Food and Beverage Analysis

Solutions that enable a safe and healthier world

Food Chemistry

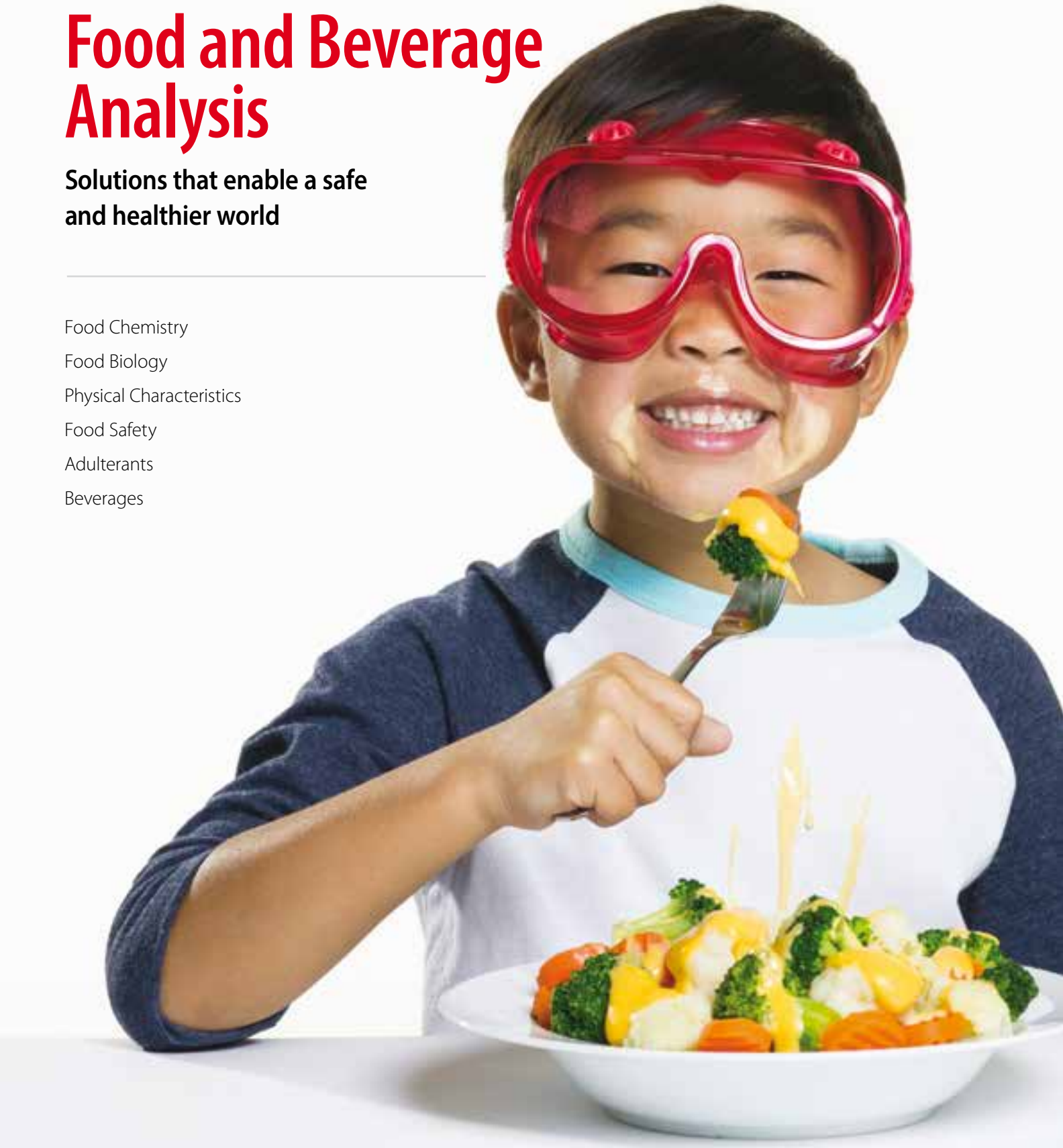
Food Biology

Physical Characteristics

Food Safety

Adulterants

Beverages



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SIGMA-ALDRICH®

FOOD AND BEVERAGE ANALYSIS

Sigma-Aldrich is dedicated to providing analytical solutions and technologies for food and beverage protection.

The analysis of food and beverage products is performed not only to determine nutritional value and quality, but also to ensure safety. Sigma-Aldrich understands the workload, detection and regulatory mandates food analysts face, and has developed specialized solutions to those very challenges. Supelco® and Fluka® analytical products allow food analysts to simplify sample preparation, cleanup and analysis steps, while increasing sensitivity to trace ingredients and harmful substances.

This brochure provides

a premier selection of proven tools and consumables that meet the needs of scientists who perform sample preparation and analysis of food and beverage ingredients and finished products

A preview of our offering includes:

- Special purpose GC columns and standards for fatty acids/fatty acid methyl esters
- **HYDRANAL®** for moisture determination by Karl Fischer titration
- **QuEChERS** materials and carbon-based SPE tubes for pesticide and metabolite residues
- **Ascentis® Express HPLC columns** for many pesticide and veterinary drug residues
- An extensive line of products for pathogen detection

Table of Contents

Food Composition / Nutritional Labeling

Food Safety

References

Books

1. Fennema's Food Chemistry, Fourth Edition, edited by Srinivasan Damodaran, Kirk L. Parkin and Owen R. Fennema, CRC Press, 2008.
2. Food Analysis, Fourth Edition, edited by S. Suzanne Nielsen, Springer, 2010.
3. Food Flavorings, Second Edition, edited by P.R. Ashurst, Blackie Academic & Professional, 1995.
4. Handbook of Alcoholic Beverages; Technical, Analytical and Nutritional Aspects, edited by Alan J. Burglass, Wiley, 2011.
5. Techniques for Analyzing Food Aroma, edited by Ray Marsili, Marcel Dekker, Inc., 1997.

Government Organizations

6. EU Association of Specialty Feed Ingredients and their Mixtures (FEFANA) website (www.fefana.org).
7. European Food Safety Authority (EFSA) website (www.efsa.europa.eu).
8. Food and Agriculture Organization of the United Nations (FAO) website (www.fao.org).
9. United States Department of Agriculture (USDA) website (www.usda.gov).
10. United States Food & Drug Administration (FDA) website (www.fda.gov/food).

Non-Government Organizations

11. American Association of Cereal Chemists (AACC) website (www.aaccnet.org).
12. American Oil Chemists' Society (AOCS) website (www.aocs.org).
13. American Society for Microbiology (ASM) website (www.asm.org).
14. Association of Official Analytical Chemists (AOAC) website (www.aoac.org).
15. Grocery Manufacturers Association (GMA) website (www.gmaonline.org).
16. The Lipid Library website (lipidlibrary.aocs.org).

CARBOHYDRATES

(Sugars and Saccharides) and Dietary Fiber

The determination of the carbohydrate and dietary fiber content of food and beverage products is one measure of nutritional quality.

- Carbohydrate content is typically determined by measuring sugars and digestible saccharides, often using HPLC
- Dietary fiber is a measure of non-digestible components, and includes non-digestible saccharides and lignin

Included are examples relevant to this area.

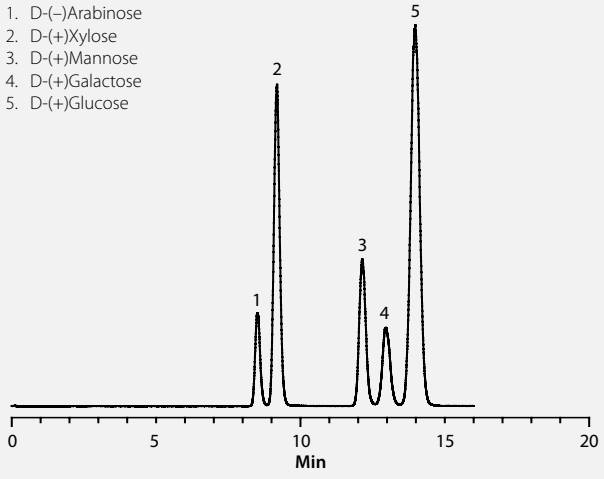
Simple Sugars

Chromatographic analysis of simple sugars can be challenging because these compounds are highly polar, uncharged and lack a chromophore. The preferred mode of HPLC separation is hydrophilic interaction chromatography (HILIC). It provides the advantage of retaining these highly polar compounds that are otherwise hard to separate. Polymeric columns bonded with aminopropyl groups offer improved stability and MS compatibility. Supelco's apHera™ NH2 column is based on covalently bonded polyamine to co-polymer which offers stability from pH 2–12, mechanical and chemical strength and high column efficiency. **Figure 1** shows the separation of several simple sugars on an apHera NH2. Other potential applications include derivatized sugars, complex carbohydrates, polar organic acids and bases.

Figure 1. HPLC Analysis of Underivatized Simple Sugars

column: apHera NH_2, 15 cm x 4.6 mm I.D., 5 µm particles (56401AST)
mobile phase: 20:80 water:acetonitrile
flow rate: 1.0 mL/min
column temp.: 25 °C
detector: ELSD, 45 °C, 3.5 psi nitrogen
injection: 10 µL
sample: each analyte at 500 µg/mL in 30:70 water:acetonitrile

1. D-(–)Arabinose
2. D-(+)Xylose
3. D-(+)Mannose
4. D-(+)Galactose
5. D-(+)Glucose

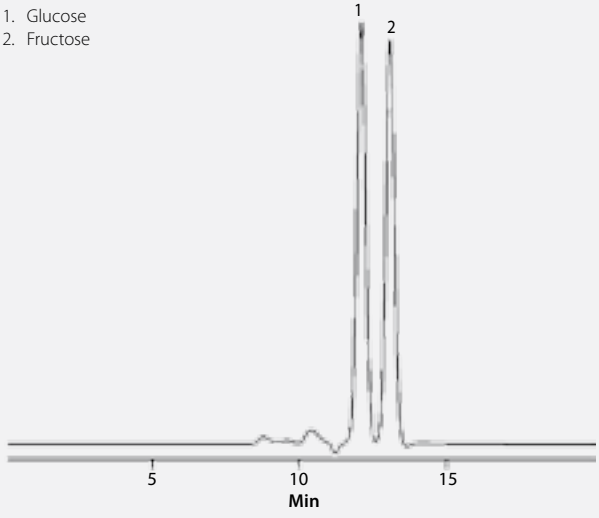


Ion exclusion HPLC is another method employed for the determination of simple sugars. Supelco's SUPELCOGEL™ resin-based ion exchange HPLC columns contain spherical particles of sulfonated polystyrene/divinylbenzene, each with specific counter ions. Each counter ion (Ca, H, Pb, K or Ag) imparts a unique selectivity for the analysis of sugars or organic acids. When the sample matrix is a beverage, limited sample preparation is required. For example, a grape juice cocktail can simply be passed through a 0.45 µm syringe filter prior to analysis. The resulting chromatogram on a column in the hydrogen (H) form is shown in **Figure 2**.

Figure 2. HPLC Analysis of Sugars in Grape Juice Cocktail

column: SUPELCOGEL C-610H, 30 cm x 7.8 mm I.D., 9 µm particles (59320-U)
mobile phase: 0.1% phosphoric acid
flow rate: 0.5 mL/min
column temp.: 30 °C
detector: RI
injection: 10 µL of a grape juice cocktail that was filtered through a 0.45 µm syringe filter

1. Glucose
2. Fructose



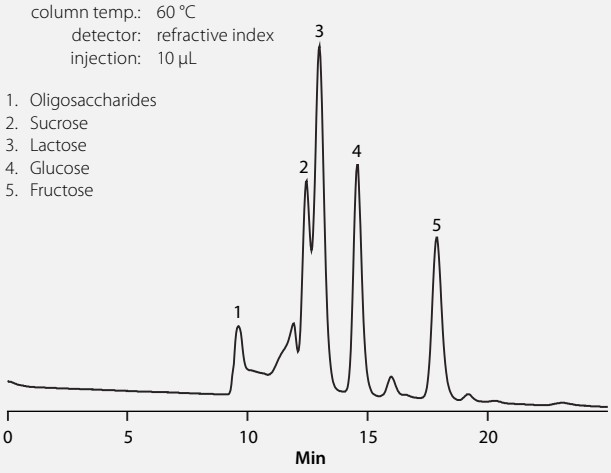
Saccharides

Because the chemical and physical properties of various saccharides are similar, they are more difficult to analyze than many other classes of compounds. HPLC is typically the mode of analysis, relying on differences in conformation, configuration and bonding mode. However, no single HPLC column or method is capable of separating all saccharides. For this reason, Supelco offers a selection of SUPELCOGEL ion exclusion columns which are specifically prepared for various saccharide analyses. **Figure 3** shows the HPLC analysis of saccharides in fruit yogurt. SUPELCOGEL C-611 columns contain two divalent cations, rather than one. This provides a different selectivity compared to the other SUPELCOGEL columns.

Figure 3. HPLC Analysis of Saccharides in Fruit Yogurt

sample/matrix: 10 g yogurt mixed with 100 mL DI water, filtered through 0.20 µm filter
column: SUPELCOGEL C-611, 30 cm x 7.8 mm I.D., 9 µm particles (59310-U)
mobile phase: 10^{-4} sodium hydroxide
flow rate: 0.5 mL/min
column temp.: 60 °C
detector: refractive index
injection: 10 µL

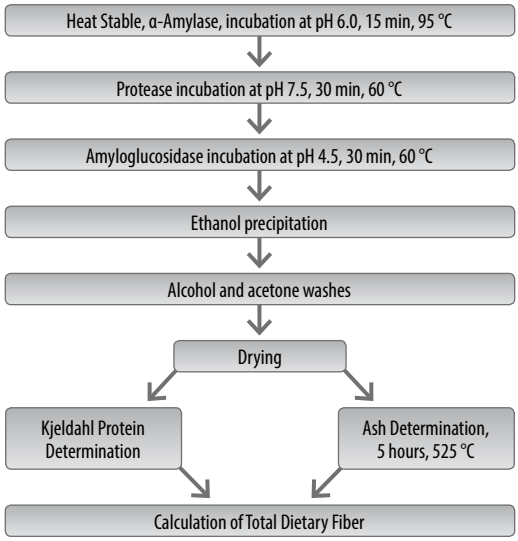
1. Oligosaccharides
2. Sucrose
3. Lactose
4. Glucose
5. Fructose



Dietary Fiber

The total dietary fiber content of foods can be determined using a combination of enzymatic and gravimetric methods. A procedure (based on AOAC Method 2009.01) using a convenient kit for performing this application is shown in **Figure 4**. Samples of dried, fat-free foods are gelatinized with heat stable amylase. The mixture is then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Ethanol is added to precipitate the soluble dietary fiber. The residue is then filtered and washed with ethanol and acetone. After drying, the residue is weighed. Half the sample is analyzed for protein, and the other half is ashed. Total dietary fiber is calculated as the weight of the residue less the weight of the protein and ash.

Figure 4. Flow Chart for Total Dietary Fiber Assay Kit



Featured Products

Cat. No.	Product Description
apHera HPLC Columns (5 µm particles)	
56102AST	apHera C18, 15 cm x 4.6 mm
56202AST	apHera C8, 15 cm x 4.6 mm
56302AST	apHera C4, 15 cm x 4.6 mm
56401AST	apHera NH_2, 15 cm x 4.6 mm
SUPELCOGEL HPLC Columns (9 µm particles)	
59305-U	SUPELCOGEL Ca, 30 cm x 7.8 mm I.D.
59320-U	SUPELCOGEL C-610H, 30 cm x 7.8 mm I.D.
59304-U	SUPELCOGEL H, 30 cm x 7.8 mm I.D.
59343	SUPELCOGEL Pb, 30 cm x 7.8 mm I.D.
59342	SUPELCOGEL K, 30 cm x 7.8 mm I.D.
59315	SUPELCOGEL Ag2, 30 cm x 7.8 mm I.D.
59310-U	SUPELCOGEL C-611, 30 cm x 7.8 mm I.D.
Analytical Standards	
47267	Monosaccharides Kit Individually packaged, 500 mg each D-(–)Arabinose; D-(+)Mannose, mixed anomers; Fructose; D-(–)Ribose; D-(+)Galactose; D-(+)Xylose; D-(+)Glucose, mixed anomers
47268-U	Disaccharides Kit Individually packaged in quantities indicated Isomaltose, mixed anomers, 100 mg; Maltose, 500 mg; Sucrose, 500 mg; α-Lactose, 500 mg
47265	Oligosaccharides Kit Individually packaged, 100 mg each Maltoheptaose, Dp7; Maltotriose, Dp3; Maltohexaose, Dp6; D-(+)Melezitose, Dp3; Maltopentaose, Dp5; D-(+)Raffinose, Dp3; Maltotetrose, Dp4; Isomaltotriose, Dp3; Stachyose, Dp4
Analytical Reagents and Solvents	
71692	Sodium hydroxide, purum p.a., >97.0%
79620	Phosphoric acid, puriss. p.a., ACS reagent, >85.0%
34967	Acetonitrile, LC-MS CHROMASOLV®, >99.9%
Total Dietary Fiber Kits	
TDF100A-1KT	Assay Kit, for 100 assays
TDFC10-1KT	Assay Control Kit, for 10 assays

FATS

(Fatty Acids and Triglycerides), Sterols and Edible Oils

Fats play an important part in the food nutrition and food chemistry areas of study. The compound classes, sample types and analytical techniques of interest include:

- Short chain, volatile fatty acids, typically analyzed in their free acid form using GC
- Larger (C8-C24+) fatty acids (such as omega fats and trans fats), typically converted to fatty acid methyl esters (FAMEs) prior to
- GC analysis
- Triglycerides, analyzed by GC
- Sterols, by GC analysis
- Edible oil characterization
- IR, specific gravity and NMR procedures

Included are examples relevant to this area.

Trans Fats

A procedure used to determine trans fat levels involves extraction, derivatization, fractionation and GC analysis. A combination of acid digestion and alkali hydrolysis is used to release the fats and oils from the sample matrix. Methylation of the fatty acids to fatty acid methyl esters (FAMEs) minimizes the influence of the active carboxyl groups, leaving dispersive and polarizable functionality as the main analyte interactions. Silver-ion (Ag-ion) fractionation is applied to the FAMEs to separate the saturates and C18 transmonoenes in fraction 1, the cis monoenes in fraction 2 and the dienes in fraction 3. GC analysis of each fraction is then performed on a specialized column able to facilitate resolution of the cis and trans monounsaturates in the oleic (C18:1) region. Chromatograms obtained from the preparation of a commercially purchased cookie sample are shown in **Figure 5**. Peaks were identified using retention time and direct comparison to standards.

Triglycerides

Because they are the main constituents of vegetable oil and animal fat, triglycerides account for most of the fats digested by humans. Most natural fats contain a mixture of individual triglycerides.

