. The flow rate is 0.13 mL/min and the column temperature is 85 °C. The column dimensions and Injection volumes are shown on the figure. The sample and peak elution order is identical to Figure 1.

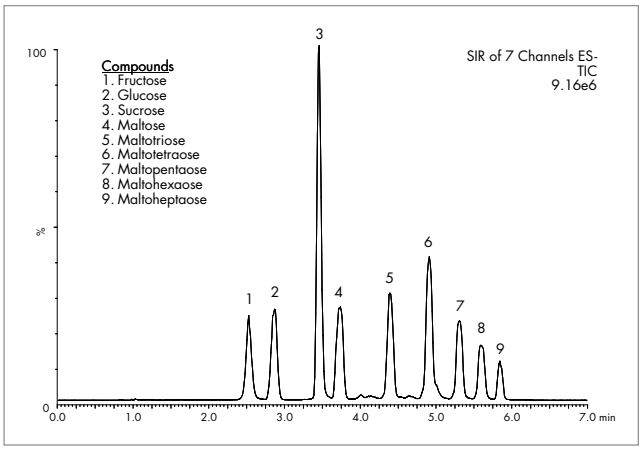


Figure 3. UPLC/MS separation of mono-, di-, and oligosaccharides. Mobile phase A is 80/20 ACN/H_2O with 0.1% NH_4OH. Mobile phase B is 30/70 ACN/H_2O with 0.1% NH_4OH. Gradient from 0 to 60 % B in 5 minutes, reset and equilibrate for 10 minutes. The flow rate is 0.17 mL/min, the column temperature is 35 °C, and the Injection volume is 0.7 µL. The concentration of each analyte is 10 µg/mL in 50/50 ACN/H_2O. The column dimensions are 2.1 x 50 mm, 1.7 µm.

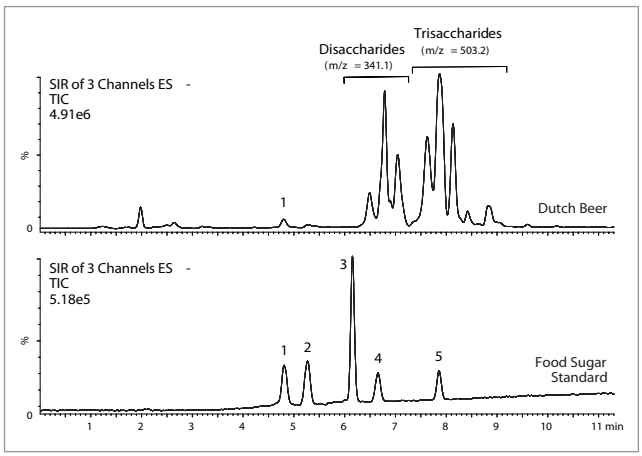


Figure 4. UPLC/MS analysis of carbohydrates in beer. Mobile phases are identical to Figure 3. Gradient from 10 to 70 % B in 10 minutes, reset and equilibrate for 25 minutes. The flow rate is 0.13 mL/min, the column temperature is 35 °C, and the Injection volume is 2 µL. The concentration of each analyte in the standard is 10 µg/mL in 50/50 ACN/H_2O. The column dimensions are 2.1 x 100 mm, 1.7 µm. Peaks: (1) fructose, (2) glucose, (3) sucrose, (4) maltose, and (5) maltotriose.

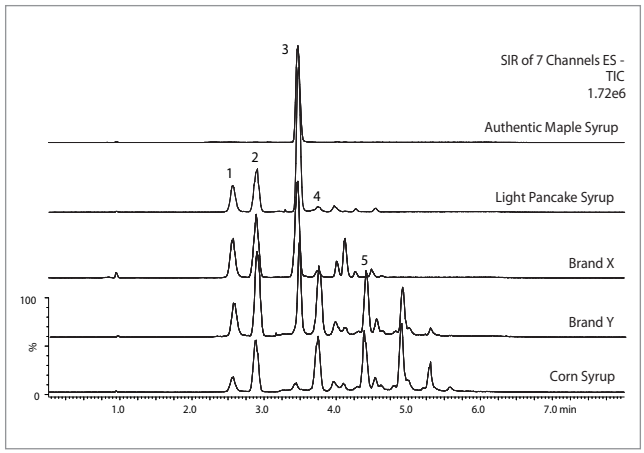


Figure 5. UPLC/MS analysis of carbohydrates in different maple syrups. Conditions are identical to Figure 3. Peaks: (1) fructose, (2) glucose, (3) sucrose, (4) maltose, and (5) maltotriose.

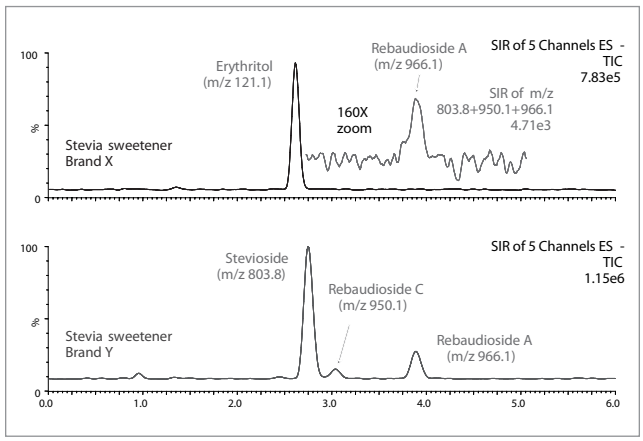


Figure 6. UPLC/MS analysis of two commercially-available stevia-related sweeteners. Isocratic mobile phase of 77.5/22.5 ACN/H_2O with 0.1% NH_4OH. The flow rate is 0.2 mL/min, the column temperature is 35 °C, and the Injection volume is 1.3 µL. The concentration of the Brand Y product is 50 µg/mL each in 50/50 ACN/H_2O. The Brand X product is prepared at 10 µg/mL in 50/50 ACN/H_2O. The column dimensions are 2.1 x 100 mm, 1.7 µm.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC BEH Amide, 1.7 µm, 2.1 x 50 mm Column	186004800
ACQUITY UPLC BEH Amide, 1.7 µm, 2.1 x 100 mm Column	186004801
ACQUITY UPLC BEH Amide, 1.7 µm, 2.1 x 150 mm Column	186004802
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720003212EN

INTRODUCTION

In this application note, an SPE method for the simultaneous extraction of fat-soluble vitamins from fortified food products was developed. The proposed method was applied for the extraction of FSVs from breakfast cereal, infant formula, and chocolate. A rapid six-minute UPLC®/MS/MS method using positive atmospheric pressure chemical ionization (APCI) was utilized for the analysis of the fat-soluble vitamin compounds.

LC CONDITIONS

System:	ACQUITY UPLC®		
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 100 mm		
Column temp.:	40 °C		
Sample temp.:	24 °C		
Flow rate:	0.6 mL/min		
Mobile phase A:	90:10 acetonitrile: water		
Mobile phase B:	Methanol		
Gradient:	Time (min)	%A	%B
	0.0	100	0
	0.5	100	0
	2.5	0	100
	4.5	0	100
	5.0	100	0
	6.0	100	0
Total run time:	6 minutes		
Injection volume:	5 µL, PLNO		

MS CONDITIONS

MS System:	Xevo® TQ-S
Ionization mode:	APCI positive
Corona current:	15 µA
Extractor:	3.0 V
Source temp.:	150 °C
Probe temp.:	550 °C
Desolvation gas:	1000 L/Hr
Acquisition:	RADAR [multiple reaction monitoring (MRM) with full scan]
Collision gas:	Argon at 3.5 x 10^{-3} mbar

SPE PROTOCOL

Food matrix + 2 mL ethanol, Vortex/sonicate

Centrifuge at 6000 RPM
Add 0.5 mL Milli-Q® water to 1 mL supernatant

SPE: Waters Oasis HLB Cartridge

CONDITION:
1 mL methanol

EQUILIBRATE:
1 mL Milli-Q water

LOAD:
0.5 mL sample matrix

WASH:
1.5 mL 5% methanol

ELUTION 1:
1 mL 1:1 IPA:ACN

ELUTION 2:
1 mL 20% ethyl acetate in ACN

Figure 1. Illustration of extraction protocol.

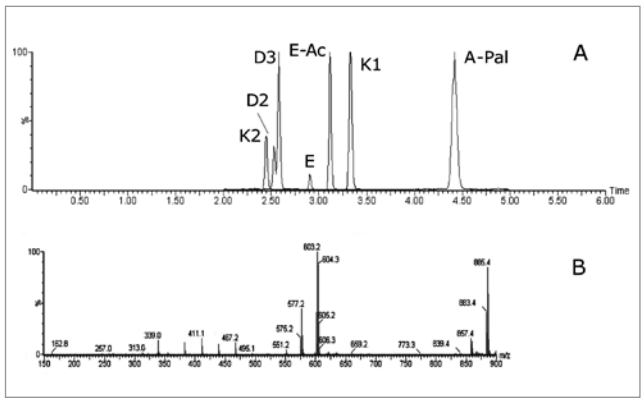


Figure 2. Acquired MRMs for the FSVs in infant formula (A) and full scan data of infant formula extract (B).

BREAKFAST CEREAL			
Vitamin	Spike concentration (ng/mL)	Intra-day variation (n=6)	Inter-day variation (n=3)
		Average %Recovery ± %RSD	Average %Recovery ± %RSD
A-palmitate	100	90.8 ± 2.2	93.5 ± 5.1
D2	100	100.6 ± 2.0	106.9 ± 5.1
D3	100	103.0 ± 2.6	104.7 ± 9.9
E	10	98.5 ± 2.1	102.3 ± 4.1
E-acetate	10	103.1 ± 1.4	107.4 ± 7.0
K1	10	100.7 ± 1.4	99.0 ± 0.6
K2	10	99.1 ± 3.0	99.6 ± 3.6

Table 3. FSVs spiked %recovery in breakfast cereal.

INFANT FORMULA			
Vitamin	Spike concentration (ng/mL)	Intra-day variation (n=6)	Inter-day variation (n=3)
		Average %Recovery ± %RSD	Average %Recovery ± %RSD
A-palmitate	100	101.8 ± 4.8	106.4 ± 4.1
D2	100	93.2 ± 3.0	99.0 ± 5.2
D3	100	91.5 ± 6.2	94.9 ± 5.0
E	10	105.1 ± 2.0	104.2 ± 6.8
E-acetate	10	94.1 ± 7.6	101.0 ± 7.4
K1	10	85.3 ± 6.0	97.3 ± 10.0
K2	10	81.8 ± 7.3	89.6 ± 8.0

Table 4. FSVs spiked %recovery in infant formula.

CHOCOLATE			
Vitamin	Spike concentration (ng/mL)	Intra-day variation (n=6)	Inter-day variation (n=3)
		Average %Recovery ± %RSD	Average %Recovery ± %RSD
A-palmitate	100	83.7 ± 6.1	83.8 ± 5.8
D2	100	82.9 ± 3.0	91.2 ± 8.1
D3	100	94.8 ± 6.6	95.9 ± 2.5
E	10	112.9 ± 6.0	103.5 ± 8.0
E-acetate	10	107.6 ± 5.0	99.0 ± 8.8
K1	10	84.6 ± 3.7	85.8 ± 8.1
K2	10	84.0 ± 1.2	85.2 ± 1.3

Table 5. FSVs spiked %recovery in chocolate.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC BEH C_{18} Column, 1.7 µm, 2.1 x 100 mm	186002352
Oasis HLB, 3 cc/60 mg Cartridge	WAT094226
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720004193EN

INTRODUCTION

Sugar analysis in foods is a standard food QC assay.

SAMPLE EXTRACTION PROCEDURE

1. Weigh out sample (~ 3g) into 50 mL centrifuge tube.
2. Add 25 mL of 50:50 acetonitrile/water and homogenize.
3. Centrifuge at 3200 rpm for 30 minutes.
4. Collect supernatant and filter using 0.45 µm PVDF syringe filter.

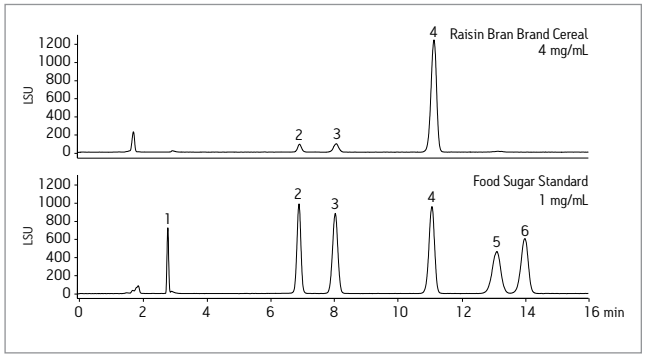
LC CONDITIONS

System:	Alliance® with 2424 EL SD
Column:	XBridge® Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Flow Profile:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Injection volume:	15.0 µL
Sample concentration:	1 mg/mL each
Column temp.:	35 °C
Needle Wash:	75:25 acetonitrile:water
Seal Wash:	50:50 acetonitrile:water

ELSD CONDITIONS

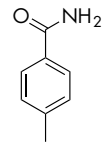
Gain:	100
Pressure:	30 psi
Drift tube temp.:	50 °C
Nebulizer:	Cooling
Data rate:	10 pps
Filter time constant:	0.2 seconds

RESULTS

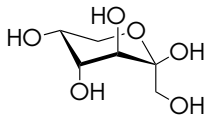


COMPOUNDS

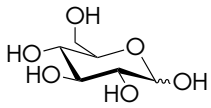
ß 1. p-Toluamide



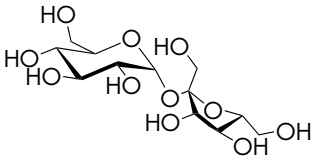
ß 2. Fructose



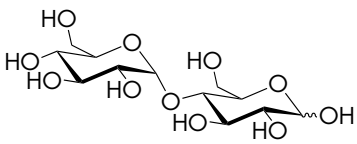
ß 3. Glucose



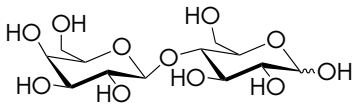
ß 4. Sucrose



ß 5. Maltose



ß 6. Lactose



ORDERING INFORMATION

Description	Part Number
XBridge Amide Column, 3.5 µm, 4.6 x 250 mm	186004870
Acrodisc® LC 13 mm, 0.45 µm, 100/pkg	WAT200512
12 x 32 LCMS Certified Glass Screw Neck Vial	600000751CV

INTRODUCTION

This is a simple assay to monitor sugar levels in milk. There is a simple sample extraction procedure provide, followed by LC/ELSD assay.