Analysis of these large compounds can be accomplished using GC, and requires a relatively high final oven temperature for elution in a reasonable time. The MET-Biodiesel column was designed for the determination of free and total glycerin in biodiesel. Its maximum temperature of 380 °C (isothermal) and 430 °C (programmed) combined with its ability to separate mono-, di- and triglycerides, make it well-suited for analysis of triglycerides in food products. **Figure 6** shows the resulting chromatogram of an extract from a butter sample. The T-number signifies the total number of carbons on the triglyceride fatty acid chains. For example, T54 could contain 3 stearic acids (C18:0), it could also contain two stearic acids (C18:0) and one oleic acid (C18:1).

Figure 5. GC Analysis of *Trans* Fats in Cookies

sample/matrix:	1 g of commercially purchased cookies was ground and subjected to acid digestion and alkali hydrolysis followed by methylation as described in AOCS Official Method Ce 1k-09
SPE tube:	Discovery® Ag-Ion SPE tubes, 750 mg/6 mL (54225-U)
conditioning:	4 mL of acetone; allow solvent to gravity drip completely through tube; discard eluant; 4 mL of hexane; allow solvent to gravity drip completely through tube; discard eluant
sample addition:	1 mL of extract; discard any eluant that drips through tube
elution:	(Fraction 1) 6 mL of hexane:acetone (96:4); collect eluant in a fresh container with slight vacuum; (Fraction 2) 4 mL of hexane: acetone (90:10); collect eluant in a fresh container with slight vacuum; (Fraction 3) 4 mL of 100% acetone; collect eluant in a fresh container with slight vacuum
eluate post-treatment:	evaporate each fraction at room temperature using nitrogen; reconstitute each fraction to 1 mL of hexane
column:	SLB®-IL111, 100 m x 0.25 mm I.D., 0.20 µm (29647-U)
oven:	168 °C
inj. temp.:	250 °C
detector:	FID, 250 °C
carrier gas:	hydrogen, 1 mL/min
injection:	1 µL, 10:1 split
liner:	4 mm I.D., split type, single taper wool packed FocusLiner™ design

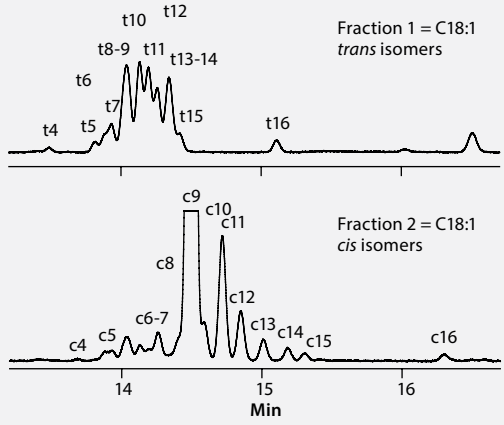
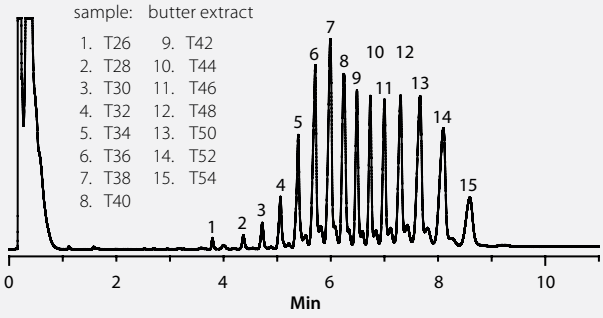


Figure 6. GC Analysis of Triglycerides in Butter

Chromatogram courtesy of Dr. M. Povolo and Dr. G. Contarini (CRA-FLC, Lodi, Italy)

column:	MET-Biodiesel, 14 m x 0.53 mm I.D., 0.16 µm with integrated 2 m x 0.53 mm I.D. guard (28668-U)
oven:	150 °C, 30 °C/min to 350 °C (15 min)
detector:	FID, 400 °C
carrier gas:	helium, 15 cm/sec
injection:	1 µL, cool on-column
sample:	butter extract

1. T26
2. T28
3. T30
4. T32
5. T34
6. T36
7. T38
8. T40
9. T42
10. T44
11. T46
12. T48
13. T50
14. T52
15. T54



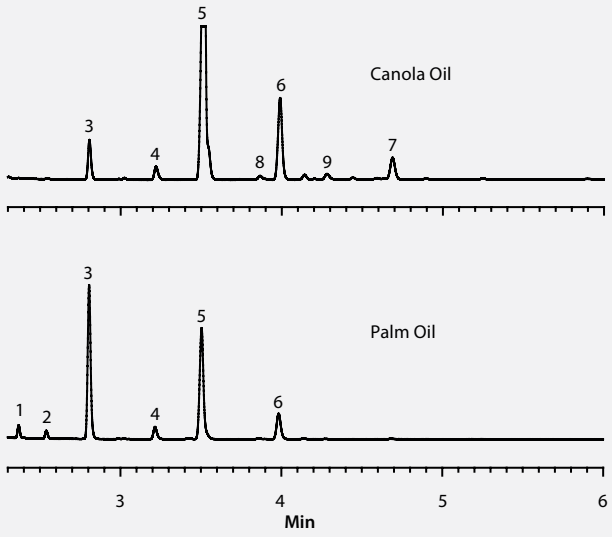
Edible Oils

The edible oil industry boasts revenue measured in the tens of billions of dollars. As such, it can be subject to criminal acts of fraud aimed at increasing profits (adding a cheaper, inferior oil to boost the volume of a premium, higher priced oil). A GC fingerprinting technique can be used to monitor product for adulteration, and also to identify the source of oils in unknown samples. Following derivatization to convert the fatty acids to FAMEs, GC analysis is performed. Two example chromatograms are shown in **Figure 7**. This quick fingerprinting technique allows the oil type and purity to be identified, by comparison of the FAME ratios in sample oils to the FAME ratios in reference oil standards.

Figure 7. GC Analysis of Canola and Palm Oils

column: SLB-IL111, 30 m x 0.25 mm I.D., 0.20 µm (28927-U)
oven: 180 °C
inj. temp.: 250 °C
detector: FID, 260 °C
carrier gas: helium, 25 cm/sec
injection: 1 µL, 50:1 split
liner: 4 mm I.D., split type, cup design
samples: characterized reference oils, methylated using BF_3-methanol prior to analysis

1. Lauric Acid Methyl Ester (C12:0)
2. Myristic Acid Methyl Ester (C14:0)
3. Palmitic Acid Methyl Ester (C16:0)
4. Stearic Acid Methyl Ester (C18:0)
5. Oleic Acid Methyl Ester (C18:1n9c)
6. Linoleic Acid Methyl Ester (C18:2n6c)
7. Linolenic Acid Methyl Ester (C18:3n3)
8. Arachidic Acid Methyl Ester (C20:0)
9. *cis*-11-Eicosenoic Acid Methyl Ester (C20:1)



Featured Products

Cat. No.	Product Description
SPE Tube	
54225-U	Discovery® Ag-Ion, 750 mg/6 mL, 30 ea
GC Columns	
23399-U	Omegawax®, 15 m x 0.10 mm I.D., 0.10 µm
24136	Omegawax, 30 m x 0.25 mm I.D., 0.25 µm
25374	Omegawax, 30 m x 0.53 mm I.D., 0.50 µm
23348-U	SP™-2560, 75 m x 0.18 mm I.D., 0.14 µm
24056	SP-2560, 100 m x 0.25 mm I.D., 0.20 µm
23362-U	SP-2560, 100 m x 0.25 mm I.D., 0.20 µm On a 5" cage to fit Agilent 6850 GC
28925-U	SLB-IL111, 15 m x 0.10 mm I.D., 0.08 µm
28927-U	SLB-IL111, 30 m x 0.25 mm I.D., 0.20 µm
28928-U	SLB-IL111, 60 m x 0.25 mm I.D., 0.20 µm
29647-U	SLB-IL111, 100 m x 0.25 mm I.D., 0.20 µm
28668-U	MET-Biodiesel 14 m x 0.53 mm I.D., 0.16 µm with integrated 2 m x 0.53 mm I.D. guard
Ascentis Express HPLC Columns (2.7 µm particles)	
53823-U	C18, 10 cm x 2.1 mm I.D.
53832-U	C8, 10 cm x 2.1 mm I.D.
53913-U	RP-Amide, 10 cm x 2.1 mm I.D.
53336-U	Phenyl Hexyl, 10 cm x 2.1 mm I.D.
53569-U	F5, 10 cm x 2.1 mm I.D.
Ascentis Express HPLC Columns (5 µm particles)	
50537-U	C18, 15 cm x 4.6 mm I.D.
50392-U	C8, 15 cm x 4.6 mm I.D.
50483-U	Phenyl Hexyl, 15 cm x 4.6 mm I.D.
50631-U	F5, 15 cm x 4.6 mm I.D.
Analytical Standards	
47885-U	Supelco® 37-Component FAME Mix 37 analytes (C4-C24), 10 mg/mL (total wt.) in methylene chloride, 1 mL Visit sigma-aldrich.com/fame for list of analytes and concentrations
46961	Canola Oil, Characterized Reference Oil, 1g
46962	Palm Oil, Characterized Reference Oil, 1 g
Analytical Reagents and Solvents	
33356	BF_3-Methanol, 10% (w/w), 20 x 1 mL
33089-U	BCl_3-Methanol, 12% (w/w), 20 x 2 mL
33355	Methanolic HCl, 3 N, 20 x 1 mL
71381	Sodium chloride, purum p.a., >99.5%
71692	Sodium hydroxide, purum p.a., >97.0%
34480	Acetone, for pesticide residue analysis
34484	*n*-Hexane, for pesticide residue analysis
34485	Methanol, for pesticide residue analysis

PROTEINS

(Amino Acids, Peptides, Proteins and Nitrogen Content)

Proteins are a major source of energy in foods. Additionally, they contain essential amino acids (such as lysine, methionine and valine) that cannot be synthesized in the body. Proteins are also major structural food components and determine the texture of meat and fish. Because of this, adding isolated proteins to foods is a technique to provide desirable appearance, texture and stability. Areas of interest include:

- Individual amino acid determination
- Peptide composition
- Protein identification and quantification, using HPLC or molecular spectroscopy techniques (IR and UV)
- Nitrogen content (Kjeldahl method) as a measure of protein content

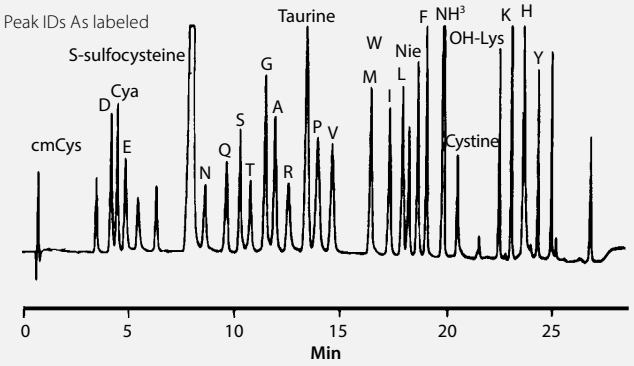
Included are examples relevant to this area.

Amino Acids

To determine the amino acid composition of a protein, the sample is first hydrolyzed to release the constituent amino acids. Analysis is typically performed using HPLC. It is sometimes helpful to derivatize the amino acids to aid in separation, and to permit detection at lower concentrations. Dabsyl chloride is an effective derivatization reagent for this application. **Figure 8** shows the HPLC separation of dabsylated amino acids.

Figure 8. HPLC Analysis of Dabsylated Amino Acids

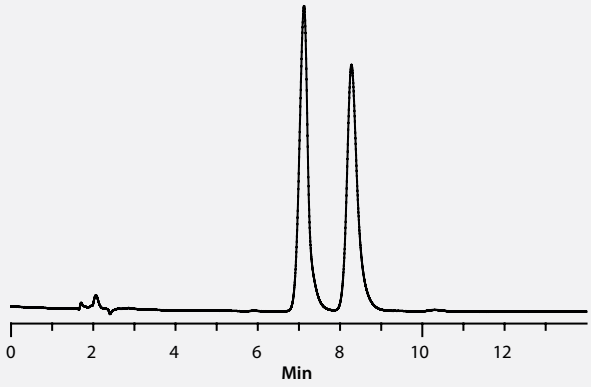
column: SUPELCOSIL™ LC-DABS, 15 cm x 4.6 mm I.D., 3 µm particles (59137)
mobile phase: A = 25 mM potassium dihydrogen phosphate (pH 6.8), B = acetonitrile:2-propanol (75:25)
gradient: 0 min: 20% B; 1 min: 20% B; 4 min: 23% B; 9 min: 23% B; 10 min: 27% B; 14 min: 27% B; 19 min: 35% B; 25 min: 60% B; 26 min: 70% B; 29 min: 70% B; 29.1 min: 20% B; 35.1 min: 20% B
flow rate: 2 mL/min
detector: UV, 436 nm
injection: 5 µL
sample: dabsylated amino acids



Some amino acids exist in nature as enantiomers, and require specialized HPLC columns for proper separation. The Astec® CHIROBIOTIC® TAG has demonstrated excellent selectivity and effective resolution of the enantiomers of several underivatized amino acids (such as serine and isoleucine) with simple mobile phases. **Figure 9** shows an excellent separation of serine enantiomers using a very simple, LC-MS compatible mobile phase.

Figure 9. HPLC Analysis of Amino Acid Enantiomers

column: Astec CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase: water:acetonitrile (30:70)
flow rate: 1 mL/min
column temp.: ambient
detection: UV, 210 nm
injection: 5 mL
sample: serine at 5 mg/mL in water:methanol (50:50)



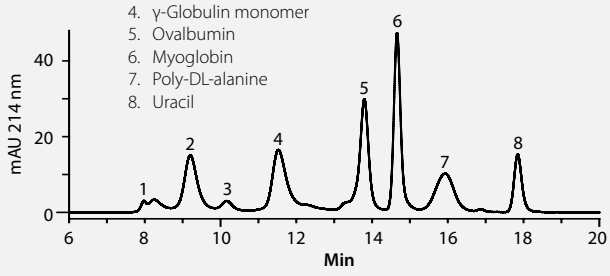
Protein Analysis using GFC

Gel filtration chromatography (GFC), also known as size exclusion chromatography, is a common technique for the resolution of macromolecules based on size and/or shape. Analytes that are large relative to the pore size of the stationary phase are excluded from the pores and elute relatively early. Analytes that are small relative to the pore size of the stationary phase are well retained in the pores, and elute later. Shape also affects retention, in that spherical or globular molecules are retained more than elongated or linear molecules. **Figure 10** shows the separation of a mixture of proteins using Discovery® BIO GFC, a product line based on uniform, spherical, high-purity silica with hydrophilic surface

Figure 10. HPLC Analysis of a Protein Mixture

column: Discovery BIO GFC 300, 30 cm x 7.8 mm I.D., 3 µm particles (567337-U)
mobile phase: 150 mM potassium phosphate monobasic, pH 7.0 (adjusted with potassium hydroxide)
flow rate: 0.7 mL/min
pressure: 1100 psi
column temp.: ambient
detector: UV, 214 nm
injection: 1 µL
sample: each at 1 g/L (except uracil, at 0.1 g/L) in mobile phase

1. Thyroglobulin aggregate
2. Thyroglobulin
3. γ-Globulin dimer
4. γ-Globulin monomer
5. Ovalbumin
6. Myoglobin
7. Poly-DL-alanine
8. Uracil



Nitrogen Content

The Kjeldahl method is often used to report the protein content of food and beverage samples based on its total nitrogen content. If the sample contains nitrite or nitrate, it must be reduced with Arnd's alloy in weak acid to produce a neutral solution prior to analysis. Kjeldahl digestion then converts nitrogen-containing organic compounds into ammonium sulfate by heating in concentrated sulfuric acid. Figure 11 shows a condensed version of the Kjeldahl reaction. Several suitable catalysts are available to speed up the decomposition:

- The mercury and selenium-free catalyst is popular for environmental and toxicological reasons
- The Missouri catalyst is an environmentally friendly alternative because it has a low copper content, although the reaction is significantly slower
- The selenium-containing catalyst according to Wieninger is used for very resistant samples, such as heteroaromatic compounds, mineral oils and fats

Free ammonia is then released by adding concentrated sodium hydroxide solution, which is evaporated by steam distillation. The amount of ammonia present, and therefore the amount of nitrogen present in the original sample, is determined by back titration.

Figure 11. Kjeldahl Reaction

Featured Products

Cat. No.	Product Description
HPLC Columns	
59137	SUPELCOSIL LC-DABS, 15 cm x 4.6 mm, 3 µm particles
14024AST	Astec CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles
53306-U	Ascentis Express Peptide ES-C18, 10 cm x 2.1 mm I.D., 2.7 µm particles
53328-U	Ascentis Express Peptide ES-C18, 15 cm x 4.6 mm I.D., 2.7 µm particles
567337-U	Discovery BIO GFC 300, 30 cm x 7.8 mm I.D., 3 µm particles
Astec CHIRALDEX® GC Columns	
75033AST	G-BP, 30 m x 0.25 mm I.D., 0.12 µm
73031AST	G-TA, 10 m x 0.25 mm I.D., 0.12 µm
73032AST	G-TA, 20 m x 0.25 mm I.D., 0.12 µm
73033AST	G-TA, 30 m x 0.25 mm I.D., 0.12 µm
73034AST	G-TA, 40 m x 0.25 mm I.D., 0.12 µm
73035AST	G-TA, 50 m x 0.25 mm I.D., 0.12 µm
Analytical Standards	
AAS18-5ML	Amino Acid Standard, 18 analytes, 5 mL Each at 2.5 µmoles/mL (except L-cystine, at 1.25 µmoles/mL) in 0.1 N HCl L-Alanine, Ammonium chloride, L-Arginine, L-Aspartic acid, L-Cystine, L-Glutamic acid, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tyrosine, L-Valine
Analytical Reagents and Solvents	
502219	Dabsyl Chloride, 500 mg
34967	Acetonitrile, LC-MS CHROMASOLV®, >99.9%
Kjeldahl Catalysts	
31835-250EA	Free of Hg and Se, 2.5 g tablets
TDFC10-1KT	Assay Control Kit, for 10 assays
31831-250EA	According to Missouri, 2.5 g tablets, without Hg, Se, Sb, Ti, Pt
31108-250EA	According to Wieninger, 2.5 g tablets, with selenium
Kjeldahl Reagents	
11066	Arnd's Alloy, contains 60% Cu and 40% Mg, 50 g
31821	Disintegrating Mixture, contains H_2SO_4 and Se, 1 L
84727	Sulfuric acid, for nitrogen determination, >97.5%
30531	Sodium hydroxide solution, for nitrogen determination, >32%

NUTRITIONAL

(other than carbohydrates, fats and proteins)

In addition to the major nutritional food components like carbohydrates, fats and proteins, several minor nutritional components are also of great interest to food analysts. Areas of interest include:

- Nutraceuticals (antioxidants, polyphenolics, catechins,
- flavonoids, natural compounds, saponins/ginsenoside and phytoestrogens)
- Vitamins, co-factors and enzymes
- Nucleotides and nucleosides
- Organic acids
- Minerals (calcium, iron, sodium, potassium, etc.)
- Calories

Included are examples relevant to this area.