SAMPLE EXTRACTION PROCEDURE

1. Dilute with 50:50 acetonitrile/water.
2. Filter using 0.45 µm PVDF syringe filter.

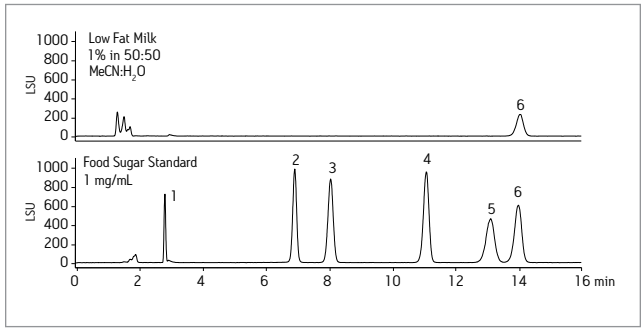
LC CONDITIONS

System:	Alliance® with 2424 EL SD
Column:	XBridge® Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Flow Profile:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Injection volume:	15.0 µL
Sample concentration:	1 mg/mL each
Column temp.:	35 °C
Needle Wash:	75:25 acetonitrile:water
Seal Wash:	50:50 acetonitrile:water

ELSD CONDITIONS

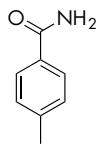
Gain:	100
Pressure:	30 psi
Drift tube temp.:	50 °C
Nebulizer:	Cooling
Data rate:	10 pps
Filter time constant:	0.2 seconds

RESULTS

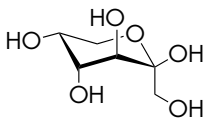


STRUCTURES

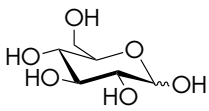
1. p-Toluamide



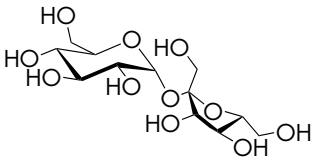
2. Fructose



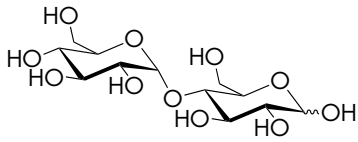
3. Glucose



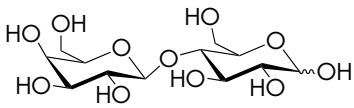
4. Sucrose



5. Maltose



6. Lactose



ORDERING INFORMATION

Description	Part Number
XBridge Amide Column, 3.5 µm, 4.6 x 250 mm	186004870
Acrodisc® LC 13 mm, 045 µm, 100/pkg	WAT200512
12 x 32 LCMS Certified Glass Screw Neck Vial	600000751CV

INTRODUCTION

This application brief contains a simple extraction protocol and LC/UV conditions for ginsenoside Rb1 in ginseng root. The gensenosides are a target of research in the root.

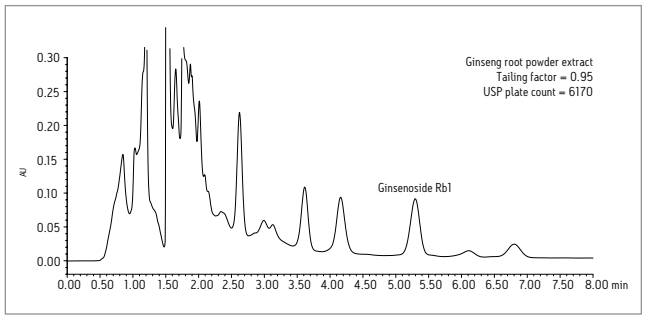
SAMPLE EXTRACTION PROCEDURE

1. Weigh 200 mg ginseng root powder into an extraction vessel.
2. Add 1 mL 80% methanol, sonicate for 5 minutes.
3. Centrifuge at 10,000 rpm for 5 minutes.
4. Collect the supernatant.
5. Repeat steps 2-4 two more times.
6. Combine the extracts, mix well.
7. Filter through 13 mm nylon 0.2 µm filter for injection.

LC CONDITIONS

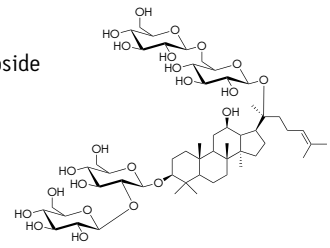
System:	Alliance® with 2998 PDA
Column:	XBridge® Amide, 3.5 µm, 4.6 x 150 mm
Mobile phase:	80:20 acetonitrile/water
Isocratic flow rate:	1.4 mL/min
Injection volume:	11.5 µL
Column temp.:	60 °C
Sample temp.:	10 °C
Needle wash:	95:5 acetonitrile:water
Seal wash:	10:90 acetonitrile:water
UV wavelength:	203 nm
Sampling rate:	20 Hz
Filter timeconstant:	0.1 seconds

RESULTS



STRUCTURE

ß Ginsenoside Rb1



ORDERING INFORMATION

Description	Part Number
XBridge Amide Column, 3.5 µm, 4.6 x 150 mm	186004869
Acrodisc® LC 13 mm, 045 µm, 100/pkg	WAT200512
12 x 32 LCMS Certified Glass Screw Neck Vial	600000751CV

INTRODUCTION

Adulteration of food and beverages is a significant problem that involves many different edible products. For this work, pineapple juice samples were analyzed using UltraPerformance Liquid Chromatography (UPLC®) for high resolution separations, photo diode array (PDA) detection, and accurate mass MS and MS/MS. Data interpretation involved the use of multi-variate analysis (MVA) and database searching in order to easily identify any key differences between authentic and adulterated pineapple juices.

PRETREATMENT

All pineapple samples were centrifuged, filtered, and diluted before analysis using Waters® ACQUITY UPLC® System with PDA detection, coupled with a Xevo® G2 Quadrupole Time-of-flight Mass Spectrometer (QTof MS).