Polyphenolic Antioxidants

The study of the putative health benefits of plant-derived polyphenolic compounds is an active research area in food chemistry. HPLC and LC-MS play an important role in the characterization of plant extracts, and both benefit from the sensitivity and resolving power provided by highly efficient Ascentis® Express HPLC columns. The example in **Figure 12** of blueberry anthocyanins on an Ascentis Express C18 is an example of the applicability of these columns for analysis of polyphenolics. The high efficiency identification of twenty-six anthocyanin derivatives in ripe wild berries is shown here.

Ascentis and Ascentis Express HPLC columns, Supelco's sample prep products, and analytical standards have been used to analyze other nutraceuticals; including vanillin, catechin, resveratrol, Withania, ginsenosides, taxols, steroildal glycosides, digoxins, silymarin, phytonutrients, spice cannabinoids, flavonoids, catechols, resorcinals, ephedrine alkaloids, hyerpericum, tamoxifen and others.

Vitamins and Organic Acids

HPLC is widely used to analyze the low molecular weight, water-soluble, thermally labile compounds that predominate as food additives. Ideal HPLC columns for this application must possess four features: choice of bonded phase for different selectivity, high efficiency for high s/n, compatibility with different mobile phase systems and detection systems, and rugged and stable to accommodate complex, real samples. Ascentis Express columns meet these requirements. The example in **Figure 13** shows Ascentis Express HILIC used to analyze a popular energy drink for four distinct classes of additives in one run: vitamins, organic acids, sugars and caffeine.

Figure 12. LC-MS Analysis of Antioxidants in Wild Blueberries

sample/matrix:	1.0 g berries
extraction:	crush berries in 1.0 mL of 1% formic acid in 50:50 methanol:water, refrigerate for 2 hours, centrifuge, collect supernatant into HPLC autosampler vial
column:	Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm (53823-U) with guard (53501-U)
mobile phase:	(A) 0.1% TFA water; (B) 0.1% TFA in (75:25 acetonitrile:water)
gradient:	2% B held 2 min; to 100% B in 40 min
flow rate:	0.2 mL/min
pressure:	1590 psi
column temp.:	35 °C
detector:	UV, 250 nm
injection:	1 µL

Peak ID	Ret. Time	m/z
Chlorogenic acid	9.650	163.0403
Delphinidin-3-galactoside	10.327	465.1058
Delphinidin-3-glucoside	10.613	465.1058
Cyanidin-3-galactoside	11.051	449.1106
Delphinidin-3-arabinoside	11.048	435.0935
Cyanidin-3-glucoside	11.378	449.1106
Petunidin-3-galactoside	11.424	479.1192
Petunidin-3-glucoside	11.707	479.1192
Cyanidin-3-arabinoside	11.795	419.0979
Petunidin-3-arabinoside	12.117	449.1086
Peonidin-3-galactoside	12.143	463.1244
Malvinidin-3-glalactoside	12.414	493.1354
Peonidin-3-glucoside	12.477	463.1244
Delphinidin-3-acylgalactoside	12.596	507.1143
Malvinidin-3-glucoside	12.675	493.1354
Peonidin-3-arabinoside	12.881	433.114
Malvinidin-3-arabinoside	13.114	463.1244
Cyanidin-3-acylgalactoside	13.314	491.1213
Delphinidin-3-acylglucoside	13.233	507.1143
Petunidin-3-acylgalactoside	13.622	521.1323
Cyanidin-3-acylglucoside	14.048	491.1213
Petunidin-3-acylglucoside	14.248	521.1323
Peonidin-3-acylgalactoside	14.348	505.137
Malvinidin-3-acylgalactoside	14.548	535.1481
Peonidin-3-acylglucoside	15.082	505.137
Malvinidin-3-acylglucoside	15.182	535.1481

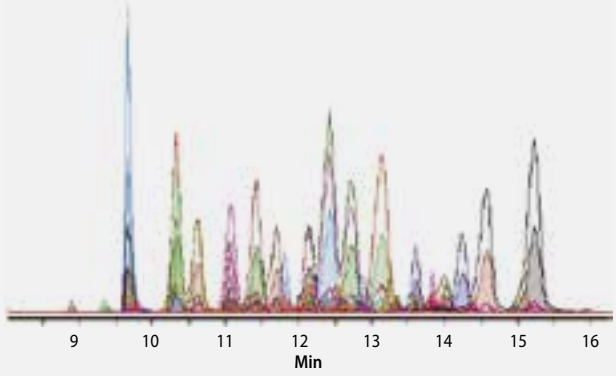
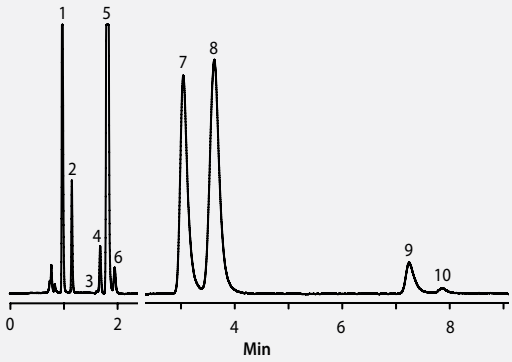


Figure 13. HPLC Analysis of Vitamins, Organic Acids, Sugars and Caffeine in an Energy Drink

column: Ascentis Express HILIC, 10 cm x 3.0 mm I.D., 2.7 µm (53970-U)
mobile phase: (A) 100 mM ammonium acetate, pH 5.0; (B) water; (C) acetonitrile; (9:1:90, A:B:C)
flow rate: 0.6 mL/min
pressure: 815 psi
column temp.: 35 °C
detector: UV, 254 nm or ELSD, 55 °C, 3.5 bar nitrogen
injection: 2 µL commercial energy drink diluted 1:9 in acetonitrile

1. Caffeine
2. Niacinamide (vitamin B_3)
3. Pyridoxine hydrochloride (vitamin B_6)
4. Benzoic acid
5. Sorbic acid
6. Riboflavin (vitamin B_2)
7. Fructose
8. Glucose
9. Sucrose
10. Taurine

UV 254 nm detection ELSD detection



Vitamins by non-Chromatographic Techniques

Fluka® offers reagents for this purpose. For example, Quantofix® test sticks are ready-to-use, rapid (<2 minutes), semi-quantitative kits for Vitamin C (ascorbic acid). They come pre-calibrated and contain all necessary equipment and reagents. Accurate and precise, the kits include color charts that are adjusted and checked using certified standard solutions. All solutions are directly traceable to primary NIST standards.

Minerals

Low levels of minerals are important for nutritional requirements, but some can be toxic at high levels. For this reason the mineral content of food and beverages is measured and controlled. Ion chromatography (IC), atomic absorption spectroscopy (AAS) and inductively coupled plasma (ICP) are the analytical techniques most often used to determine the mineral content of foods.

TraceCERT® certified reference materials (CRM) are developed and produced in an accredited laboratory, fulfilling both ISO 17025 and ISO Guide 34 requirements. All are traceable to at least two independent references (i.e. NIST, BAM or SI unit kg) and include comprehensive documentation. A picture of a TraceCERT CRM and its accompanying documentation is shown in **Figure 14**.

TraceSELECT® acids, bases and salts are designed for sample preparation and analysis in the ppm and ppb level.

Figure 14. TraceCERT CRM and Documentation

Featured Products

Cat. No.	Product Description
Ascentis Express HPLC Columns (2.7 µm particles)	
53823-U	C18, 10 cm x 2.1 mm I.D.
53832-U	C8, 10 cm x 2.1 mm I.D.
53913-U	RP-Amide, 10 cm x 2.1 mm I.D.
53939-U	HILIC, 10 cm x 2.1 mm I.D.
53970-U	HILIC, 10 cm x 3.0 mm I.D.
53569-U	F5, 10 cm x 2.1 mm I.D.
Ascentis Express HPLC Columns (5 µm particles)	
50537-U	C18, 15 cm x 4.6 mm I.D.
50392-U	C8, 15 cm x 4.6 mm I.D.
50289-U	HILIC, 15 cm x 4.6 mm I.D.
50631-U	F5, 15 cm x 4.6 mm I.D.
Vitamin Analytical Standards	
47861	Riboflavin (Vitamin B_2), 1000 mg
47865-U	Nicotinamide, amide derivative of Niacinamide (Vitamin B_3), 1000 mg
47862	Pyridoxine Hydrochloride (Vitamin B_6), 1000 mg
47863	L-Ascorbic acid (Vitamin C), 1000 mg
Analytical Solvents	
34967	Acetonitrile, LC-MS CHROMASOLV®, >99.9%
Test Strips	
37203-1EA	Quantofix Ascorbic Acid (Vitamin C) Test Sticks, pack of 100 sticks
***Trace*CERT CRMs for IC**	
39865-100ML	Calcium, 1000 mg/L in nitric acid, 100 mL
53337-100ML	Potassium, 1000 mg/L in nitric acid, 100 mL
43492-100ML	Sodium, 1000 mg/L in nitric acid, 100 mL
IC Eluents	
36486-1L	Sodium bicarbonate, 0.1 M in water, 1 L
56169-1L	Sodium carbonate, 0.1 M in water, 1 L
***Trace*CERT CRMs for AAS and ICP**	
19051-100ML	Calcium, 1000 mg/L in nitric acid, 100 mL
43149-100ML	Iron, 1000 mg/L in nitric acid, 100 mL
06335-100ML	Potassium, 1000 mg/L in nitric acid, 100 mL
00462-100ML	Sodium, 1000 mg/L in nitric acid, 100 mL
Inorganic Acids	
02650-1L	Nitric acid, *Trace*SELECT Ultra, ~65%, 1 L
84385-500ML	Nitric acid, *Trace*SELECT, ~69.5%, 1 L

NON-NUTRITIONAL INGREDIENTS AND ADDITIVES

Some materials with no nutritional value are also added to food and beverage products. These ingredients and additives are designed to make the food smell better, taste better, last longer and/or look better. Areas of interest include:

- Flavor and Fragrance (raw materials, aroma, volatiles, essential oils, enantiomers, rancidity via iodine number or bromine number)
- Artificial sweeteners
- Preservatives (other than antioxidants) such as sorbate, benzoate, parabens and nitrates
- Dyes

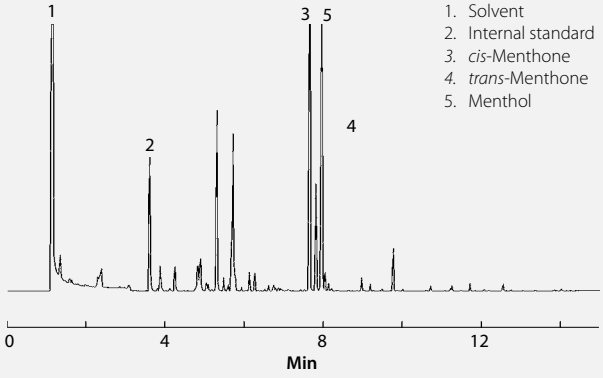
Included are examples relevant to this area.

Flavor and Fragrance Analysis

Headspace solid phase microextraction (SPME), coupled with analysis on a capillary GC column, is an ideal approach for characterizing quality and composition of flavor and fragrance components. In **Figure 15**, SPME and headspace extraction was applied to measure the mint flavor in a chocolate cookie candy. Menthol, the primary component in peppermint oil, is very efficiently adsorbed by a 100 µm coating of polydimethylsiloxane (PDMS) on an SPME fiber. Even though menthol has a high boiling point, it is easily extracted at 45 °C with a short extraction time while minimizing interference by other components in the chocolate. By using headspace SPME to quantify menthol, the percentage of peppermint oil in mint-flavored chocolate can be easily determined.

Figure 15. Peppermint Oil in Chocolate Cookie Bar

sample/matrix:	4 g peppermint cookie bar
SPME fiber:	100 µm polydimethylsiloxane fused silica (57300-U)
extraction:	headspace, 45 °C for 1 min
desorption process:	250 °C for 5 min
column:	Equity®-5, 30 m x 0.25 mm I.D., 0.25 µm (28089-U)
oven:	60 °C (1 min), 10 °C/min to 230 °C
inj. temp:	250 °C
detector:	FID, 250 °C
carrier gas:	helium, 35 cm/sec
injection:	splitless (splitter closed 3 min)
liner:	0.75 mm I.D., SPME type, straight design

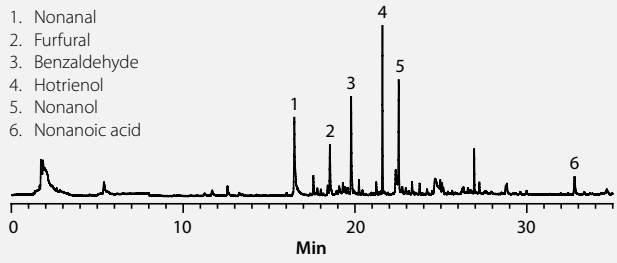


SPME can also be used to chemically fingerprint the volatile components of natural foods, such as honey, as a procedure for determining origin. An example chromatogram is shown in **Figure 16**. This is possible because both the composition of volatile compounds, as well as their ratios to one-another, are influenced by the floral source.

Figure 16. GC Analysis of Volatile Compounds in Honey

Chromatogram courtesy of Federica Bianchi and Marilena Musci (University of Parma, Italy)

sample/matrix:	5 g honey + 5 mL bidistilled water in a 20 mL vial, allowed to equilibrate at 50 °C for 15 min with magnetic stirring
SPME fiber:	2 cm 50/30 µm divinylbenzene/carboxen on polydimethylsiloxane (57348-U)
extraction:	headspace, 50 °C for 40 min with magnetic stirring
desorption process:	250 °C for 2 min
column:	SUPELCOWAX® 10, 30 m x 0.25 mm I.D., 0.25 µm (24079)
oven:	35 °C (8 min), 6 °C/min to 60 °C, 4 °C/min to 160 °C, 20 °C/min to 200 °C (1 min)
detector:	MSD (interface at 230 °C), m/z 35–300
carrier gas:	helium, 1 mL/min
liner:	0.75 mm I.D., direct (SPME) type, straight design



Chiral Flavor and Fragrance Compounds

While not shown, chiral chromatography is often used to identify and quantify enantiomeric flavor and fragrance compounds. Supelco® offers an impressive list of chiral GC columns through its Astec® and DEX™ product lines.

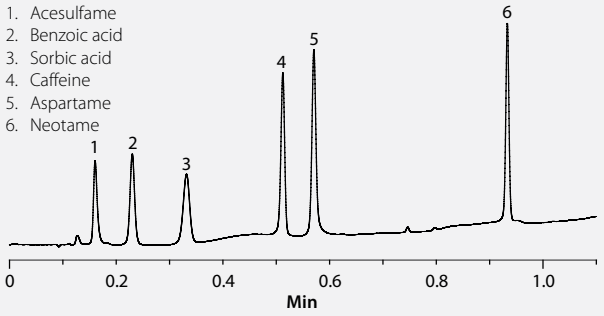
Artificial Sweeteners and Preservatives

Synthetic compounds that duplicate the taste of sugar, but contain less energy, are often added to diet foods and beverages. The logic is to maintain the desired taste, but reduce the caloric value. To achieve a targeted sweetness, artificial sweeteners are often used in specific combinations to mimic the sweetness observed from natural sugars. Other compounds that have the ability to inhibit microbial growth are added to prolong shelf life. Because artificial sweeteners and preservatives are both considered additives, they are often regulated. Therefore, their identifications and concentrations must be determined. **Figure 17** shows the HPLC separations of three artificial sweeteners (acesulfame, aspartame and neotame), two preservatives (benzoic acid and sorbic acid) and caffeine in a diet soda sample.

Figure 17. HPLC Analysis of Diet Soda

column: Ascentis® Express RP-Amide, 3 cm x 4.6 mm I.D., 2.7 µm particles (53921-U)
mobile phase: (A) 100 mM ammonium acetate, pH 5.6, titrated with acetic acid; (B) water; (C) acetonitrile
gradient: 20% A constant; 5 to 60% C in 1 min; hold at 60% C for 0.1 min
flow rate: 3 mL/min
column temp.: 40 °C
detector: UV at 214 nm
injection: 1 µL
sample: diet soda 100–500 µg/mL in buffer

1. Acesulfame
2. Benzoic acid
3. Sorbic acid
4. Caffeine
5. Aspartame
6. Neotame



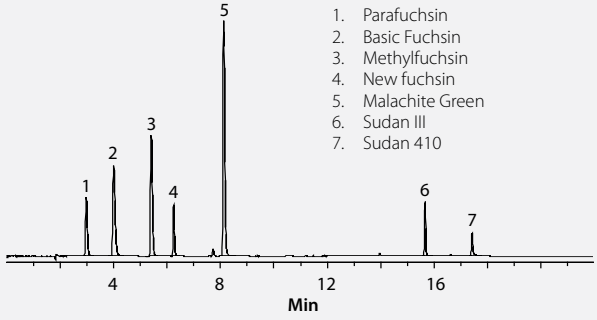
Food Dyes

A sensitive HPLC method can be used for quality control testing of dyes and the identification of by-products. Supelco's Ascentis Express HPLC columns provide outstanding sensitivity and resolution for such applications. **Figure 18** shows a UV chromatogram of seven dyes after resolution, sensitivity and peak symmetry were optimized for all analytes. An organic phase mixture of methanol:acetonitrile (10:90) with a final gradient composition of 98% organic mobile phase resulted in the best overall peak shapes with minimal tailing of compounds.

Figure 18. HPLC Analysis of Food Dyes

column: Ascentis Express C8, 10 cm x 4.6 mm I.D., 2.7 µm particles (53837-U)
mobile phase: (A) water with 0.1% formic acid; (B) acetonitrile:methanol (90:10)
gradient: 25% B 0–1.5 min; to 98% B by 15 min; 98% B 15–22 min; to 25% B by 25 min
flow rate: 0.8 mL/min
column temp.: 55 °C
detector: UV DAD, 200–950 nm
injection: 3 µL
sample: 7 dyes dissolved in a mixture of methanol:acetonitrile

1. Parafuchsin
2. Basic Fuchsin
3. Methylfuchsin
4. New fuchsin
5. Malachite Green
6. Sudan III
7. Sudan 410



Featured Products

Cat. No.	Product Description
SPME Fiber Assemblies	
57348-U	2 cm 50/30 µm divinylbenzene/carboxen on polydimethylsiloxane, 3 ea
GC Columns	
28564-U	SLB®-5ms, 20 m x 0.18 mm I.D., 0.18 µm
28576-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm
28471-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm
28473-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.50 µm
28089-U	Equity-5, 30 m x 0.25 mm I.D., 0.25 µm
24343	SUPELCOWAX 10, 15 m x 0.10 mm I.D., 0.10 µm
24079	SUPELCOWAX 10, 30 m x 0.25 mm I.D., 0.25 µm
24284	SUPELCOWAX 10, 30 m x 0.25 mm I.D., 0.50 µm
73033AST	Astec CHIRALDEX® G-TA, 30 m x 0.25 mm I.D., 0.12 µm
24304	β-DEX 120, 30 m x 0.25 mm, 0.25 µm
Ascentis Express HPLC Columns (2.7 µm particles)	
53823-U	C18, 10 cm x 2.1 mm I.D.
53832-U	C8, 10 cm x 2.1 mm I.D.
53837-U	C8, 10 cm x 4.6 mm I.D.
53913-U	RP-Amide, 10 cm x 2.1 mm I.D.
53921-U	RP-Amide, 3 cm x 4.6 mm I.D.
53336-U	Phenyl Hexyl, 10 cm x 2.1 mm I.D.
53939-U	HILIC, 10 cm x 2.1 mm I.D.
53569-U	F5, 10 cm x 2.1 mm I.D.
Ascentis Express HPLC Columns (5 µm particles)	
50537-U	C18, 15 cm x 4.6 mm I.D.
50392-U	C8, 15 cm x 4.6 mm I.D.
50483-U	Phenyl Hexyl, 15 cm x 4.6 mm I.D.
50289-U	HILIC, 15 cm x 4.6 mm I.D.
50631-U	F5, 15 cm x 4.6 mm I.D.
Analytical Standards	
17992	Disperse Blue 35
29173	Disperse Orange 1
11074	Disperse Red 1
11344	Disperse Yellow 3
40446	Pararot
51383	Sudan I
07937	Sudan II
68562	Sudan III
67386	Sudan IV
91282	Sudan Red G
Analytical Reagents and Solvents	
17836	Ammonium acetate, for HPLC, >99.0%
34967	Acetonitrile, LC-MS CHROMASOLV®, >99.9%

WATER/MOISTURE CONTENT

Karl Fischer (KF) titration is the most important and reliable direct method to determine the water content of foods in a selective manner. Using HYDRANAL® reagents, high throughput, stable end points and accurate results can all be achieved in KF moisture analysis of a broad variety of food samples. HYDRANAL began when pioneering chemists Eugen Scholz and Helga Hoffmann replaced the noxious pyridine commonly used for KF with imidazole. Today, HYDRANAL is the world-wide leader in pyridine-free reagents for KF titration, and sets the industry standard for quality, capacity, speed, safety and reliability.

Benefits of this product line include:

- Effective on all types of food substances
- Rapid, accurate and precise measurements
- Measures the complete water content, including chemically combined water, but not other volatile substances (an advantage over drying methods)
- Includes many auxiliary reagents for enhancing sample solubility and speed of the titration

Over the years, Sigma-Aldrich® has built a large knowledge base. Fluka's HYDRANAL service labs in Germany and in the United States provide customer support in the use of HYDRANAL reagents and KF techniques. This technical help includes solving problems such as sample solubility, side reactions, selecting the appropriate KF reagent for specific needs and challenging samples. Supportive literature is offered in the form of various brochures, a manual and a multimedia guide on CD.

Included are examples relevant to this area.

Water Content of Chocolate

Chocolate and milk chocolate samples with high fat content need pre-treatment before the water determination can be carried out. Directly before the titration, the chocolate sample should be ground or grated. The sample should not be left exposed to ambient air once it is grated, otherwise its water content will change according to the room conditions. In order to dissolve the fat and finely dispersed chocolate samples in the Karl Fischer working medium, the addition of chloroform to the working medium is recommended. Alternatively, the titration can be carried out at 50 °C. Recommended sample size is approximately 1 g. Before starting the titration and after sample addition, a stirring time of two to three minutes should be applied. Titration duration is about three minutes, using the reagents described in **Table 1**.

Water Content of Cheese

Cheese does not dissolve in methanol and therefore does not release its water content in classical KF solvents. The addition of formamide to the working medium as a solubilizer is necessary. Carrying out the titration at elevated temperatures is also recommended. The sample should be finely grated or cut into small pieces. This type of sample will give a turbid solution with the working medium after stirring for 5–10 minutes. The titration should take about 2–3 minutes (sample size 0.2 g / powder funnel) using the reagents described in **Table 2**. Coulometric KF titration is not recommended for this sample type.

Water Content of Ice Cream

The sample should be melted and carefully homogenized before the analysis. To improve the dissolution of the fat content, the titration should be carried out in HYDRANAL-LipoSolver CM, which contains chloroform. Titration time is about 2–3 minutes (sample size 0.05 g / syringe without needle) using the reagents described in **Table 3**. Coulometric KF titration is not recommended for this sample type.

Water Content of Gum

Fruit gum dissolves very slowly in the alcoholic media of the KF reagents. It is recommended to cut a 0.3 g sample into small pieces with a knife or scissors. Addition of formamide to the working medium is required for sample dissolution. If the titration is carried out at 50 °C, dissolution time is around 3 minutes, with an additional 3–4 minutes for titration using the reagents described in **Table 4**.

Table 1. Reagents for Chocolate and Milk Chocolate

Technique	Titrating Agent	Working Medium
One-Component		
Option 1	HYDRANAL-Composite 5	HYDRANAL-Methanol Rapid:HYDRANAL-Chloroform (1:1)
Option 2	HYDRANAL-Composite 5	HYDRANAL-Methanol dry:HYDRANAL-Chloroform (1:1)
Option 3	HYDRANAL-Composite 5	HYDRANAL-LipoSolver CM
Two-Component		
Option 1	HYDRANAL-Titrant 5	HYDRANAL-Solvent:HYDRANAL-Chloroform (1:1)
Option 2	HYDRANAL-Titrant 5	HYDRANAL-Solvent CM

Table 2. Reagents for Cheese

Technique	Titrating Agent	Working Medium
One-Component		
Option 1	HYDRANAL-Composite 5	HYDRANAL-Methanol dry:HYDRANAL- Formamide dry (1:1)
Option 2	HYDRANAL-Composite 5	HYDRANAL-Methanol Rapid:HYDRANAL- Formamide dry (1:1)
Two-Component		
Option	HYDRANAL-Titrant 5	HYDRANAL-Solvent:HYDRANAL-Formamide dry (1:1)

Table 3. Reagents for Ice Cream

Technique	Titrating Agent	Working Medium
One-Component		
Option 1	HYDRANAL-Composite 5	HYDRANAL-LipoSolver CM
Option 2	HYDRANAL-Composite 5	HYDRANAL-Methanol dry:HYDRANAL- Chloroform (1:1)
Option 3	HYDRANAL-Composite 5	HYDRANAL-Methanol Rapid:HYDRANAL- Chloroform (1:1)
Two-Component		
Option 1	HYDRANAL-Titrant 5	HYDRANAL-Solvent CM
Option 2	HYDRANAL-Titrant 5	HYDRANAL-Solvent:HYDRANAL-Choroform (2:1)

Table 4. Reagents for Fruit Gum with Sugar Crust

Technique	Titrating Agent	Working Medium
One-Component		
Option 1	HYDRANAL-Composite 5	HYDRANAL-Methanol dry:HYDRANAL- Formamide dry (2:1)
Option 2	HYDRANAL-Composite 5	HYDRANAL-Methanol Rapid:HYDRANAL- Formamide dry (2:1)
Two-Component		
Option	HYDRANAL-Titrant 5	HYDRANAL-Solvent:HYDRANAL-Formamide dry (1:1)

Featured Products

Cat. No.	Product Description
34805	HYDRANAL-Composite 5, 1 L
37855	HYDRANAL-LipoSolver CM, 1 L
34741	HYDRANAL-Methanol dry, 1 L
37817	HYDRANAL-Methanol Rapid, 1 L
34724	HYDRANAL-Formamide dry, 1 L
37863	HYDRANAL-Chloroform, 1 L
34801	HYDRANAL-Titrant 5, 1 L
34800	HYDRANAL-Solvent, 1 L
34812	HYDRANAL-Solvent CM, 1 L

GENETICALLY MODIFIED ORGANISMS

(GMO)

Some crop types have undergone genetic engineering to impart desirable traits, such as gaining resistance to pests, herbicides and weather, improving shelf life, and increasing specific nutritional indicators. Because of concerns over the safety of food, forage, and feedstuff produced from GMO plants, their use is strictly regulated in a number of countries. Sigma-Aldrich® offers a wide range of certified reference materials (CRMs) for the most widely used GMOs. These products are manufactured by the IRMM (Institute of Reference Materials and Measurements) which is a part of the joint research center (JRC) of the European Commission. Some of these products are shown in **Figure 19**.

Available GMO CRMs include cotton, maize (corn), potato, rapeseed, soya bean and sugar beet. GMO CRMs are produced gravimetrically, by mixing GMO-containing material with GMO-free material in different ratios to achieve the target level. Included is one example relevant to this area of food and beverage analysis.

Figure 19. GMO CRMs

Photo courtesty of Thomas Linsinger (JRC, European Union)

Soya Bean GMO CRMs

The appearance of soya bean plants, such as those shown in **Figure 20**, may be identical whether the plant is natural or a GMO. For this reason, analytical techniques such as polymerase chain reaction (PCR) and gel electrophoresis must be performed. The source (natural or GMO) can be determined because the pattern obtained from the natural plant will differ from the pattern obtained from the GMO. Additionally, the ratio of natural and GMO in a sample can also be determined if CRMs exist at various levels. For example, CRMs for Soya 305423 are available at four levels (one level being a blank). This allows four data points (<0.08%, 0.5%, 1% and 10%) for quantitation as well as identification. Also of note is that more than one GMO may exist for a given plant type (for example, Soya 305423 and Soya 356043 are different engineered soya bean GMOs).

Figure 20. Soya Bean Sprouts

Featured Products

Cat. No.	Product Description
Cotton GHB119	
ERMBF428A-1G	Contains 0% Cotton GHB119, 1 g
ERMBF428B-1G	Contains 1% Cotton GHB119, 1 g
ERMBF428C-1G	Contains 10% Cotton GHB119, 1 g
Maize 98140	
ERMBF427A-1G	Contains 0% Maize 98140, 1 g
ERMBF427B-1G	Contains 0.5% Maize 98140, 1 g
ERMBF427C-1G	Contains 2% Maize 98140, 1 g
ERMBF427D-1G	Contains 10% Maize 98140, 1 g
Potato EH92-527-1	
ERMBF421A-1G	Contains 0% Potato EH92-527-1, 1 g
ERMBF421B-0.5G	Contains 100% Potato EH92-527-1, 0.5 g
Soya 305423	
ERMBF426A-1G	Contains <0.08% Soya 305423, 1 g
ERMBF426B-1G	Contains 0.5% Soya 305423, 1 g
ERMBF426C-1G	Contains 1% Soya 305423, 1 g
ERMBF426D-1G	Contains 10% Soya 305423, 1 g
Soya 356043	
ERMBF425A-1G	Contains <0.05% Soya 356043, 1 g
ERMBF425B-1G	Contains 0.1% Soya 356043, 1 g
ERMBF425C-1G	Contains 1% Soya 356043, 1 g
ERMBF425D-1G	Contains 10% Soya 356043, 1 g
Sugar Beet H7-1	
ERMBF419A-1G	Contains 0% Sugar Beet H7-1, 1 g
ERMBF419B-1G	Contains 100% Sugar Beet H7-1, 1 g

PHYSICAL CHARACTERISTICS

The measurement of certain physical properties is often used to determine the identity or purity of a substance. To ensure the accuracy of these measurements, the measurement equipment needs to be regularly calibrated using accurate analytical standards with known properties. Sigma-Aldrich offers a wide range of physical property standards for various applications important in food analysis.

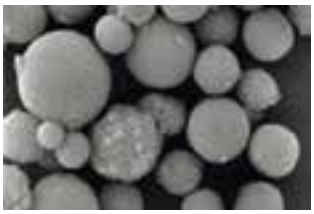
- The products cover a wide range of physical properties, such as, melting point, density, conductivity, viscosity, turbidity, particle size, color, thermal conductivity, mechanical, morphological, optical and isotope measurement
- A growing number of products are certified reference materials (CRMs), supplied by recognized accredited manufacturers like Paragon, Whitehouse Scientific, H&D Fitzgerald and the IRMM

Included are examples relevant to this area.

Viscosity Standards

Sigma-Aldrich's extensive product portfolio of certified viscosity standards is produced by Paragon Scientific (UK) in an ISO 17025 certified laboratory in strict accordance with ASTM D2162 (Standard Practice for basic calibration of master viscosity standard oils). All standards are traceable to NIST, and are supplied with a certificate stating the viscosity values and density at various temperatures, uncertainties and expiry date. Kinematic viscosity and dynamic viscosity are presented for all temperatures.

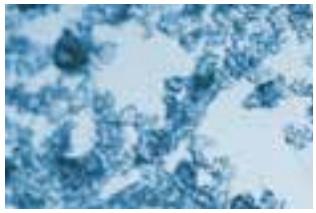
Particle Size Standards



These NIST (National Institute of Standards and Technology, USA) and NPL (National Physical Laboratory, UK) traceable standards were commissioned by the Bureau of Certified References (BCR) and are measured by a large international team, including the 20 top laboratories in the field of particle size. For the certification of the glass particles, several unambiguous primary methods such as microscopy, sieving, sedimentation and Coulter counter were used. The standards are delivered in sets of 10 vials in quantities suitable for any method of analysis without further subdivision. These products are manufactured and certified by the recognized specialists from Whitehouse Scientific.

Melting Point Standards

Melting point is used to identify compounds and estimate purity. Sigma-Aldrich offer a range of melting point standards to help ensure



reliable performance of melting point instruments. Replicate measurements allow reporting of an uncertainty value, +/- 0.3 °C to +/- 0.5 °C. Measurements are made in the thermodynamic mode, with traceability to primary reference material.

Featured Products

Cat. No.	Product Description
Melting Point Standards	
01422-1G	79–81 °C (Naphthalene), 1 g
76170-5G	121–123 °C (Benzoic acid), 5 g
Density Standards	
15889-10ML-F	1623 Kg/m^3, 10 mL
44964-10ML-F	692 Kg/m^3, 10 mL
Conductivity Standards	
60138-250ML	14.7 mS/m, 250 mL
60136-250ML	141 mS/m, 250 mL
Viscosity Standards	
93835-500ML	N2, kinematic 2.216 mm^2/s (cSt), dynamic 1.770 mPa (cP), 500 mL
63484-500ML	N10, kinematic 17.01 mm^2/s (cSt), dynamic 14.43 mPa (cP), 500 mL
Turbidity Standards	
70036-1EA-R	1 and 10 NTU (2 pcs)
70037-1EA-R	100 and 1000 NTU (2 pcs)
Particle Size Standards	
42459-10X0.1G	1–10 µm, 10 x 0.1 g ea
80847-10X0.25G	10–100 µm, 10 x 0.25 g ea
Color Standards	
86293-1SET-F	Solutions BY, according to Ph. Eur., set of 7 x 2 mL ampules
82995-1SET-F	Solutions GY, according to Ph. Eur., set of 7 x 2 mL ampules
Thermal Conductivity Standards	
BCR724B-1EA	Glass ceramic, 21 mm x 13.9 mm O.D. rod, 1 ea
BCR724C-1EA	Glass ceramic, 22 mm x 25.9 mm O.D. rod, 1 ea
Mechanical Standards	
BCR116-3.2KG	Limestone powder, for shear testing, 3.2 Kg
Morphological Standards	
BCR069-10G	Quartz, 14–90 µm, for Stokes' diameter, 10 g
BCR130-50G	Quartz, 50–220 µm, for volume diameter, 50 g
BCR302-20G	Microcrystalline cellulose, for water sorption, 20 g
Optical Standards	
BCR400-1EA	Tomato paste color tile, 10 cm x 10 cm, 1 ea
Isotope Measurement Standards	
ERMAE701-1SET	^{41}Ca/^{40}Ca in 0.6 M HNO_3, for isotope abundance ratio, 1 set
ERMAE642-4ML	^{37}Cl in water, for isotope amount content, 4 mL

FAST AND SENSITIVE GLUTEN DETECTION

Now offering AOAC Certified Gluten Testing ELISA Kits and Lateral Flow Strips

Now offering Gluten ELISA kits and lateral flow strips to help meet the FDA's 20 parts per million (PPM) or less gluten-free label requirements to ensure your labeling accuracy.

Gluten ELISA Kits Provide:

- Quantitative low levels of gluten in food ingredients - detection levels of less than 20 PPM
- Detection of gluten levels in prepared and processed foods and beverages
- Standard curve based on the levels of gluten found in cooked breads
- Detection of gluten levels in rye and barley
- Validation and certification as a Performance Tested Methods(sm) (#051101) by AOAC Research Institute

Gluten Testing Strips Provide:

- Detection limits of 10 PPM
- Results in less than 20 minutes
- Rapid detection of gluten in raw ingredients, prepared foods and on surfaces
- Validation and certification as a Performance Tested Methods(sm) (#051101) by AOAC Research Institute

Gluten Product Offering:

Cat. No.	Product Description
SE110019	Gluten ELISA Kit
SE110021	Gluten Testing Strip Kit

MEAT SPECIES IDENTIFICATION KITS

Preventing adulteration of meat with less desirable or objectionable meat species is important for economic, regulatory, health and ethnic reasons. Our ELISA meat species kits have been formatted and refined for ease of use to meet or exceed USDA-FSIS protocol standards.