UPLC CONDITIONS

System:	ACQUITY UPLC		
Column:	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm		
Column temp.:	45 °C		
Injection volume:	3 µL		
Flow rate:	0.4 mL/min		
Mobile phase A:	10 mM ammonium acetate in water		
Mobile phase B:	Acetonitrile		
Gradient:	Time (min)	%A	%B
	0.00	99	1
	0.75	99	1
	2.00	95	5
	3.00	95	5
	6.50	45	55
	8.50	10	90
	9.00	10	90
	9.10	99	1

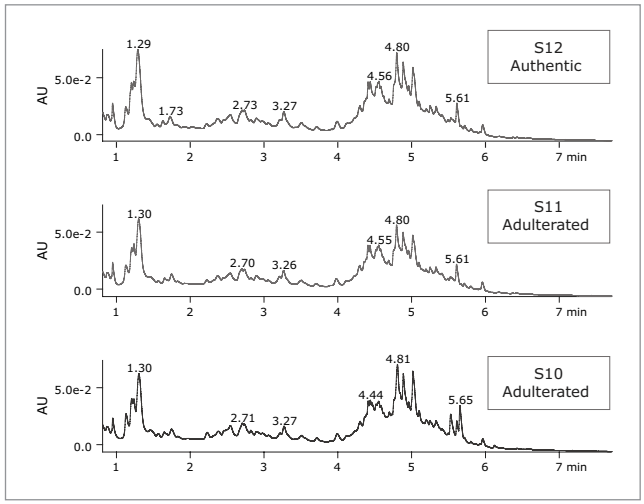
UV CONDITIONS

UV System:	ACQUITY® PDA Detector
Range:	210 to 500 nm
Sampling rate:	20 pts/s

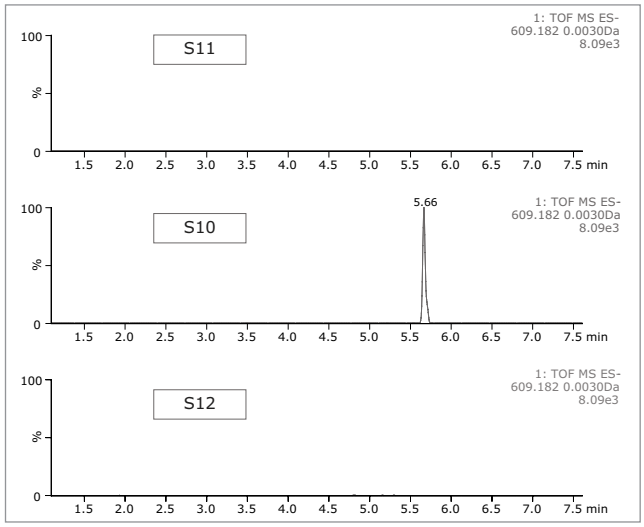
QTof MS CONDITIONS

MS system:	Xevo G2 QTof MS
Ionization mode:	ESI Negative (ESI-)

RESULTS



UV comparison (extracted wavelength – 283 nm) of three pineapple juices: S10, S11, and S12 (authentic sample).



Extracted ion chromatograms (XIC) for m/z 609.1816 (hesperidin) in S10, S11, and S12.

ORDERING INFORMATION

Description	Part Number
ACQIUTY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm	186003539

Ref: Waters Application Note 720004173EN

INTRODUCTION

Polyphenols are widely recognized as functional components of food and beverage products, such as tea, wine, and fruits. Polyphenols are reported to play a potential role in protecting human health from degenerative and cardiovascular diseases.

SAMPLE EXTRACTION PROCEDURE

Each juice sample was filtered through a 0.45 µm filter and diluted with an equal amount of water.

LC CONDITIONS

System:	ACQUITY UPLC H-Class
Mobile phase A:	Water + 0.1% acetic acid
Mobile phase B:	Acetonitrile + 0.1% acetic acid

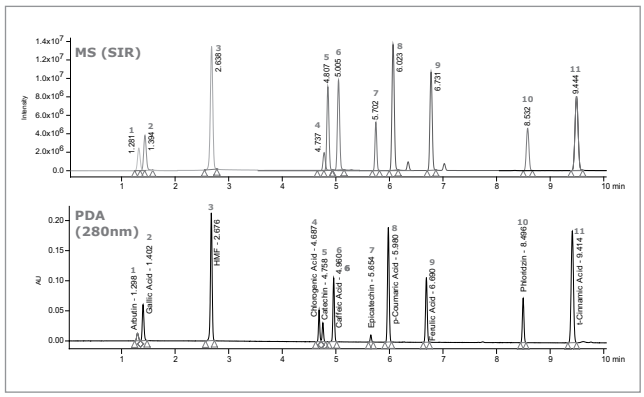
METHOD 1

Column:	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm		
Column temp:	45 °C		
Flow rate:	0.65 mL/min		
Total run time:	15.0 minutes		
Gradient:	Time (min)	%A	%B
	Initial	99	1
	1.0	99	1
	10.0	70	30
	12.0	5	95
	12.1	99	1
	15.0	99	1

METHOD 2

Column:	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 50 mm		
Column temp.:	45 °C		
Flow rate:	0.80 mL/min		
Total run time:	7.0 minutes		
Gradient:	Time (min)	%A	%B
	Initial	99	1
	0.5	99	1
	4.0	70	30
	5.5	5	95
	5.6	99	1
	7.0	99	1

RESULTS



Simultaneous acquisition of PDA and MS (SIR) data for a 10 ppm standard mix in a single analysis.

		Retention times (min)		% RSD for Method 1 (MS)	
Peak	Compound	Method 1	Method 2	Retention time	Peak area
1	Arbutin	1.30	0.57	0.10	0.26
2	Gallic acid	1.40	0.64	0.06	0.20
3	5-hydroxy-methyl-2-furaldehyde (HMF)	2.68	1.23	0.19	0.26
4	Chlorogenic acid	4.69	2.12	0.29	0.37
5	Catechin	4.76	2.13	0.29	0.37
6	Caffeic acid	4.96	2.22	0.27	0.34
7	Epicatechin	5.65	2.45	0.22	0.31
8	p-Coumaric acid	5.98	2.59	0.20	0.27
9	Ferulic acid	6.69	2.84	0.17	0.24
10	Phloridzin	8.50	3.39	0.13	0.18
11	t-Cinnamic acid	9.41	3.78	0.11	0.75

Retention times and repeatability results.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 50 mm	186003538
ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm	186003539

Ref: Waters Application Note 720004069EN

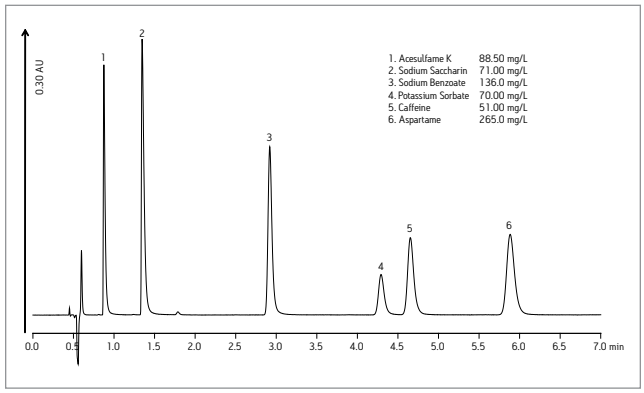
INTRODUCTION

The soft drink market is an important worldwide business that generates profits for several major manufacturers. To ensure consistency of products and to satisfy quality control requirements, accurate quantification of additives is essential. Six additives that are commonly used are sodium benzoate and potassium sorbate as preservatives; acesulfame K; aspartame and saccharin for sweetness (diet beverages); and caffeine. Some or all of these compounds may be present depending on the formulation of a particular beverage.

LC CONDITIONS

System:	ACQUITY UPLC H-Class
Run time:	7.0 minutes
Column:	ACQUITY UPLC BEH Phenyl, 1.7 µm, 2.1 x 100 mm
Column temp.:	35 °C
Mobile phase:	Waters' Beverage Mobile Phase (included in Kit)
Flow rate:	0.5 mL/min
Injection volume:	1 µL
Detection:	UV at 214 nm

RESULTS



Chromatogram of standard three.

Analyte	RT (min)	%RSD RT	Theoretical Amount	Measured Amount	%RSD Amount	% Deviation
Acesulfame K	0.87	0.04	88.5	89.0	0.24	0.56
Sodium Saccharin	1.35	0.03	71.0	71.3	0.21	0.42
Sodium Benzoate	2.92	0.01	136.0	136.3	0.28	0.22
Potassium Sorbate	4.30	0.01	70.0	70.7	0.32	1.00
Caffeine	4.67	0.01	51.0	51.3	0.30	0.59
Aspartame	5.90	0.01	265.0	265.4	0.42	0.15

Reproducibility data for seven injections of a QC standard.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC BEH Phenyl, 1.7 µm, 2.1 x 100 mm	186002885
Waters' Beverage Analysis Kit	176002534

Ref: Waters Application Note 720004016EN

INTRODUCTION

Micronutrient malnutrition is a global threat affecting more than one third of the world population. Of special significance is the fortification of vitamins in various food supplies. Food and drinks are the major source of vitamins and with the growing awareness for a balanced diet, consumption of fortified food has substantially increased.

LC CONDITIONS

System:	ACQUITY® SQD with PDA detector
Column:	ACQUITY UPLC® HSS Cyano, 1.8 µm, 2.1 x 50 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Column temp.:	30 °C
Gradient:	10% (B) for 0.36 minutes, 10-30% (B) in 3.6 minutes, hold at 30% (B) for 0.36 minutes, re-equilibrate at 10% (B) for 1.8 minutes between injections
Flow rate:	0.58 mL/min
Detection:	UV at 260 nm
Injection volume:	3 µL
Strong needle wash:	50/50 acetonitrile/water
Weak needle wash:	10/90 acetonitrile/water

These UPLC conditions were scaled directly from the 5 µm HPLC method using the ACQUITY UPLC Columns Calculator. The calculator can be used to scale these conditions back to the HPLC conditions, for both the 5 µm and 2.5 µm materials.

MS CONDITIONS

MS System:	Waters SQD
Ionization mode:	ESI positive
Acquisition range:	Single Ion Recording (SIR)
Capillary voltage:	3.19 kV
Cone voltage:	50 V
Desolvation gas:	600 L/hr
Cone gas:	0 L/hr
Source temp.:	100 °C
Desolvation temp.:	350 °C

Sample	Weight
Soy flour (cSRM)	104.2 mg
Soy tablet (cSRM)	111.9 mg
Soy protein isolate (cSRM)	365.6 mg
Soy protein concentrate (cSRM)	973.7 mg
Soy-based infant formula (commercially-available)	1119.2 mg

Table 1. Samples used for analysis

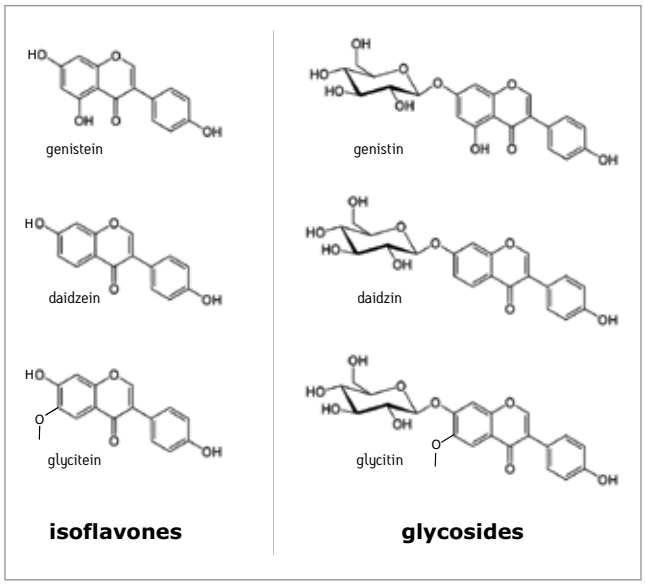


Figure 1. Structures of the three main soy isoflavones (genistein, daidzein, and glycitein) and their corresponding glycosides (genistin, daidzin, and glycitin).

SAMPLE PREPARATION

Standard Solution: Prepared from daidzin (25 ppm), glycitin (25 ppm), genistin (15 ppm), daidzein (25 ppm), glycitein (25 ppm), and genistein (15 ppm) using 10/90 acetonitrile/water diluent.

Samples

Candidate Standard Reference Materials were obtained from the National Institute of Standards and Technology. Each sample was weighed into 12 ml centrifuge tubes (Table 1). For each tube, 4 mL of 80/20 methanol/water was added followed by sonication for 1 hour. Tubes were centrifuged for 2 minutes at 3000 rpm. A 2 mL aliquot of supernatant was collected from each tube and filtered using a 0.45 µm GHP syringe filter prior to analysis. The remainder of the sample in each tube was hydrolyzed using 150 µL of 2 N sodium hydroxide. After mixing for 10 minutes, the solutions were neutralized with 50 µL of glacial acetic acid. The sample was again centrifuged for 5 minutes at 3000 rpm, with the collected supernatant filtered using a 0.45 µm GHP syringe filter prior to analysis.

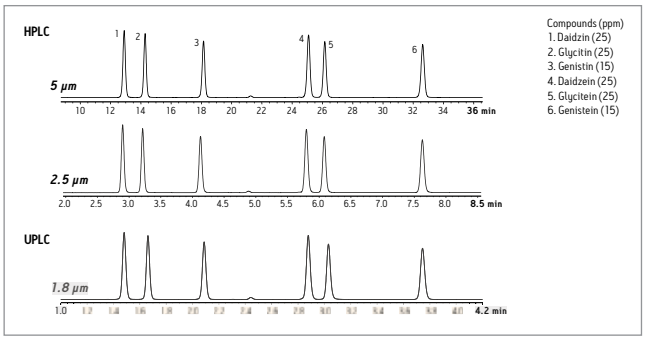


*Figure 2. HPLC and UPLC separations (UV) of soy isoflavone standards on cyano columns: XSelect® HSS Cyano, 5 µm, 4.6 x 150 mm (top); XSelect HSS Cyano **XP**, 2.5 µm, 4.6 x 75 mm (middle); and ACQUITY UPLC HSS Cyano, 1.8 µm, 2.1 x 50 mm (bottom). The gradient profiles, flow rates, and Injection volumes were scaled for each method using the ACQUITY UPLC Columns Calculator. The flow rates were 1.0 mL/min, 2.0 mL/min, and 0.58 mL/min, respectively.*

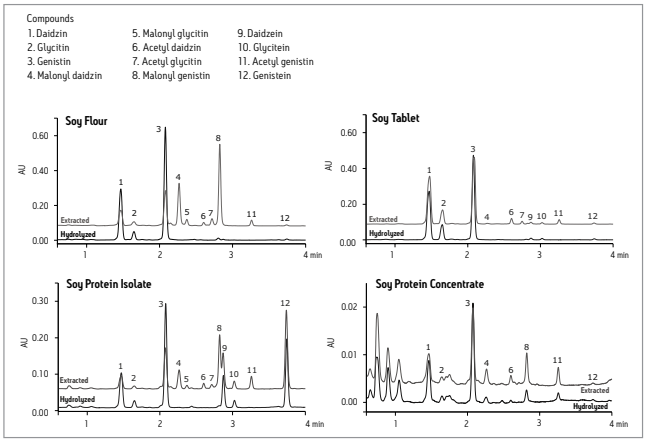


Figure 3. UPLC analysis of extracted (top chromatograms) and hydrolyzed (bottom chromatograms) candidate Standard Reference Materials (cSRMs).