For more information, visit
sigma-aldrich.com/meatspecies

ELISA meat species kits ensure:

- Meat quality and composition is confirmed
- Meat meets USDA/FSIS protocols
- Meat is unadulterated
- Meat is properly labeled

Cooked Meat Species Kits – USDA Official Method

Cat. No.	Product Description	Product Details
SE110001	Beef cooked meat species ELISA kit	**Limit of detection:** 1.0% in canned, cooked, or processed foods (USDA protocol) **Specificity:** All blood fed tissues, species specific as indicated **# of tests per kit:** Up to 40 samples in duplicate (20 samples using USDA procedure) **Hands-on-time:** ~ 30 minutes (for sample preparation/extraction) 3 hours for immunoassay procedures **Kit format:** 96 microwell plate system with single strip format (single strips of 8 wells)
SE110005	Horse cooked meat species ELISA kit	
SE110002	Pork cooked meat species ELISA kit	
SE110003	Poultry cooked meat species ELISA kit	
SE110004	Sheep cooked meat species ELISA kit	
SE110008	3-species (beef, pork, and poultry) cooked meat species ELISA kit	
SE110009	4-species (beef, pork, poultry, and sheep) cooked meat species ELISA kit	
SE110006	Deer cooked meat species ELISA kit	

Raw Meat Species Kits – USDA Official Method

Cat. No.	Product Description	Product Details
SE110014	Horse raw meat species ELISA kit	**Limit of Detection:** <1.0% for meat and poultry (all species) <5.0% for milk (relevant species only) **Specificity:** Species specific serum albumin **# of tests per kit:** Up to 40 samples in duplicate **Hands-on-time:** 10 minutes (for sample preparation/extraction) 60 minutes (for immunoassay procedures) **Kit format:** 96 microwell plate system with single strip format (single strips of 8 wells)
SE110011	Pork raw meat species ELISA kit	
SE110012	Poultry raw meat species ELISA kit	
SE110013	Sheep raw meat species ELISA kit	
SE110010	Beef raw meat species ELISA kit	
SE110016	4-species (beef, pork, poultry, and sheep) raw meat species ELISA kit	
SE110015	3-species (beef, pork, and poultry) raw meat species ELISA kit	

Next Generation Meat Species ELISA Kits – MAFF Official Method

Cat. No.	Product Description	Product Details
SE110017	Ruminant MELISA kit	**Limit of Detection:** <0.5% muscle tissue in feed samples (at normal dilution) <0.5% muscle in MBM samples (with high sensitivity extraction) <15 ppm in 138°C tissue samples (with high sensitivity extraction) **Specificity:** Troponin-I (muscle), species specific as indicated **# of tests per kit:** Up to 42 samples in duplicate **Hands-on-time:** 30 minutes (for preparation/extraction) 90 minutes (for immunoassay) **Kit format:** 96 microwell plate system with single strip format (single strips of 8 wells)
SE110018	Pork MELISA kit	

Lateral Flow Assay for Fish Species Identification

Cat. No.	Product Description	Product Details
SE110020	Pangasius Testing Strip kit	**Qualitative test requiring only 0.5 g of tissue** **Hands-on-time:** ~10 minutes (strip is ready to read)

MICROBIOLOGY QUALITY CONTROL

Food contaminated by microorganisms (bacteria and yeasts), viruses and protozoa can cause severe disease in humans. There are two categories of foodborne diseases. First, food poisoning is caused by the presence of microbial toxins in food products, e.g. byStaphylococcus aureus, Clostridium perfringens (both produce enterotoxins which elicit enteric disease such as diarrhea), andClostridium botulinum (botulism is the most severe type of food poisoning).

Second, the growth of microorganisms in the body after eating contaminated food, e.g. by Salmonella spp. (salmonellosis) andCampylobacter jejuni (high fever, abdominal cramps). Many human pathogens are transmitted by fecal contaminated water, the most important being Salmonella typhi (typhoid fever) and Vibrio cholerae (cholera).

Microbiological test procedures for the examination of foods and beverages have been standardized and regulated, but nearly every country has its own regulations. Most of the culture media, additives, dyes and indicators, which Sigma-Aldrich® offers, conform to Swiss regulations, or other norms like IOS. Product lines include:

- Culture media and base ingredients
- Chromogenic media for differentiation of microbes based on colony color
- Test kits (biochemical, immunological and molecular biological methods) for pathogen detection, identification and confirmation
- Vitroid™ certified reference microorganisms to test the performance of microbiological assays

Included are examples relevant to this area.

Chromogenic Media

For simple and fast detection of bacteria, use media with a chromogenic substrate such as Salmon-GAL, X-Gal and X-glucuronide. Certain enzymes, produced by some bacteria, cleave these substrates, resulting in the different coloration of certain bacteria colonies. This allows microbiologists to clearly see the presence of the targeted microorganism. **Figure 21** shows the clearly visible color change associated with bacterial growth. Most chromogenic media is intended for detection of pathogenic bacteria, including Sigma-Aldrich's media for Clostridium perfringens, the first of their kind.

Figure 21. Colony Growth on Chromogenic Medium

Test Kits for Classic Biochemical Methods

Convenient discs and strips, such as those shown in **Figure 22**, can be used in the identification and confirmation of microorganisms. Based on rapid screening methods like the detection of enzymes with chromogenic substrates, indicators or on complex building reactions, they are faster and easier to use than conventional detection techniques. Also, the sensitivity to certain inhibitory substances can be used to identify organisms. The sterility indicator strips are used to monitor sterilization and are important for controlling the sterilization process.

Figure 22. Discs and Strips for Rapid Classical Biochemical Tests

Test Kit for Innovative Immunological Methods

The easy-to-use Staphylo Monotec Test Kit Plus is a rapid agglutination test for differentiation between Staphylococcus aureus and other Staphylococcus species. With this kit, three characteristic properties (coagulase, protein A and capsular serotype 5 polysaccharide) of S. aureus can be detected in one step with high reliability. This results in increased sensitivity and specificity in detecting methicillin resistant Staphyloccocus aureus. **Figure 23** shows the Staphylo Monotec Test Kit Plus in use.

Figure 23. Positive Agglutination Reaction

Test Kits for Innovative Molecular Biological Methods

HybriScan™ rapid test kits are based on an rRNA sandwich hybridization system which is less expensive and more robust than polymerase chain reaction (PCR) methods. They are also more accurate (only detects living cells), quicker (time savings of up to 10 days over cultivation-based assays) and can be performed with standard laboratory equipment. The processing of a HybridScan assay is shown in **Figure 24**. These kits can be used for the identification and quantification of spoilage and pathogenic microorganisms in foods and beverages. They are ideal for the comprehensive and reliable routine control of raw materials and concentrates in all production steps, up to the quality check of finished goods.

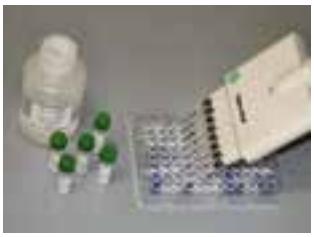


Figure 24. HybriScan Assay Showing Color Reaction prior to Photometric Quantification

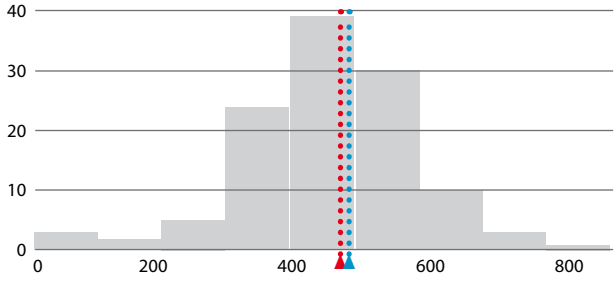
One such kit is the HybriScan D Beer kit, which can detect all beer-spoiling contaminants within two hours and in a single assay. After two hours (pre-enrichment for 24 hours, if necessary) the brewery could have its first reliable results. The robustness of the HybriScan assay enables detection of bacterial contaminations in brewer's yeast and leads to efficient use of this valuable resource. All bacteria, not just beer-spoiling contaminants, can be detected and quantified with this innovative rapid test system.

Vitroid Certified Reference Microorganism Discs

Through the Vitroid line, Sigma-Aldrich is the first company to produce certified reference test strains under double accreditation. They are certified according to ISO 17025, produced under ISO

Guide 34, and meet UKAS guidelines. These products contain a precisely defined and certified cfu level in a dried state on small discs. **Figure 23** shows an example of cfu distribution data.

Figure 25. Vitroid cfu Distribution (480 CFU/100 mL, Uncertainty 7.56, Certified Uncertainty 22.5)



Strains from internationally recognized cell culture repositories (ATCC and NCTC) are used, while the Vitroid stabilization technology provides high organism viability. As little as 100 µL is required to solubilize a disc. Alternatively, a disc can be placed directly on the surface of a medium plate. **Figure 26** shows examples of Vitroid discs in a container and on a plate. Upon solubilization, the standard is ready to use without a recovery or pre-incubation step.

Vitroid discs can easily be implemented into any microbiology laboratory's procedures, with little or no modification, to deliver a quantified dose of a microorganism. Their use provides an economical means for microbiologists to test the performance and limitations of their methods.

Figure 26 Vitroid Discs before Rehydration in Solution (left) and on a Plate (right)

Featured Products

Cat. No.	Product Description
Culture Media and Base Ingredients	
22091	Tryptic Soy Agar (TSA)
22092	Tryptic Soy Broth (TSB)
70138	Brain Heart Infusion Agar (BHI Agar)
53286	Brain Heart Infusion Broth (BHI Broth)
77187	Peptone Water, Phosphate-Buffered (BPW)
70139	Potato Glucose Agar (PDA)
05040	Agar
48722	Gelatin from Porcine Skin
70161	Yeast Extract
70166	Skim Milk Powder*
95039	Tryptone
22090	Casein Hydrolysate
70164	Meat Extract
70167	Malt Extract
Chromogenic Media	
12398	CP™ ChromoSelect Agar for *Clostridium perfringens*
73009	HiCrome™ ECC Agar for *Escherichia coli* and Coliforms
81938	HiCrome Coliform Agar for *Escherichia coli* and Coliforms
Test Kits	
88597	Catalase Test (Hydrogen Peroxide, 3%)
49940	ONPG Disks
40560	Oxidase Strips
50448	Staphylo Monotec Test Kit Plus
62533	HybriScan ***D*** Beer
68301	HybriScan ***D*** Drinks
02349	HybriScan ***D*** Total Bacterial Count
61397	HybriScan ***D*** Yeast
Vitroid Certified Reference Test Strain Discs	
RQC20106	*Clostridium perfringens*, NCTC Strain 10240, 500 cfu
RQC01705	*Escherichia coli*, ATCC Strain 11775, 200 cfu
RQC02008	*Legionella pneumophilia*, ATCC Strain 12821, 50,000 cfu
RQC01901	*Listeria monocytogenes*, ATCC Strain 19115, 30 cfu
RQC02301	*Salmonella goldcoast*, NCTC Strain 13175, 30 cfu
RQC13002	*Staphylococcus aureus* susp. Aureus, ATCC Strain 6538, 50 cfu

*Not available in the U.S.A.

PESTICIDE AND METABOLITE RESIDUES

Analysis of pesticide levels in food and beverage products is important not only to insure low levels for human consumption, but also to avoid international trade problems. At the present time, more than 1000 pesticide and metabolite residue compounds are identified as associated with food crops, either in current use or used in the past.

Included are examples relevant to this area.

QuEChERS Extraction/Cleanup Prior to Analysis of Pesticides in Oranges

This Quick-Easy-Cheap-Effective-Rugged-Safe (QuEChERS) method simplifies the sample preparation steps required for extraction of pesticides from foods of plant origin. The extraction is done using an extraction solvent (such as acetonitrile) and pre-weighed extraction/buffer salts. After a centrifugation step, the extract is cleaned with bulk SPE materials and additional salts. **Figure 27** shows the GC-MS chromatogram for an orange sample that was spiked with pesticides prior to extraction. **Figure 28** shows LC-MS/MS MRM transition chromatograms of a spiked orange extract. Cleanup was performed using Supel™ QuE Z-Sep/C18 sorbent, an innovative material that is better at fat removal than traditional PSA/C18 material.

Figure 27. GC Analysis of a Spiked Orange Sample

sample/matrix:	15 g ground-up orange spiked with 29 pesticides and 1 internal standard
extraction:	add 15 mL of 1% acetic acid in acetonitrile; add contents of Supel QuE acetate tube (55234-U); shake by hand for 1 min; centrifuge at 3300 rpm for 2 min; take a 2 mL aliquot; add contents of 2 Supel QuE PSA tubes (55287-U); centrifuge at 3300 rpm for 2 min; take a 1 mL aliquot; evaporate to 0.1 mL; reconstitute to 1.0 mL with toluene
column:	SLB®-5ms, 30 m x 0.25 mm I.D, 0.25 µm (28471-U)
oven:	100 °C (1 min), 10 °C/min to 300 °C (5 min)
inj. temp.:	250 °C
detector:	MSD, 300 °C; selected ion monitoring (SIM), 7 monitoring groups used
carrier gas:	helium, 1 mL/min constant
injection:	1 µL, pulsed (20 psi until 0.20 min), splitless (1.0 min)
liner:	4 mm I.D., splitless type, single taper design

1. Methamidiphos
2. Dichlorvos
3. Acephate
4. Propoxur
5. Ethoprophos (I.S.)
6. Hexachlorobenzene
7. γ-BHC
8. Diazinon
9. Chlorothalonil
10. Methyl chlorpyrifos
11. Carbaryl
12. Dichlofluanid
13. Chlorpyrifos
14. *p*-Dichlorobenzophenone
15. Cyprodinil
16. Pencanazole
17. Tolylfluanid
18. Heptachlor epoxide
19. Captan
20. Thiabendazole
21. Folpet
22. *cis*-Chlordane
23. Imazalil
24. 4,4′-DDE
25. Dieldrin
26. Endosulfan sulfate
27. Dicofol
28. *cis*-Permethrin
29. *trans*-Permethrin
30. Coumaphos

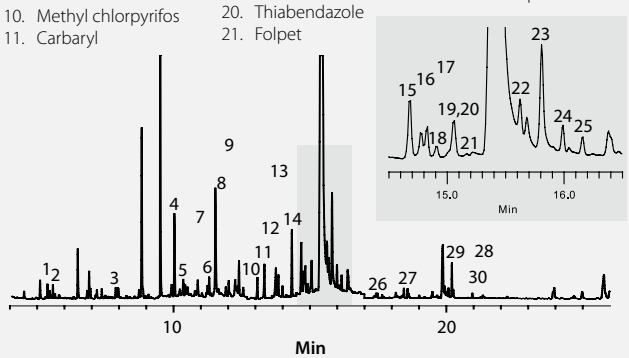
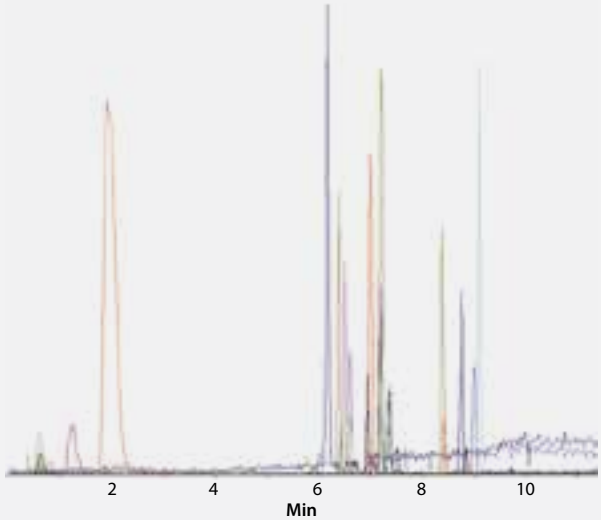


Figure 28. LC-MS/MS MRM Transition Chromatograms of a Spiked Orange Extract

sample/matrix:	10 g of pureed oranges (homogenized with rind); spike at 50 ppb (add 16.75 µL of a custom made pesticide mix, each analyte at 30 µg/mL)
extraction:	add 10 mL acetonitrile; shake for 1 minute; add contents of a Supel QuE citrate extraction tube (55227-U); shake immediately for 1 minute; centrifuge at 3200 rpm for 5 minutes; transfer 0.7 mL of the acetonitrile layer into a Supel QuE Z-Sep/C18 cleanup tubes (55284-U); shake for 1 minute; centrifuge at 5000 rpm for 5 minutes; transfer 0.2 mL of the supernatant into an empty 1.5 mL centrifuge tube; add 0.2 mL of water; centrifuge at 5000 rpm for 2 minutes
column:	Ascentis® Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase:	(A) 10 mM ammonium acetate in water; (B) 10 mM ammonium acetate in acetonitrile
gradient:	hold at 30% B for 1 min; 30% to 80% B in 2 min; hold at 80% B for 4 min; hold at 100% B for 3 min; hold at 30% B for 3 min
flow rate:	0.3 mL/min
pressure:	2730 psi
column temp.:	30 °C
detector:	MS/MS, ESI positive

1. Methomyl (0.60 min)
2. Trichlorfon (1.22 min)
3. Carbendazim (1.94 min)
4. Aldicarb (2.70 min)
5. Parathion-methyl (5.87 min)
6. Methabenzthiazuron (6.16 min)
7. Naled (6.37 min)
8. Methidathion (6.38 min)
9. Clethodim (6.42 min)
10. Phosmet (6.50 min)
11. Ametryn (6.58 min)
12. Sethoxydim (6.61 min)
13. Anilazine (6.78 min)
14. Fenxehamid (6.94 min)
15. Mecarbam (7.00 min)
16. Oryzalin (7.06 min)
17. Diflunezuron (7.18 min)
18. Fenoxycarb (7.19 min)
19. Iprobenfos (7.21 min)
20. Quinalphos (7.23 min)
21. Edifenphos (7.28 min)
22. Etrimfos (7.33 min)
23. Fenthion (7.34 min)
24. Fenitrothion (7.36 min)
25. Diazinon (7.37 min)
26. Tolclofos (7.52 min)
27. Phorate (7.53 min)
28. Chlorpyrifos-methyl (7.67 min)
29. EPN (7.68 min)
30. Terbufos (8.14 min)
31. Ethion (8.36 min)
32. Lufenuron (8.40 min)
33. Spiromesifen (8.74 min)
34. Octhilinone (8.74 min)
35. Pyraclostrobin (8.75 min)
36. Carbophenothion (8.83 min)
37. Flufenoxuron (9.02 min)
38. Fenpyroximate (9.10 min)



Dual-Layer SPE Tube Cleanup and GC Analysis of Pesticides in Spinach

Highly pigmented foods, such as spinach, require cleanup prior to analysis for pesticide residues. A dual-layer SPE tube containing graphitized carbon and aminopropyl on silica (NH2) is often used.

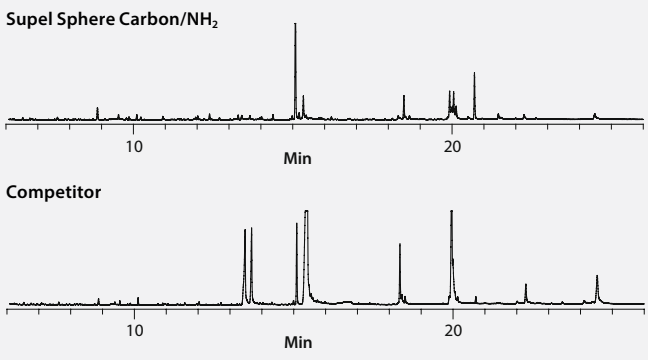
- Carbon removes chlorophyll pigments and sterols
- NH2 removes fatty acids, polar pigments, organic acids, and sugars

Removing these matrix materials reduces interferences that can lead to ion suppression in LC-MS, inlet contamination in GC-MS, and column contamination for both techniques.

Following extraction of a spinach sample, cleanup was performed using either a Supel Sphere SPE tube (uses spherical material for both layers), or a traditional SPE tube (uses granular material). Tubes with spherical materials provide fast, more consistent flows compared to tubes containing granular material, and results in more contact time between extracts and sorbents. The resulting chromatograms are displayed in **Figure 29**. As shown, the spherical material removes more unwanted matrix interference than the irregular material.