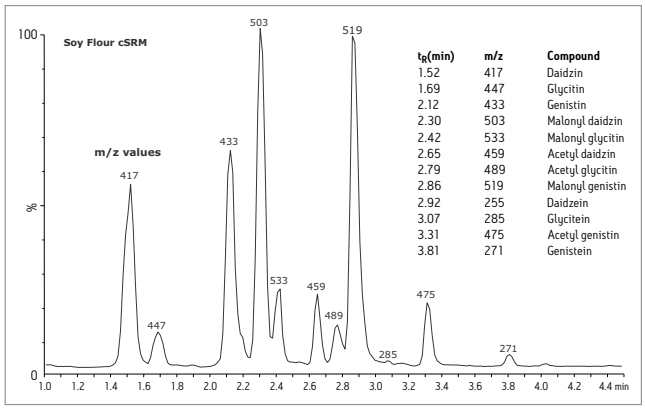


Figure 4. ESI+ LC/MS confirmation of peak identity for the Soy Flour cSRM, using single ion recording (SIR).

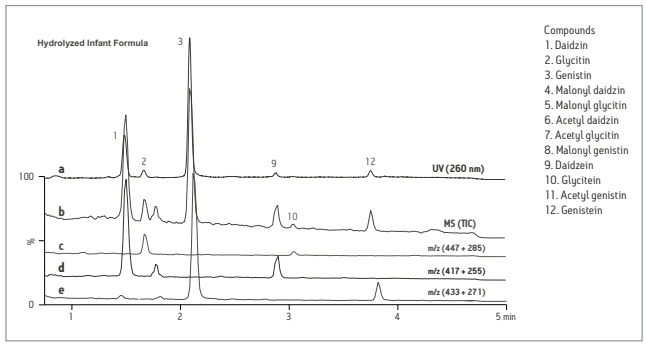


Figure 5. UPLC analysis of isoflavones in a commercially-available, soy-based infant formula (after hydrolysis); a) UV [260 nm], b) MS - Total Ion Chromatogram [TIC], c) extracted ion chromatogram for m/z 447 + 285 [glycitin and glycitein], d) extracted ion chromatogram for m/z 417 + 255 [daidzin and daidzein], and e) extracted ion chromatogram for m/z 433 + 271 [genistin and genistein].

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC HSS Cyano, 1.8 µm, 2.1 x 50 mm	186005986
XSelect HSS Cyano ***XP*** Column, 2.5 µm, 4.6 x 75 mm	186006194

Ref: Waters Application Note 720004193EN

INTRODUCTION

In this application note, we will show data on sugar content for several fruit juices along with their glucose/fructose ratios (G/F). Also we shall show the effect on these ratios of spiking orange juice with high fructose corn syrup (HFCS) at various levels.

LC CONDITIONS

System:	ACQUITY® UPLC H-Class			
Runtime:	10.0 minutes			
Column:	ACQUITY UPLC BEH Amide Column, 1.7 µm, 2.1 x 100 mm			
Column temp.:	85 °C			
Mobile phase A:	0.05% Triethylamine (TEA) dissolved in water			
Mobile phase B:	0.05% Triethylamine (TEA) dissolved in acetone			
Injection volume:	3 µL			
Gradient:	Time (min)	Flow Rate (mL/min)	%A	%B
	Initial	0.25	10	90
	1.0	0.25	10	90
	10.0	0.25	50	50
	10.1	0.25	10	90

ELSD CONDITIONS

Nitrogen flow:	40 psi
Drift tube:	55 °C
Nebulizer:	Cooling
Acquisition:	10 pts/sec
Gain:	50
Curve fit:	Quadratic

SAMPLE PREPARATION

Samples of various fruit juices were purchased at a local market. Aliquots of these juices were centrifuged at 4000 rpm for 30 minutes. The supernatant was collected and diluted 1:50 with 40:60 water/acetone and injected in triplicate.

Samples of orange juice were spiked with varying levels of either 55 HFCS or 42 HFCS and prepared as described above to determine the effect on the glucose /fructose and fructose / sucrose (F/S) ratios (mean of three measurements).

RESULTS

Juice	Fructose	Glucose	Sucrose	Sorbitol	Ratio Glucose/ Fructose
Orange	1.60	1.51	3.86	ND	0.94
White Grape	6.65	6.19	ND	ND	0.93
Pear	6.95	1.27	0.62	1.82	0.18
Apple	5.30	1.41	1.46	0.51	0.27
Pineapple	2.73	2.85	3.37	ND	1.04

Table 2. Sugar content of various fruit juices; units are g/100 mL; ND=not detected.

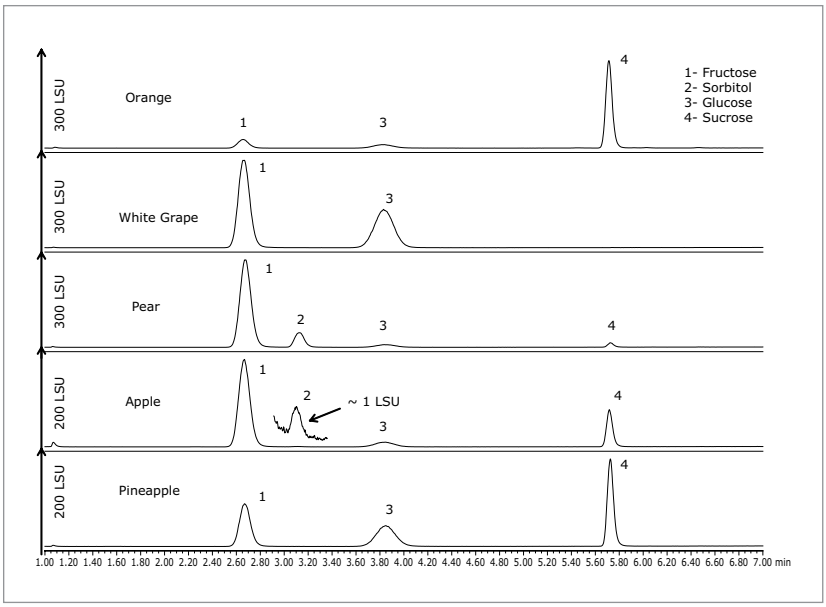


Figure 3. Fruit juice profiles.