Figure 29. Extracts of Spiked Spinach (full scan mode, same Y-axis)

sample/matrix:	10 g homogenized spinach in a 50 mL centrifuge tube (55248-U)
extraction:	add 10 mL acetonitrile; shake for 1 minute; add content of a Supel QuE acetate extraction tube (55234-U); shake for 1 minute; centrifuge at 3200 rpm for 5 minutes; transfer the supernatant to a 12 mL tube containing 1 g of anhydrous magnesium sulfate; shake for 1 minute; centrifuge at 3200 rpm for 5 minutes; transfer 5 mL of the supernatant to a glass test tube and evaporate at 40 °C to 1 mL; add 250 µL of toluene
SPE tube:	Supel Sphere Carbon/NH_2 Tube (54283-U)
conditioning:	10 mL of acetonitrile:toluene (75:25)
sample addition:	from extraction above
elution:	20 mL of acetonitrile:toluene (75:25)
column:	SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm (28576-U)
oven:	70 °C (2 min), 15 °C/min to 325 °C (5 min)
inj. temp.:	Programmed, 60 °C (0.28 min), 600 °C/min to 325 °C (5 min)
detector:	MS, SIM mode
carrier gas:	helium, 1 mL/min constant
injection:	10 µL LVI, PTV solvent vent, rapid injection speed; split vent flow: 100 mL/min (5 psi) until 0.28 min, 60 mL/min at 2.78 min
liner:	4 mm I.D., split type, wool packed FocusLiner™ with single taper design



Extraction/Cleanup and GC Analysis of Pesticides in Various Produce Types

The detection of organophosphorous (OP) pesticides is sometimes better accomplished using a selective nitrogen-phosphorous detector (NPD) instead of a MS. Several produce types were spiked with a mixture of 63 OP pesticides, to a 10 ng/g level. The extraction method involved mixing each sample with 10 mL of acetonitrile and a mixture of dry salts (4 g of magnesium sulfate and 1 g of sodium chloride). Extract cleanup was performed using Supelclean ENVI-Carb-II/PSA dual-layer SPE tubes prior to GC-NPD analysis. Recovery data for several representative pesticides is shown in **Table 5**.

Table 5. % Recovery (%RSD) of Several OP Pesticides

Pesticide	Cabbage	Onion	Mushroom	Apple
Methamidophos	76% (10%)	70% (45%)	70% (5%)	58% (3%)
Dichlorvos	masked	57% (8%)	89% (7%)	76% (6%)
Ethoprophos	63% (9%)	66% (11%)	77% (9%)	75% (7%)
Methyl parathion	65% (2%)	66% (11%)	81% (7%)	77% (6%)
Profenfos	71% (3%)	77% (11%)	92% (7%)	82% (6%)
Coumafos	78% (8%)	69% (8%)	94% (12%)	88% (6%)

Featured Products

Cat. No.	Product Description
Supel QuE QuEChERS Products	
55234-U	Acetate Tube, 12 mL, 50 ea
55227-U	Citrate Extraction Tube, 12 mL, 50 ea
55296-U	Z-Sep+ cleanup tube, 12 mL, 50 ea
55284-U	Z-Sep/C18 cleanup tube, 2 mL, 100 ea
55228-U	PSA Tube, 12 mL, 50 ea
55229-U	PSA/C18 Tube, 12 mL, 50 ea
55286-U	PSA/C18/ENVI-Carb Tube, 12 mL, 50 ea
55230-U	PSA/ENVI-Carb Tube 1, 12 mL, 50 ea
55248-U	Empty Tube, 50 mL, 50 ea
SPE Tubes	
54283-U	Supel Sphere Carbon/NH_2, 6 mL, 30 ea
55119-U	Supelclean ENVI-Carb-II/PSA, 500 mg/300 mg/6 mL, 30 ea
54035-U	Supelclean ENVI-Carb/NH_2, 500 mg/500 mg/6 mL, 30 ea
57094	Supelclean ENVI-Carb, 500 mg/6 mL, 30 ea
52579-U	Supelclean PSA, 500 mg/6 mL, 30 ea
Ascentis Express HPLC Columns (2.7 µm particles)	
53822-U	C18, 5 cm x 2.1 mm I.D.
53823-U	C18, 10 cm x 2.1 mm I.D.
53832-U	C8, 10 cm x 2.1 mm I.D.
53913-U	RP-Amide, 10 cm x 2.1 mm I.D.
53569-U	F5, 10 cm x 2.1 mm I.D.
GC Columns	
28564-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm
28576-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm
28471-U	SLB-5ms, 30m x 0.25 mm I.D., 0.25 µm
28473-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.50 µm
Analytical Reagents and Solvents	
17836	Ammonium acetate, for HPLC, >99.0%
34967	Acetonitrile, LC-MS CHROMASOLV®, >99.9%

VETERINARY DRUG RESIDUES

The use of several types of drugs may be integral to the process of raising food producing animals and fish. It is possible that drug residues may remain in the final food or beverage product, even after processing steps. Because elevated amounts of some of these drugs may cause detrimental health effects to humans (including being carcinogens and allergy-triggers), it is important to monitor their levels. These compound types include:

- Antibiotics (aminoglycosides, chloramphenicol, fluoroquinolones, malachite green dye, etc.) used to inhibit microbial growth
- Feed additives (hormones and steroids) used to increase yield

Included are examples relevant to this area.

Extraction/Cleanup and HPLC Analysis of Fluoroquinolones in Beef Kidney

One difficulty in the analysis of drugs in complex matrices is that the sample preparation process tends to capture many non-target compounds along with the target analytes. The presence of these interferences may lead to ion-suppression effects when LC-MS is used as the analytical technique. It is necessary to select cleanup steps that remove the interferences from extracts, without also removing target analytes. One approach is to use a mixed mode SPE material, such as Discovery® DSC-MCAX. The two ligands blended in this mixed mode hydrophobic-cation exchange material are C8 (provides reversed-phase interaction) and benzene sulfonic acid (provides strong cation exchange interaction). This unique combination of ligands allows aggressive washing steps to remove interferences without loss of target analytes.

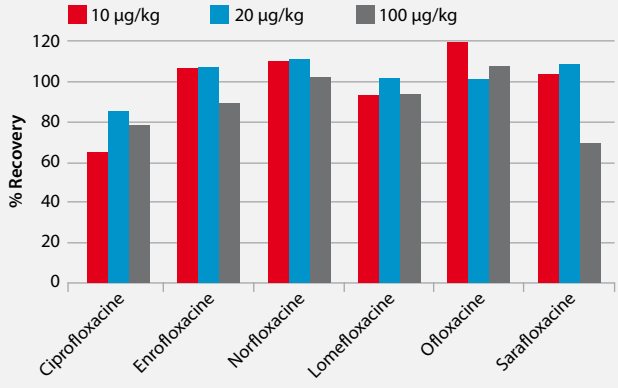
Beef kidney samples were spiked at several levels with six fluoroquinolones, extracted, cleaned up and then analyzed. The recovery results at three spiking levels are shown in **Figure 30**. Five of the six compounds gave acceptable recoveries. Ciprofloxacine recovery was lower due to observed ion-suppression effects.

Extraction/Cleanup and HPLC Analysis of Chloramphenicol in Milk

Another approach for the removal of interferences without the loss of target analytes is through the use of molecular imprinted polymer (MIP) materials. MIPs are highly crosslinked polymer-based molecular recognition elements. Their primary feature is that they can be engineered to be highly selective to compounds that are structurally similar. The benefit is increased affinity for the specific compound structure they are engineered for, and decreased affinity for other structures.

Figure 30. Recoveries of Fluoroquinolones from Beef Kidney at Three Spiking Levels

sample/matrix:	2 g beef kidney sample spiked with 6 fluoroquinolones; homogenize with 30 mL of 50 mM sodium hydrophosphate (pH 7.4); centrifuge at 5000 rpm for 10 min; filter; adjust to pH 3 using phosphoric acid
SPE tube:	Discovery DSC-MCAX, 50 mg/1 mL (52781-U)
conditioning:	1 mL methanol; 1 mL pH 3 phosphate buffer
sample addition:	1 mL kidney extract
washing:	1 mL 50 mM phosphate buffer at pH 3; 1 mL methanol; dry tube under vacuum for 2 min
elution:	1 mL 5% NH_3 in methanol
eluate post-treatment:	evaporate at 35 °C under nitrogen; reconstituted with 150 µL of 50% acetonitrile-0.1% formic acid
column:	Ascentis® C18, 5 cm x 2.1 mm I.D., 3 µm particles (581300-U)
mobile phase:	(A) 0.1% formic acid in water; (B) acetonitrile
gradient:	0 min: 5% B; 7 min: 15% B; 7.2 min: 80%B; 8.2 min: 5% B; 11.2 min: 5% B
flow rate:	0.5 mL/min
column temp:	25 °C
detector:	MS/MS, ESI (+)
injection:	3 µL



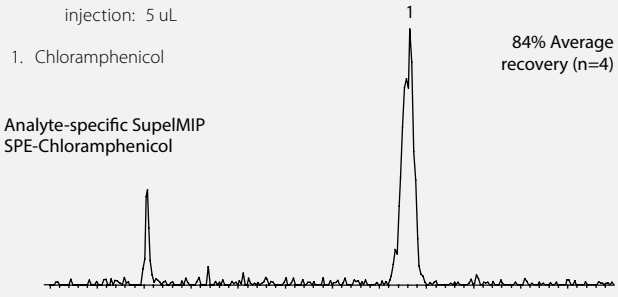
Confirmatory methods for chloramphenicol use LC-MS as the analytical technique due to the sensitivity required (0.3 µg/kg), plus the additional benefit of analyte identity confirmation. **Figure 31** shows chromatograms comparing extracts of spiked milk samples following cleanup using either a chloramphenicol-specific MIP material, or a non-specific, generic polymeric material. **Figure 32** shows mass spectra (m/z 100–650) of the 3.65–4.00 min region that encompasses chloramphenicol. For this application, it is evident on both the chromatograms and the mass spectra, that the MIP material removes more interference in comparison to the generic polymeric material.

Figure 31. Comparison of Chromatograms from Two Cleanup Methods

Conditions (chloramphenicol-specific MIP material)

sample/matrix:	centrifuge whole pasteurized milk (purchased from local supermarket) at 5000 rpm for 15 min; collect lower aqueous lower layer; spike with chloramphenicol
SPE tube:	SupelMIP® SPE-Chloramphenicol, 25 mg/10 mL LRC (53210-U)
conditioning:	1 mL methanol, 1 mL DI water
sample addition:	1 mL of the pre-treated milk sample
washing:	2 x 1 mL MS-grade water; 1 mL 5% acetonitrile in 0.5% acetic acid; 2 x 1 mL MS-grade water; 1 mL 20% acetonitrile in 1% ammonium hydroxide; dry for 15 min under gentle vacuum; 3 x 1 mL dichloromethane; dry for 1 min under gentle vacuum
elution:	2 x 1 mL methanol:acetic acid:MS-grade water (89:1:10, v/v/v); combine eluate
eluate post-treatment:	evaporate at 50 °C to dryness under nitrogen; reconstitute with 150 µL of 100 mM ammonium acetate:water:acetonitrile (10:60:30)
column:	Ascentis C18, 10 cm x 2.1 mm I.D., 3 µm particles (581301-U)
mobile phase:	100 mM ammonium acetate:water:acetonitrile (10:60:30)
flow rate:	0.2 mL/min
column temp:	35 °C
detector:	MS, ESI (–), m/z 320-323
injection:	5 uL

1. Chloramphenicol



Conditions (non-specific hydrophilic polymeric material)

sample/matrix:	spike 5 mL of milk with 40 ng chloramphenicol; add 15 mL 10% trichloracetic acid in water to precipitate proteins; vortex; heated at 65 °C for 1 hour; cool to room temperature; centrifuge at 3000 rpm for 15 min; filter supernatant over glass wool (rinse filter with 10 mL DI water); adjust to pH 5 with 0.1 M sodium acetate
SPE tube:	conventional hydrophilic polymer SPE, 500 mg/12 mL
conditioning:	3 mL methanol; 4 mL DI water; 4 mL 10 mM hydrochloric acid
sample addition:	entire pre-treated milk extract
washing:	4 mL MS-grade water; 2 mL 5% methanol; 2 mL 50% methanol
elution:	2 mL methanol
eluate post-treatment:	evaporate at 50 °C to dryness under nitrogen; reconstitute with 0.4 mL DI water; liquid-liquid extract with 0.6 mL acetonitrile:dichloromethane (4:1, v/v); centrifuge at 7000 rpm for 5 min; transfer upper organic layer to a fresh tube; repeat liquid-liquid extraction procedure two additional times on the lower aqueous layer; combine all three organic layers; evaporate at 60 °C to dryness under nitrogen; reconstitute with 0.2 mL of 100 mM ammonium acetate:water:acetonitrile (10:60:30); filter through a 0.2 µm nylon filter

All other conditions are the same as above.

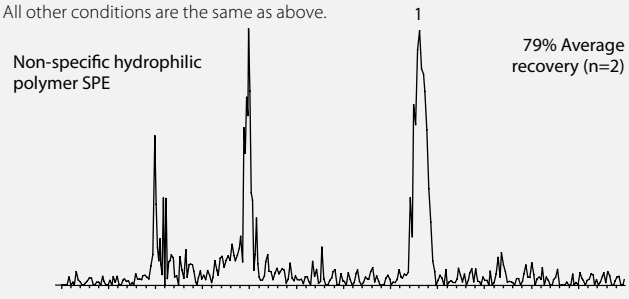
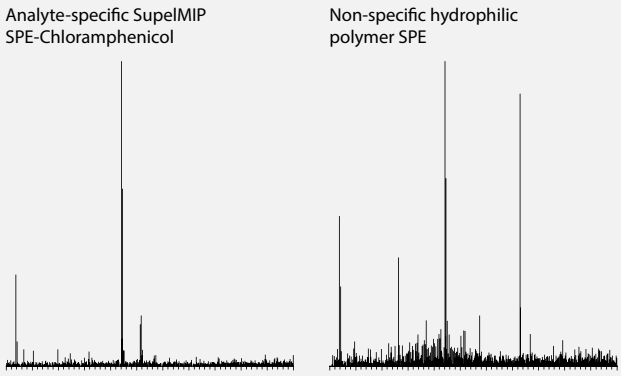


Figure 32. Comparison of Mass Spectra from Two Cleanup Methods



Featured Products

Cat. No.	Product Description
Supel™ QuE QuEChERS Products	
55234-U	Acetate Tube, 12 mL, 50 ea
55227-U	Citrate Extraction Tube, 12 mL, 50 ea
55296-U	Z-Sep+ cleanup tube, 12 mL, 50 ea
55284-U	Z-Sep/C18 cleanup tube, 2 mL, 100 ea
55228-U	PSA Tube, 12 mL, 50 ea
55229-U	PSA/C18 Tube, 12 mL, 50 ea
55286-U	PSA/C18/ENVI-Carb™ Tube, 12 mL, 50 ea
55230-U	PSA/ENVI-Carb Tube 1, 12 mL, 50 ea
55248-U	Empty Tube, 50 mL, 50 ea
SPE Tubes	
52781-U	Discovery DSC-MCAX, 50 mg/1 mL, 108 ea
52783-U	Discovery DSC-MCAX, 100 mg/3 mL, 54 ea
52786-U	Discovery DSC-MCAX, 300 mg/6 mL, 30 ea
54812-U	Supelclean™ ENVI-Carb Plus, 400 mg/1 mL Reversible Tube, 30 ea
55119-U	Supelclean ENVI-Carb-II/PSA, 500 mg/300 mg/6 mL, 30 ea
54035-U	Supelclean ENVI-Carb/NH_2, 500 mg/500 mg/6 mL, 30 ea
57094	Supelclean ENVI-Carb, 500 mg/6 mL, 30 ea
52579-U	Supelclean PSA, 500 mg/6 mL, 30 ea
53210-U	SupelMIP SPE-Chloramphenicol, 25 mg/10 mL LRC, 50 ea
Ascentis Express HPLC Columns (2.7 µm particles)	
53823-U	C18, 10 cm x 2.1 mm I.D.
53832-U	C8, 10 cm x 2.1 mm I.D.
53913-U	RP-Amide, 10 cm x 2.1 mm I.D.
53336-U	Phenyl Hexyl, 10 cm x 2.1 mm I.D.
53569-U	F5, 10 cm x 2.1 mm I.D.
GC Columns	
28564-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm
28576-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm
28471-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm
28473-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.50 µm
Analytical Reagents and Solvents	
17836	Ammonium acetate, for HPLC, >99.0%
34967	Acetonitrile, LC-MS CHROMASOLV®, >99.9%

TOXINS

(other than pesticide/drug residues)

Many government agencies require food and beverage products to be monitored for the presence of toxins that have detrimental effects on consumer health. Toxins can migrate into crops if they are grown in contaminated air or water. Another source is naturally occurring organisms (certain fungal, bacterial or algal growths emit toxins) that contaminate crops they co-exist with. Toxins may also be found in meat/dairy/poultry products if the animals were exposed to contaminated feed. Many of these toxins remain potent even after food processing/packaging steps, and remain in the final products. Areas of interest include:

- Mycotoxins/aflatoxins (in bread, cheese, cereal grains, oil seeds and tree nuts from mold and fungi)
- Allergens (particularly in dairy, egg, peanut, tree nuts, fish, shellfish, soy and wheat)
- Dioxins/furans/PCBs (on crops; in meats/dairy/poultry from contaminated feed)
- Perchlorate (in crops from contaminated groundwater; in meats/dairy from contaminated feed)
- Phycotoxins (in shellfish from algae)
- Phytochemicals such as furanocoumarins (in crops)
- Heavy metals such as mercury, lead, cadmium, arsenic, copper and nickel (primarily in fish from contaminated water)

Included are examples relevant to this area.

Mycotoxins

Mycotoxins are a diverse group comprised of hundreds of secondary metabolic products of various fungal species. Several show marked toxicity in humans. Contamination of the food supply with mycotoxins is increasingly prevalent, and can occur during growth, harvest, transportation, processing or storage. The removal of contaminated products from the food chain is a primary means of eliminating human exposure. Sensitive and accurate detection of very low levels of these compounds is critical to identify contaminated product.

Supel Tox SPE tubes are designed for fast and simple cleanup of mycotoxins. Few steps are needed, resulting in less variability of results. **Figure 33** depicts recovery and reproducibility data for four aflatoxins in peanut paste samples, comparing sample cleanup using Supel Tox SPE tubes to sample cleanup using immunoaffinity SPE tubes. The LC-MS analysis of 14 mycotoxins from a cereal sample is shown in **Figure 34**.

Figure 33. Cleanup of Aflatoxins in Peanut Paste

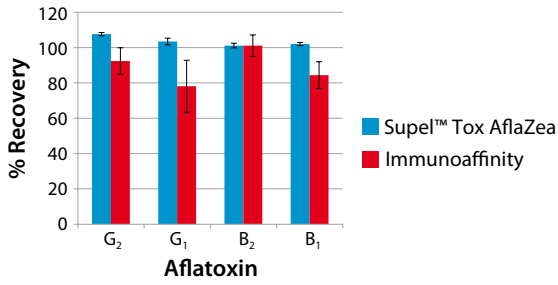
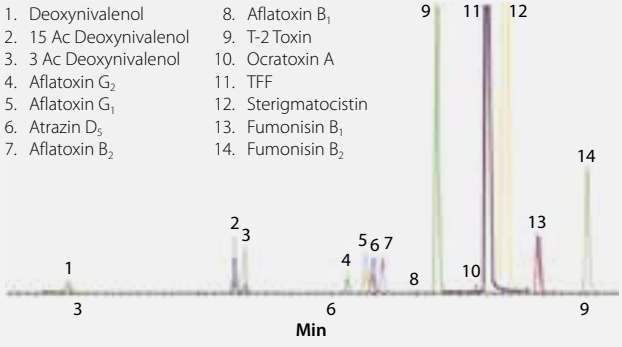


Figure 34. LC-MS Analysis of Mycotoxins in Cereal

Chromatogram courtesy of Enio Belotti (All. Water and Life Lab s.r.l., Entratico (BG), Italy)

sample/matrix:	spike 5 grams of cereal with 14 mycotoxins; extract with 20 mL acetonitrile:1% formic acid in water (75:25); shake for 1 min; centrifuge; filter through a 0.45 µm syringe filter
column:	Ascentis® Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase:	(A) 1 mM ammonium acetate, 0.5% acetic acid in water; (B) 1 mM ammonium acetate, 0.5% acetic acid in methanol
gradient:	0 min: 5% B; 0.5 min: 10%B; 12 min: 95%B; 15 min: 95%B
flow rate:	400 µL/min
column temp.:	40 °C
detector:	MS/MS, ESI(+) and ESI (–)
injection:	2 µL

1. Deoxynivalenol
2. 15 Ac Deoxynivalenol
3. 3 Ac Deoxynivalenol
4. Aflatoxin G_2
5. Aflatoxin G_1
6. Atrazin D_5
7. Aflatoxin B_2
8. Aflatoxin B_1
9. T-2 Toxin
10. Ocratoxin A
11. TFF
12. Sterigmatocistin
13. Fumonisin B_1
14. Fumonisin B_2



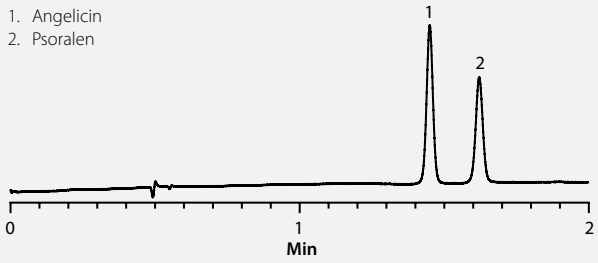
Furanocoumarins

As a chemical defense against a variety of mammalian and insect predators, some plants produce furanocoumarins (a class of phytochemicals consisting of a furan ring fused with coumarin). In humans, furanocoumarins can interfere with the metabolism of a number of drugs. Angelicin and psoralen are the two most common furanocoumarins, and are structurally similar. Analysis of these two analytes using HPLC is shown in **Figure 35**.

Figure 35. HPLC Analysis of Angelicin and Psoralen

column: Ascentis Express F5, 10 cm x 4.6 mm I.D., 2.7 µm particles (53590-U)
mobile phase: water:methanol (40:60)
flow rate: 2.0 mL/min
pressure: 360 bar
column temp.: 40 °C
detector: UV, 254 nm
injection: 2 µL
sample: 25 µg/mL each in 90:10 water:methanol

1. Angelicin
2. Psoralen



Heavy Metals

Industrial effluent streams can introduce heavy metal contamination into aqueous systems. Heavy metals can then migrate to any plant or animal life which uses this water. Fish and other aquatic life are obviously the most affected. Because heavy metals do not break down, they will bio-accumulate up the food chain. Atomic absorption spectroscopy (AAS) and inductively coupled plasma (ICP) are the analytical techniques most often used to determine the presence and levels of heavy metals in foods. Fluka® offers multiple certified reference materials (CRMs) and high quality acids, bases and salts for the calibration and operation of AAS and ICP instruments.

TraceCERT® certified reference materials (CRM) are developed and produced in an accredited laboratory, fulfilling both ISO 17025 and ISO Guide 34 requirements. All are traceable to at least two independent references (i.e. NIST, BAM or SI unit kg) and include comprehensive documentation. A picture of a TraceCERT CRM and its accompanying documentation is shown in **Figure 36**.

TraceSELECT® acids, bases and salts are designed for sample preparation and analysis in the ppm and ppb level.

Figure 36. TraceCERT CRM and Documentation

Featured Products

Cat. No.	Product Description
Supel Tox SPE Tubes	
55314-U	AflaZea, 6 mL, 30 ea
55316-U	DON, 6 mL, 30 ea
55308-U	Tricho, 6 mL, 30 ea
55315-U	FumoniBind, LRC, 25 ea
55318-U	OchraBind, LRC, 25 ea
55307-U	TrichoBind, LRC, 25 ea
Ascentis Express HPLC Columns (2.7 µm particles)	
53823-U	C18, 10 cm x 2.1 mm I.D.
53832-U	C8, 10 cm x 2.1 mm I.D.
53913-U	RP-Amide, 10 cm x 2.1 mm I.D.
53336-U	Phenyl Hexyl, 10 cm x 2.1 mm I.D.
53569-U	F5, 10 cm x 2.1 mm I.D.
53590-U	F5, 10 cm x 4.6 mm I.D.
Ascentis Express HPLC Columns (5 µm particles)	
50537-U	C18, 15 cm x 4.6 mm I.D.
50392-U	C8, 15 cm x 4.6 mm I.D.
50483-U	Phenyl Hexyl, 15 cm x 4.6 mm I.D.
50631-U	F5, 15 cm x 4.6 mm I.D.
GC Columns	
28564-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm
28576-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm
28471-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm
28473-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.50 µm
Mycotoxin Calibration Standards	
46303	Aflatoxin Mix, 4 analytes at various concentrations in methanol, 5 mL Aflatoxin B_1, 1 µg/mL; Aflatoxin B_2, 0.3 µg/mL Aflatoxin G_1, 1 µg/mL; Aflatoxin G_2, 0.3 µg/mL
32771-1ML	Aflatoxin B_2-$^{13}C_{17}$, at 0.5 µg/mL in acetonitrile, 1 mL
34132-2ML	3-Acetyldeoxynivalenol, 100 µg/mL in acetonitrile, 2 mL
34133-2ML	15-Acetyldeoxynivalenol, 100 µg/mL in acetonitrile, 2 mL
34124-2ML	Deoxynivalenol, 100 µg/mL in acetonitrile, 2 mL
34128-1ML	Deoxynivalenol-$^{13}C_{15}$, 25 µg/mL in acetonitrile, 1 mL
34139-2ML	Fumonisin B_1, 50 µg/mL in acetonitrile:water, 2 mL
34142-2ML	Fumonisin B_2, 50 µg/mL in acetonitrile, 2 mL
32606-1ML	Fumonisin B_3, 50 µg/mL in acetonitrile/water, 1 mL
34131-2ML	Nivalenol, 100 µg/mL in acetonitrile, 2 mL
34037-2ML	Ochratoxin A, 10 µg/mL in acetonitrile, 2 mL
46914-U	Patulin, 100 µg/mL in chloroform, 1 mL
46916-U	Zearalenone, 50 µg/mL in acetonitrile, 1 mL
Analytical Reagents and Solvents	
17836	Ammonium acetate, for HPLC, >99.0%
34967	Acetonitrile, LC-MS CHROMASOLV®, >99.9%
34966	Methanol, LC-MS CHROMASOLV, >99.9%
***Trace*CERT CRMs for AAS and ICP**	
01969-100ML	Arsenic, 1000 mg/L in nitric acid, 100 mL
36379-100ML	Cadmium, 1000 mg/L in nitric acid, 100 mL
68921-100ML	Copper, 1000 mg/L in nitric acid, 100 mL
41318-100ML	Lead, 1000 mg/L in nitric acid, 100 mL
28941-100ML	Mercury, 1000 mg/L in nitric acid, 100 mL
28944-100ML	Nickel, 1000 mg/L in nitric acid, 100 mL
Inorganic Acids	
02650-1L	Nitric acid, *Trace*SELECTUltra, ~65%, 1 L
84385-500ML	Nitric acid, *Trace*SELECT, ~69.5%, 1 L

PROCESSING/PACKAGING CONTAMINANTS

Chemical reactions that occur during food processing steps may produce undesirable contaminants. Other contaminants may migrate from packaging containers into food and beverage products. Therefore, food analysts routinely monitor finished products to safeguard that the levels of these contaminants are below regulatory limits. Areas of interest include:

- Phthalates and adipates (from plastic food containers)
- Bisphenol A, BADGE, BFDGE and NOGE (from metal cans lined with epoxy-based lacquers; from rigid plastic beverage bottles)
- Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) (from microwave popcorn bags, fast food wrappers, candy wrappers and pizza box liners)
- Semicarbazide (from the soft plastic seals in twist-off metal lids on glass containers of jams, honeys, sauces, ketchup, baby foods, fruit juice, mayonnaise, mustard, pickles, etc.)
- Benzene (from decarboxylation of sodium or potassium benzoate preservatives in the presence of ascorbic acid, when exposed to heat and light)
- Nitrosamines (in meat from breakdown of sodium nitrite preservative if overcooked; in fish from a reaction of sodium nitrite preservative and dimethylamine, from fish feed)
- Furans (from breakdown of amino acids, sugars, vitamin C and PUFAs during thermal treatment of foods in bottles, cans and jars)
- PAHs (from smoking/heating meat; in fats/oils due to their lipophilic nature)
- Acrylamides (from deep-frying foods)
- 3-MCPD (in refined vegetable oil, and products made with refined vegetable oil)
- Disinfection product residues and solvents (from cleaning of equipment)
- Irradiation by-products (formed during irradiation process to destroy microorganisms)

Included are examples relevant to this area.

Phthalates

Food and beverage products that are packaged in plastic containers may contain trace levels of phthalate esters, compounds used in the manufacture of plastics. Depending on the matrix, a cleanup step may be necessary to remove fat and oil components from the extract prior to GC-MS analysis. A quicker approach is to employ an extraction technique that is more selective for phthalate esters, and less selective for fats and oils. High temperature headspace solid phase microextraction (SPME) can be used for the extraction of phthalate esters from oily food matrices. The use of matrix-matched calibration standards and internal standards is imperative to alleviate matrix issues that may exist with some types of oily matrices. A chromatogram of phthalate esters extracted from the spiked chicken flavored oil included with a ramen noodle kit is presented in **Figure 37**.

Using internal standard corrected response factors, the phthalate ester levels (in µg/kg) were calculated for unspiked and spiked samples. Also calculated were % recovery, average % recovery and %RSD values. These results are summarized in **Table 6**. Several phthalate esters were found in the unspiked sample, most likely caused by the plastic packaging.

Acrylamide

An unintentional consequence of the cooking process when high carbohydrate, low protein foods are fried, roasted or baked at high temperatures is the formation of acrylamide. It is believed the mechanism of formation is the reaction of asparagine and a carbonyl-containing compound. This mechanism involves formation of a Schiff base followed by decarboxylation and elimination of either ammonia or a substituted imine to yield acrylamide. Temperature plays an important role in the reaction.

A procedure developed by Grob, et. al. (Mitt. Lebensm Hyg. 2002; 93; 638-652) provides a means for the extraction and GC analysis of acrylamide in foods. **Figure 38** provides a summary of this method. Sigma-Aldrich® offers a ready-to-use kit that makes acrylamide determination faster and easier. The Acrylamide Kit contains all the necessary standards and solvents for twelve determinations of acrylamide using the Grob method. Concentrations and specifications of each solution are designed specifically for this method. The GC analysis of the three-component acrylamide mixture is shown in **Figure 39**.

Figure 37. Phthalates in Spiked Chicken Flavored Oil

sample/matrix:	add analytes and internal standards to a 15 mL vial; mix; evaporate hexane; reconstitute with 1 g oil from a ramen noodle chicken flavor packet; vortex for 2 min; heat until headspace at 90 °C
SPME fiber:	100 µm PDMS fiber (57300-U)
extraction:	headspace, 90 °C (measured in the headspace) for 30 min
desorption process:	260 °C for 4 min
column:	SLB®-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U)
oven:	60 °C (1 min), 10 °C/min to 330 °C (10 min)
detector:	MSD, interface at 330 °C, SIM
carrier gas:	helium, 0.6 mL/min constant
liner:	0.75 mm I.D., SPME type, straight design (2637501)

1. Dimethyl phthalate (DMP)
2. Diethyl phthalate (DEP)
3. Diisobutyl phthalate (DIBP)
4. Dibutyl phthalate (DBP)
5. bis(2-Methoxyethyl) phthalate (DMEP)
6. bis(4-Methyl-2-pentyl) phthalate (BMPP)
7. bis(2-Ethoxyethyl) phthalate (DEEP)
8. Dipentyl phthalate (DPP)
9. Dihexyl phthalate (DHP)
10. Butylbenzyl phthalate (BBP)
11. bis(2-*n*-Butoxyethyl) phthalate (DBEP)
12. Dicyclohexyl phthalate (DCHP)
13. bis(2-Ethylhexyl) phthalate (DEHP)
14. Diphenyl phthalate
15. Dioctyl phthalate (DNOP)
16. Dinonyl phthalate (DNP)

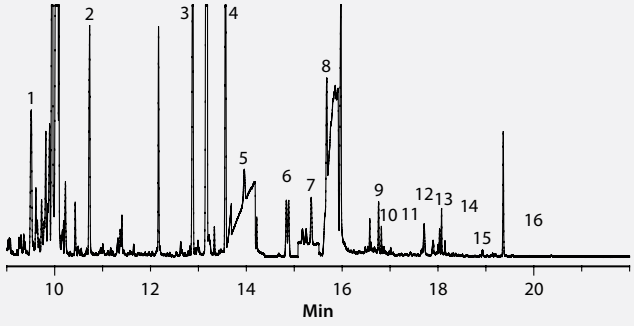


Table 6. Recovery and Reproducibility: Chicken Flavored Oil (Spiked Samples at 500 µg/kg)

Analyte	Unspiked Sample Level (µg/kg)	Three Spiked Samples Avg. Recovery	%RSD
Dimethyl phthalate	0	102%	1
Diethyl phthalate	0	96%	1
Diisobutyl phthalate	253	98%	2
Dibutyl phthalate	328	95%	4
bis(2-Methoxyethyl) phthalate	0	62%	5
bis(4-Methyl-2-pentyl) phthalate isomer 1	0	74%	2
bis(4-Methyl-2-pentyl) phthalate isomer 2	0	72%	1
bis(2-Ethoxyethyl) phthalate	0	64%	4
Dipentyl phthalate	0	105%	3
Dihexyl phthalate	0	94%	2
Butylbenzyl phthalate	63	71%	4
bis(2-*n*-Butoxyethyl) phthalate	0	128%	6
Dicyclohexyl phthalate	0	91%	5
bis(2-Ethylhexyl) phthalate	986	79%	26
Diphenyl phthalate	0	103%	10
Dioctyl phthalate	0	91%	12
Dinonyl phthalate	0	101%	20

Figure 38. Summary of Grob Method

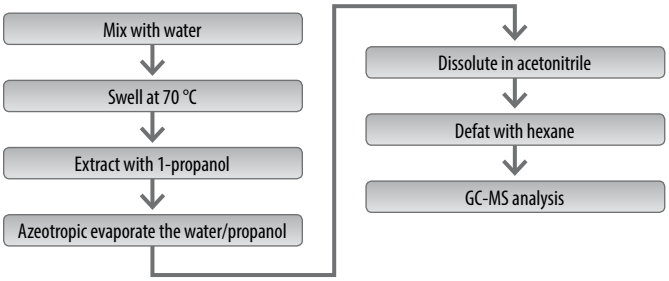
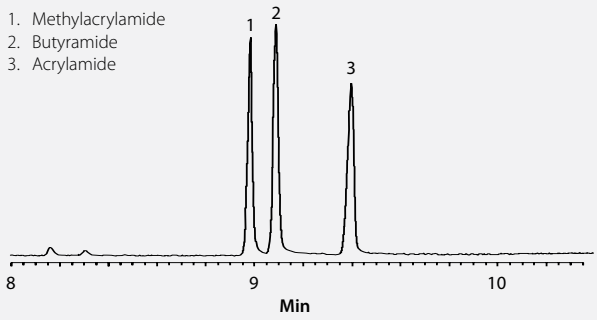


Figure 39. GC-MS Analysis of Acrylamides

column: SUPELCOWAX® 10, 30 m x 0.25 mm I.D., 0.25 µm (24079)
oven: 70 °C (1 min), 15 °C/min to 220 °C (2 min)
carrier gas: helium, 20 cm/sec
detector: MS
injection: 1 µL, on column

1. Methylacrylamide
2. Butyramide
3. Acrylamide



Featured Products

Cat. No.	Product Description
SPME Fiber Assemblies	
57300-U	100 µm PDMS for manual holder, 24 ga, 3 ea
57301	100 µm PDMS, for autosampler holder, 24 ga, 3 ea
57341-U	100 µm PDMS, for autosampler holder, 23 ga, 3 ea
57342-U	100 µm PDMS, for manual holder, 23 ga, 3 ea
GC Columns	
28564-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm
28576-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm
28471-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm
28473-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.50 µm
24343	SUPELCOWAX 10, 15 m x 0.10 mm I.D., 0.10 µm
24079	SUPELCOWAX 10, 30 m x 0.25 mm I.D., 0.25µm
24284	SUPELCOWAX 10, 30 m x 0.25 mm I.D., 0.50 µm
Ascentis Express HPLC Columns (2.7 µm particles)	
53823-U	C18, 10 cm x 2.1 mm I.D.
53832-U	C8, 10 cm x 2.1 mm I.D.
53913-U	RP-Amide, 10 cm x 2.1 mm I.D.
53336-U	Phenyl Hexyl, 10 cm x 2.1 mm I.D.
53569-U	F5, 10 cm x 2.1 mm I.D.
Ascentis Express HPLC Columns (5 µm particles)	
50537-U	C18, 15 cm x 4.6 mm I.D.
50392-U	C8, 15 cm x 4.6 mm I.D.
50483-U	Phenyl Hexyl, 15 cm x 4.6 mm I.D.
50631-U	F5, 15 cm x 4.6 mm I.D.
Phthalate Ester Standards	
36927	Butylbenzyl phthalate, 250 mg
36736	Dibutyl phthalate, PESTANAL®, 1 g
36908	Dicyclohexyl phthalate, PESTANAL, 250 mg
53008	Diethyl phthalate, PESTANAL, 5 mL
36738	Dimethyl phthalate, PESTANAL, 1 g
31301	Dioctyl phthalate, PESTANAL, 250 mg
442867	Dipentyl phthalate, 1000 mg
36617	Diphenyl phthalate, PESTANAL, 1 g
36735	bis(2-Ethylhexyl) phthalate, PESTANAL, 1 g
36934	bis(2-Methoxyethyl) phthalate, PESTANAL, 250 mg
Deuterated Phthalate Ester Analogs	
34169	Dibutyl phthalate-3,4,5,6-d_4, 25 mg
34186	Dicyclohexyl phthalate-3,4,5,6-d_4, 25 mg
34185	Diethyl phthalate-3,4,5,6-d_4, 25 mg
34167	Dihexyl phthalate-3,4,5,6-d_4, 25 mg
34204	Diisobutyl phthalate-3,4,5,6-d_4, 25 mg
34194	Dipentyl phthalate-3,4,5,6-d_4, 25 mg

ADULTERANTS

Unintentional food and beverage contamination can occur at all stages in the food cycle. This includes growing/raising of crops/animals, as well as the various processing, packaging and storage steps. It is unfortunate that contaminants can also be purposely introduced, for financial gain or to intentionally cause harm.