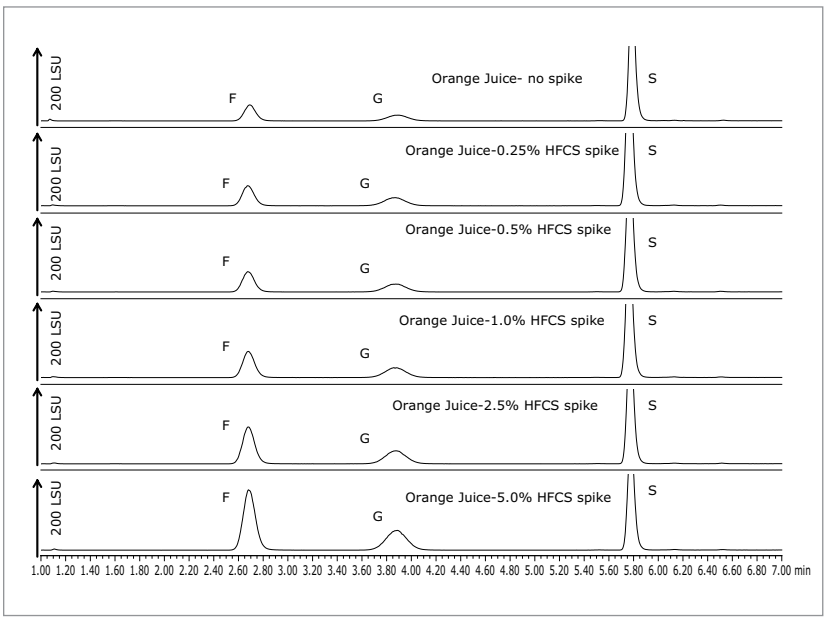


Figure 4. Samples of orange juice spiked with varying levels of 55% HFCS.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC BEH Amide Column, 1.7 µm, 2.1 x 100 mm	186004801
LCMS Certified Vials	600000751CV

Ref: Waters Application Note 720004404EN

INTRODUCTION

The feasibility of simultaneous determination of vitamins A and E in infant formulas by UltraPerformance Convergence Chromatography™ (UPC$^{2®}$) is demonstrated in this work.

PRETREATMENT

Vitamin A (retinyl acetate and retinyl palmitate) and vitamin E (alpha-tocopheryl acetate and alpha-tocopherol) were extracted from infant formula (IF) samples by liquid-liquid extraction.

LC CONDITIONS

System: ACQUITY UPC^2
Column: ACQUITY UPC^2 HSS C_{18}, SB, 1.8 µm, 3.0 x 100 mm
Mobile phase A: CO_2
Mobile phase B: Methanol

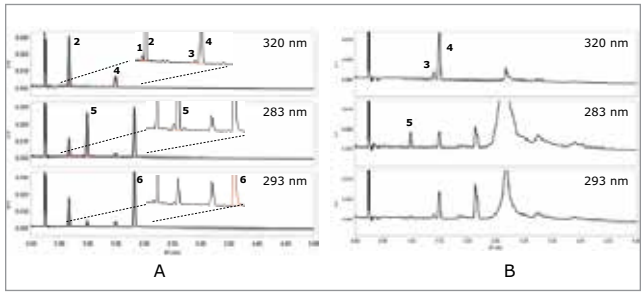
Compound	Range (µg/mL)*	R^2	Equation[b]	LOQ (µg/mL)*
Retinyl acetate	0.4 to 5.5	0.9977	Y=9186x + 1107	0.5
Retinyl palmitate	0.2 to 3.5	0.9953	Y=4212x + 98	0.3
Alpha-tocopheryl acetate	17.5 to 245.0	0.9988	Y=199x +289	13.0
Alpha-tocoph-erol	15.8 to 221.0	0.9987	Y=315.7x + 80.7	12.0

Compound	Repeatability (n=6)		Recovery (%) (n=3)
	Mean ± SD (µg/g)[a]	RSD (%)	Mean ± SD
Retinyl acetate	5.34 ± 0.04	0.7	91 ± 0.8
Retinyl palmitate	13.6 ± 0.3	2.4	–
Alpha-tocopheryl acetate	130.0 ± 1.0	1.1	94 ± 1.4
Alpha-tocopherol	82.3 ± 3.1	3.8	–

Repeatability and recovery result obtained on spiked infant formula samples.

[a] The values are expressed in µg vitamin per gram of infant formula powder.

RESULTS



Typical chromatograms of vitamins A and E by UPC^2 with PDA detection. (A) standards; (B) Infant formula sample. Peaks: 1 cis-retinyl acetate, 2 all trans-retinyl acetate, 3 cis-retinyl palmitate, 4 all trans-retinyl palmitate, 5 alpha-tocopheryl acetate, and 6 alpha-tocopherol.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPC^2 HSS C_{18}, SB, 1.8 µm, 3.0 x 100 mm	186006623

Ref: Waters Technology Brief 720004538EN

INTRODUCTION

the power of Waters® ACQUITY® UltraPerformance Liquid Chromatography (UPLC®), with UV detection provides a rapid and simple method that enables the simultaneous analysis of 10 watersoluble vitamin compounds, along with caffeine, and six common food dyes, using a water/methanol gradient in one 7.5 minutes run.

LC CONDITIONS

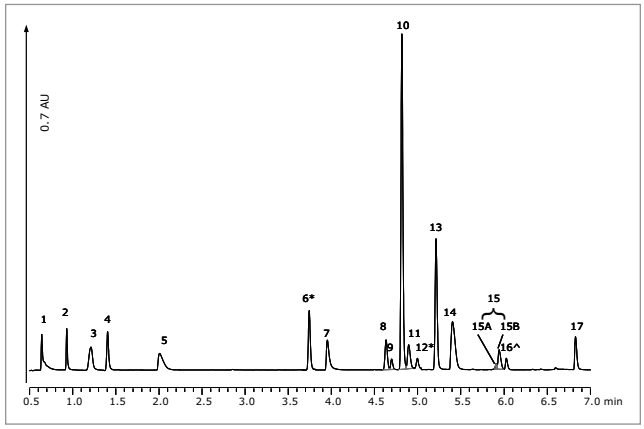
System:	ACQUITY UPLC®
Column:	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm
Column temp:	30 °C
Sample temp:	4 °C
Flow rate:	0.45 mL/min
Mobile phase A:	Water (0.1% formic acid)
Mobile phase B:	Methanol (0.1% formic acid)
Weak needle wash:	3:1:1 water/methanol/acetonitrile (1000 µL)
Strong needle wash:	5:1:1 acetonitrile/isopropanol/water (500 µL)
Gradient run time:	7.5 minutes
Injection volume:	2 µL, full-loop injection
Gradient:	Time (min) %A

Time (min)	%A
0.00	99
1.50	99
1.60	95
3.00	80
5.50	45
5.60	45
5.80	02
7.50	02
7.60	99

PDA CONDITIONS

Detector:	ACQUITY-extended PDA
Wavelengths:	630 nm, 270 nm, and 205 nm
Resolution:	1.2 nm
Filter response:	0.1 s
Sampling rate:	20 points/s
Exposure time:	Auto

RESULTS



*Chromatogram showing solvent standards with concentrations of approximately 5 ng/µL. All extracted at 270 nm, except compounds 6, 12, and 16. * Extracted at 205 nm. Extracted at 630 nm.*

Compound Number	Compound Name	RT (min)	UV extracted wavelength (nm)
1	Thiamine (B1)	0.64	270
2	Ascorbic acid (C)	0.93	270
3	Nicotinic acid (B3-OH)	1.21	270
4	Nicotinic acid (B3-OH)	1.40	270
5	Pyridoxine (B6)	2.01	270
6	Calcium pantothenate (B5)	3.74	205
7	FD&C Yellow No. 5 (E102)	3.97	270
8	Cyanocobalamin (B12)	4.63	270
9	Folic acid (B9)	4.70	270
10	Caffeine	4.81	270
11	FD&C Yellow No. 6 (E110)	4.89	270
12	Biotin (B7)	4.99	205
13	Riboflavin (B2)	5.21	270
14	FD&C Red No. 40 (E129)	5.40	270
15	FD&C Green No. 3 (E143)	5.94	270
16	FD&C Blue No. 1 (E133)	6.02	630
17	FD&C Blue No. 1 (E133)	6.83	270

Identification of compounds in Figure 1 chromatogram, with retention time and UV extracted wavelength information. (Water-soluble vitamin compounds, food dyes, other).

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC HSS T3 Column, 1.8 µm, 2.1 x 100 mm	186003539

Ref: Waters Application Note 720003188EN

INTRODUCTION

LC/MS/MS technology has begun to be more widely accepted for the quantitative analysis of fortified vitamins in food products.[2-5] The advantages in selectivity and sensitivity, along with the ability to analyze multiple analytes in a single injection make this technology highly suitable for this application.

LC CONDITIONS

System:	ACQUITY UPC®
Column:	ACQUITY® HSS T3 C_{18}, 1.8 µm, 1.0 X 100 mm
Column temp.:	60 °C
Injection volume:	10 µL
Flow rate:	0.15 mL/min
Mobile phase A:	Water + 0.05% HCOOH and 0.01% HFBA
Mobile phase B:	Methanol with 10 mM NH_4OH
Strong wash:	Methanol
Weak wash:	Water

Gradient:

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.15	99	1	6
0.40	0.15	99	1	6
6.00	0.15	40	60	6
6.50	0.15	1	99	2
7.50	0.15	1	99	6
7.60	0.15	99	1	6
9.00	0.15	99	1	6

MS CONDITIONS

Mass spectrometer:	Xevo® TQ-S
Ionization mode:	ESI +
Capillary voltage:	2.5 kV
Desolvation temp.:	500 °C
Desolvation gas flow:	750 L/h
Source temp.:	150 °C
Cone gas:	300 L/h

MRM TRANSITIONS

The MRM transitions, cone voltage, and collision energy selected for each of the water-soluble vitamins and their internal standards are shown in Table 1, along with the expected compound retention time.

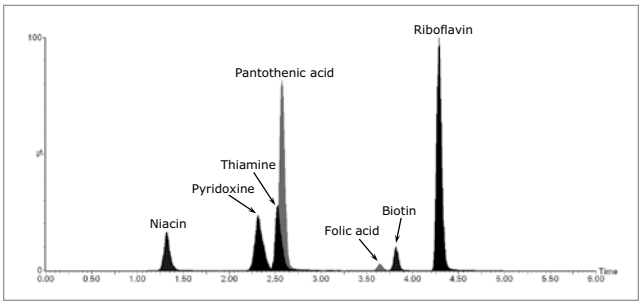
Compound	MRM Transition	Rt	Cone (V)	Collision (V)
Niacinamide	123.0 > 80.0	1.32	30	35
$^{2}H_4$-Niacinamide	127.0 > 84.0	1.30	30	35
Nicotinic acid	123.9 > 80.0	1.22	30	25
$^{2}H_4$-Nicotinic acid	128.0 > 84.1	1.22	30	25
Pantothenic Acid	220.1 > 90.1	2.58	12	25
$^{13}C_3$,^{15}N-Pantothenic acid	224.3 > 93.9	2.58	12	25
Thiamine	265.3 > 122.0	2.53	24	40
$^{13}C_3$-Thiamine	268.3 > 122.0	2.53	24	40
Pyridoxine	170.2 > 151.7	2.32	30	25
$^{13}C_4$-Pyridoxine	174.2 > 155.7	2.32	30	25
Biotin	245.3 > 97.0	3.82	22	24
$^{13}C_5$-Biotin	250.3 > 232.0	3.82	22	24
Riboflavin	377.2 > 242.8	4.29	40	30
$^{13}C_4$,$^{15}N_2$-Riboflavin	383.2 > 248.6	4.29	40	30
Folic acid	442.3 > 295.0	3.64	20	25
$^{13}C_5$-Folic acid	447.3 > 295.0	3.64	20	25

Table 1. MRM transitions, retention times, and tuning parameters for the water-soluble vitamins and their internal standards.

SAMPLE PREPARATION

Precisely weighed amounts of sample were made up according to the (proprietary) standard operating procedure (SOP) for the method. The amount depended upon the specific product to be analyzed. Products included both ready-to-feed and powdered formulations. Isotopically labeled standards for each of the vitamins were added. Following thorough mixing of the samples, 25 mL of 1% ascorbic acid was added to the samples. Following another thorough mixing, 80 µL of 30% ammonium hydroxide was added. The samples were mixed again and allowed stand for 10 minutes. An aliquot of the supernatant from the settled samples was filtered through 0.45 µm PTFE directly into autosampler vials.

RESULTS



MRM chromatograms for each of the vitamins for the analysis of the NIST SRM 1849a.

Vitamin	NIST SRM 1849a Amount ± range	Mean	RSD	Accuracy	n
Biotin (µg/kg)	1990.0 ± 130.0	2140.0	3.0%	108%	11
Folic acid (µg/kg)	2290.0 ± 60.0	2320.0	2.2%	101%	19
Niacin (mg/kg)	109.0 ± 10.0	109.0	1.9%	100%	19
Pantothenic acid (mg/kg)	68.2 ± 1.9	69.8	2.0%	102%	19
Pyridoxine (mg/kg)	13.5 ± 0.9	13.7	1.9%	101%	19
Riboflavin (mg/kg)	20.4 ± 0.5	20.7	2.8%	101%	19
Thiamine (mg/kg)	12.6 ± 1.0	13.2	2.3%	105%	19

Expected amount and acceptable range for the NIST SRM 1849a along with the calculated mean values, RSD, and accuracy for 19 separate analyses over an eight-month period. The first eight preparations used a different internal standard RSD for biotin, therefore only analyses with the final internal standard for biotin were included (n=11).

Vitamin	Day 1 (n=4)	Day 2 (n=4)	Day 3 (n=4)	Day 4v (n=4)
Biotin (µg/kg)	1.1	1.4	2.7	0.7
Folic acid (µg/kg)	1.8	2.1	2.8	1.6
Niacin (mg/kg)	0.7	1.3	1.9	0.5
Pantothenic acid (mg/kg)	0.9	1.8	2.1	0.4
Pyridoxine (mg/kg)	0.8	1.7	1.7	0.4
Riboflavin (mg/kg)	1.7	1.4	2.3	1.2
Thiamine (mg/kg)	0.0	2.0	1.2	1.3

Intra-day precision for four separate days. Values are the percentage relative standard deviation (% RSD) for the NIST SRM 1849a. For days 1 through 3, n=4; for day 4, n=8.

Vitamin	Concentration of lowest level standard (ng/mL)
Biotin	1.18
Folic acid	1.14
Niacin	14.17
Pantothenic acid	8.40
Pyridoxine	1.78
Riboflavin	1.40
Thiamine	2.01

Concentration levels for the lowest level working standard.

ORDERING INFORMATION

Description	Part Number
ACQUITY UPLC HSS T3 Column, 1.8 µm, 1 x 100 mm	186003536

Ref: Waters Technology Brief 720004690EN

A

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V

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