Current areas of interest include:

- Melamine; an adulterant to boost protein value
- Honey; dilution with an inferior product to boost volume
- Essential oils; dilution with an inferior product to boost volume
- Spices; dyes as adulterants to achieve a desired color

Included are examples relevant to this area.

Melamine Adulterant

In 2008, it was discovered that some pet food, infant formula and milk products were contaminated with melamine and related compounds, which are harmful to humans and mammalian pets.

An investigation revealed that melamine was intentionally added to raw materials, such as wheat gluten and rice protein, to inflate nitrogen content; often the sole measure of protein content. Because these raw materials appeared to contain more protein than they did, they could be sold at a higher price.

The GC analysis of a spiked dog food sample is shown in **Figure 40**. Complete extraction conditions are included, as well as the percent recoveries calculated by subtracting values in an unspiked sample from values in the spiked sample. **Figure 41** shows the LC-MS/MS analysis of melamine in milk, a difficult matrix that requires careful sample prep to ensure valid results. The use of a strong cation exchange material during the extraction resulted in an 85% recovery from a spiked sample.

Figure 40. GC Analysis of Melamine and Related Compounds in Dog Food

sample/matrix:	spike 0.5 g dog food sample with 4 analytes at 10 µg/g; mix thoroughly with 20 mL of diethylamine:water:acetonitrile (10:40:50); sonicate for 30 min; centrifuge for 10 min; filter; evaporate to dryness; add Sylon™ BFT and pyridine; add internal standard to a level of 1000 ng/mL; incubate at 70 °C for 45 min to form trimethylsilyl (TMS) derivatives
column:	SLB®-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
oven:	115 °C (3 min), 10 °C/min to 325 °C (6 min)
inj. temp.:	250 °C
detector:	MS (interface at 325 °C, SIM
carrier gas:	helium, 1 mL/min
injection:	1 µL, splitless
liner:	4 mm I.D., splitless type, single taper design

1. Cyanuric acid (97%)
2. Ammelide (105%)
3. 2,6-Diamino-4-chloropyrimidine (I.S.)
4. Ammeline (77%)
5. Melamine (73%)

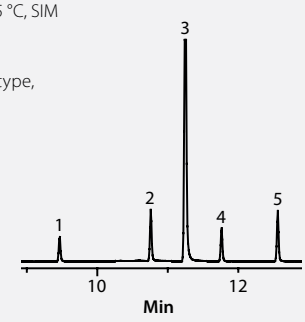
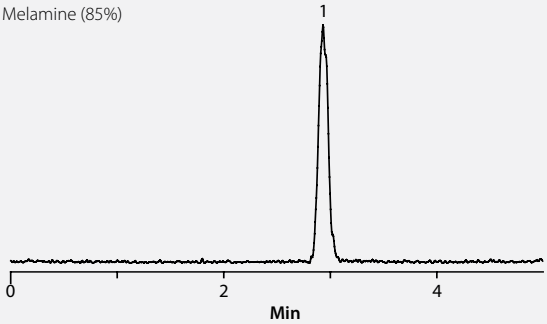


Figure 41. LC-MS/MS Analysis of Melamine in Milk

sample/matrix:	spike 5 mL of milk with melamine at 100 ng/mL; add 5 mL of phosphate buffer (100 mM, pH 2.5) and 1 mL acetonitrile; place in ultrasonic bath for 5 min; centrifuge at 3500 rpm for 10 min; recover middle supernatant layer
SPE tube:	Discovery® DSC-SCX, 500 mg/6 mL (52688-U)
conditioning:	3 mL methanol; 3 mL 100 mM phosphate buffer (pH 2.5)
sample addition:	2.2 mL of the sample extract
washing:	3 mL of phosphate buffer (100 mM, pH 3); 3 mL of methanol
elution:	4 mL of 5% ammonia in methanol
eluate post-treatment:	evaporate to dryness at 50 °C in a water bath under a flow of nitrogen at 5 psi; reconstitute in 1 mL of 10 mM ammonium formate in 90:10 acetonitrile:water
column:	Ascentis® Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase:	(A) 10 mM ammonium formate in 90:10 acetonitrile:water; (B) 10 mM ammonium formate in 70:30 acetonitrile:water
gradient:	0 min: 0 %B; 5 min: 100 %B; 10 min: 0 %B; 15 min: 0 %B
flow rate:	0 min: 200 µL/min; 5 min: 400 µL/min; 10 min: 400 µL/min; 15 min: 200 µL/min
column temp.:	30 °C
detector:	MS/MS, MRM at m/z 127/85 and 127/68
injection:	2 µL

1. Melamine (85%)



Essential Oil Product Dilution

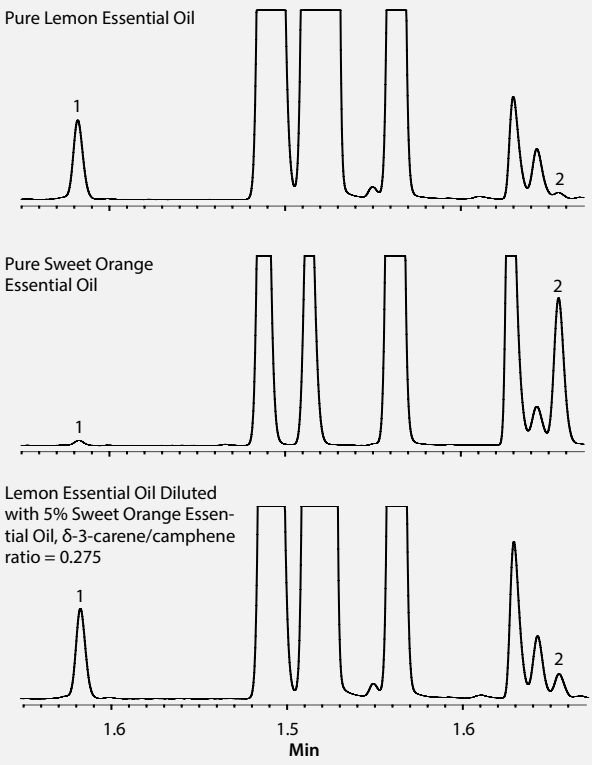
Citrus essential oils are used during the manufacture of foods and beverages to add flavor and aroma. These complex raw materials are mixtures of more than 200 components. Of significance is that citrus essential oils are similar in the components they contain; their major differences being the ratios of these components. It is these different ratios which imparts unique olfactory properties, and makes some oils more valuable than others. For example, in Italy in 1999, 1 kg of winter lemon essential oil cost between 12.9 and 16.5 Euro, whereas 1 kg of sweet orange oil cost approximately 0.7 Euro.

Figure 42. Comparison of Pure and Adulterated Essential Oils

Chromatograms courtesy of Prof. Luigi Mondello (Univ. of Messina, Messina, Italy)

column:	SLB-5ms, 10 m x 0.10 mm ID, 0.10 µm (28465-U)
oven:	40 °C, 30 °C/min to 85 °C, 80 °C/min to 320 °C
inj.:	320 °C
det.:	FID, 320 °C
carrier gas:	hydrogen, 70 cm/sec
injection:	0.4 µL, 300:1 split
sample:	as specified in hexane

1. Camphene
2. δ-3-carene



GC analysis can be used to detect the dilution of expensive lemon essential oil with low-cost sweet orange oil by measuring the ratio of δ-3-carene to camphene. This is because:

- Sweet orange essential oil contains 0.1% of δ-3-carene and practically no camphene
- Lemon essential oil contains trace levels of δ-3-carene and ~0.06% camphene

The δ-3-carene/camphene ratio cannot exceed a value of 0.140 for the sample to be considered pure lemon essential oil. **Figure 42** shows the GC analysis of pure lemon essential oil, pure sweet orange essential oil and lemon essential oil diluted with 5% sweet orange essential oil. For the latter, a δ-3-carene/camphene value of 0.275 was obtained, indicating the oil is diluted.

Featured Products

Cat. No.	Product Description
SPE Tubes	
52688-U	Discovery® DSC-SCX, 500 mg/6 mL, 30 ea
52689-U	Discovery DSC-SCX, 1 g/6 mL, 30 ea
GC Columns	
28564-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm
28576-U	SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm
28471-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm
28473-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.50 µm
Ascentis Express HPLC Columns (2.7 µm particles)	
53823-U	C18, 10 cm x 2.1 mm I.D.
53832-U	C8, 10 cm x 2.1 mm I.D.
53913-U	RP-Amide, 10 cm x 2.1 mm I.D.
53336-U	Phenyl Hexyl, 10 cm x 2.1 mm I.D.
53939-U	HILIC, 10 cm x 2.1 mm I.D.
53569-U	F5, 10 cm x 2.1 mm I.D.
Ascentis Express HPLC Columns (5 µm particles)	
50537-U	C18, 15 cm x 4.6 mm I.D.
50392-U	C8, 15 cm x 4.6 mm I.D.
50483-U	Phenyl Hexyl, 15 cm x 4.6 mm I.D.
50289-U	HILIC, 15 cm x 4.6 mm I.D.
50631-U	F5, 15 cm x 4.6 mm I.D.
Analytical Standards	
52549-250MG	Melamine, >99.0%, 250 mg
45613-250MG	Ammeline, PESTANAL®, 250 mg
16614-250MG	Cyanuric acid, >98.0%, 250 mg
C33204-5G	2,6-Diamino-4-chloropyrimidine, 98%, 5 g
Analytical Reagents and Solvents	
33148	BSTFA+TMCS, 99:1 (Sylon BFT), 20 x 1 mL ampules
17843	Ammonium formate, for HPLC, >99.0%
34967	Acetonitrile, LC-MS CHROMASOLV®, >99.9%
34966	Methanol, LC-MS CHROMASOLV, >99.9%

FAPAS® PROFICIENCY TESTING SCHEMES

Get assurance that you are delivering quality results demanded by your customers by participating in global FAPAS food PT schemes. We offer a variety of the most popular PT schemes on the market; which will allow you to utilize our extensive distribution network and meet your ISO 17025 requirements.

FAPAS is part of the Food & Environment Research Agency (FERA), which is recognized as the world's market leader in the provision of laboratory Proficiency Testing to the Food sector.

FAPAS Products

Cat. No.	Product Description
FAP00018	Aflatoxins B & G &/or total in maize FAPAS® Proficiency Testing Study
FAP00015	Aflatoxins B & G &/or total in peanut FAPAS® Proficiency Testing Study
FAP00020	Aflatoxins B & G &/or total & OTA in paprika FAPAS® Proficiency Testing Study
FAP00021	Al, As (total), Cd, Pb, Hg (total) in soya flour FAPAS® Proficiency Testing Study
FAP00011	Artificial colours in soft drink FAPAS® Proficiency Testing Study
FAP00031	Artificial colours in sugar confectionery (boiled sweets) FAPAS® Proficiency Testing Study
FAP00022	As (inorganic), As (total), Cd, Hg (total), Pb (all high levels) in powdered rice FAPAS® Proficiency Testing Study
FAP00013	Benzoic acid, quinine, acesulfame K, aspartame in tonic water FAPAS® Proficiency Testing Study
FAP00001	Brix, pH, citric acid, sorbic acid, cyclamate, saccharin in orange juice FAPAS® Proficiency Testing Study
FAP00010	Brix, pH, citric acid, sorbic acid, cyclamate, saccharin in soft drink FAPAS® Proficiency Testing Study
FAP00006	Ca, Fe, Mg, P, K, Na, Zn in breakfast cereal FAPAS® Proficiency Testing Study
FAP00012	Caffeine, benzoic acid, total sugars, phosphoric acid in cola drink FAPAS® Proficiency Testing Study
FAP00009	Caffeine in coffee (ground) - one regular, one decaffeinated FAPAS® Proficiency Testing Study
FAP00034	DON in wheat flour FAPAS® Proficiency Testing Study
FAP00033	FB1, FB2 & total fumonisins (as a sum of FB1 & FB2) in maize flour FAPAS® Proficiency Testing Study
FAP00003	Formaldehyde in 3% aq. acetic acid FAPAS® Proficiency Testing Study
FAP00032	Illegal dyes (e.g. sudan) in hot pepper sauce FAPAS® Proficiency Testing Study
FAP00008	Moisture, ash, nitrogen, starch, sodium, total dietary fibre by AOAC in breadcrumbs FAPAS® Proficiency Testing Study
FAP00007	Moisture at 130, ash, nitrogen, total dietary fibre by AOAC in cereal FAPASFAPAS®Proficiency Testing Study
FAP00016	Multi-mycotoxins: Afla B1, DON, ZON, OTA, FB1 & FB2, total fumonisins (as sum of FB1,FB2), T-2 & HT-2 toxins & as a sum of T-2 & HT-2 toxins in maize FAPAS® Proficiency Testing Study
FAP00019	Multi-mycotoxins: Afla B1, DON, ZON & OTA in maize FAPAS® Proficiency Testing Study
FAP00004	Nitrate in cabbage purée FAPAS® Proficiency Testing Study
FAP00005	Nitrate in lettuce purée FAPAS® Proficiency Testing Study
FAP00023	Nitrate in spinach purée FAPAS® Proficiency Testing Study
FAP00024	Pesticide residues in strawberry purée FAPAS® Proficiency Testing Study
FAP00002	Pesticide residues (list 2) in brown rice FAPAS® Proficiency Testing Study
FAP00002B	Pesticide residues (list 2) in brown rice - blank sample FAPAS® Proficiency Testing Study
FAP00029	Pesticide residues (list 3) in orange purée FAPAS® Proficiency Testing Study
FAP00029B	Pesticide residues (list 3) in orange purée - blank sample FAPAS® Proficiency Testing Study
FAP00028	Pesticide residues (list 3) in spinach purée FAPAS® Proficiency Testing Study
FAP00028B	Pesticide residues (list 3) in spinach purée - blank sample FAPAS® Proficiency Testing Study
FAP00026	Pesticides in cucumber purée FAPAS® Proficiency Testing Study
FAP00026B	Pesticides in cucumber purée - blank sample FAPAS® Proficiency Testing Study
FAP00027	Pesticides in green tea FAPAS® Proficiency Testing Study
FAP00027B	Pesticides in green tea - blank sample FAPAS® Proficiency Testing Study
FAP00025	Pesticides in lettuce purée FAPAS® Proficiency Testing Study
FAP00025B	Pesticides in lettuce purée - blank sample FAPAS® Proficiency Testing Study
FAP00035	Soya, soya protein in wheat flour FAPAS® Proficiency Testing Study
FAP00030	Sulphur dioxide in dried apricot (water/fruit slurry) FAPAS® Proficiency Testing Study

BEVERAGE TESTING

Foods are solids or semi-solids that typically require some physical manipulation prior to extraction procedures. Additionally, these extracts may need some level of clean-up prior to analysis. Because beverages are liquid, sample preparation is often much simpler; usually involving direct injection, headspace, solid phase microextraction (SPME), solid phase extraction (SPE) or liquid-liquid extraction (LLE). Areas of interest include:

- Overall composition
- Alcohol or caffeine content
- Nutraceuticals, antioxidants, saponins/ginsenoside
- Vitamins, sweeteners
- Aroma, sulfur compounds, off-flavors
- Impurities

Included are examples relevant to this area.

Resveratrol in Red Wine

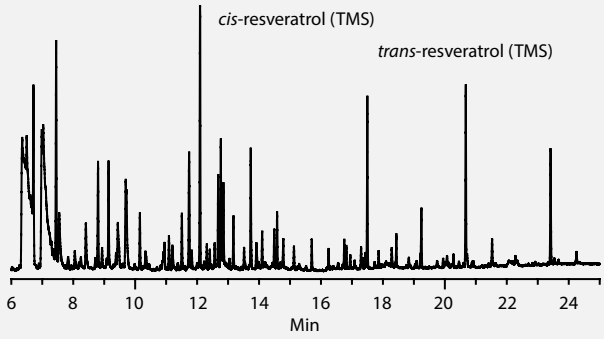
Resveratrol is a phytoallexin produced by grapes and other plants to increase resistance to fungal infection. It is suspected that human consumption of resveratrol may reduce the risk of certain cancers, heart disease and other age-related disorders. Red wine, which is produced by fermentation of juice on the crushed grapes, has been found to contain a greater amount of resveratrol than white wine, which is produced by fermentation of the juice alone. Because resveratrol contains three hydroxyl functional groups, derivatization is necessary prior to GC analysis.

A highly sensitive, simple and quantitative procedure to perform this application uses SPME involving on-fiber derivatization. Extraction is accomplished by immersion of the SPME fiber into the sample. Derivatization directly on the SPME fiber is accomplished by subsequently exposing it to the vapors of a silyating reagent. Finally, GC analysis is performed.

Figure 43 shows the GC analysis of an unspiked California merlot. Results for trans-resveratrol are 22.6 µg/L in the unspiked sample, and 134.7 µg/L in a spiked sample (chromatogram not shown). This calculates out to a 110% recovery.

Figure 43. GC Analysis of Resveratrol in Red Wine

sample/matrix:	3 mL of red wine (California merlot) diluted 3:1 in 12% ethanol:water
SPME fiber:	85 µm polyacrylate
extraction:	immerse fiber into extract at room temperature for 15 min while stirring at 400 rpm; derivatize by immersing fiber in a 4 mL vial containing 5 µL of Sylon™-BFT for 20 min
desorption process:	280 °C for 2 min
column:	SLB®-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
oven:	100 °C (1 min), 10 °C/min to 325 °C (3 min)
detector:	MS (interface at 325 °C), m/z 40-450
carrier gas:	helium, 1 mL/min
liner:	0.75 mm I.D., direct (SPME) type, straight design



Bisphenol A in Bottled Water

Bisphenol A (BPA) is one of the chemicals used to make both polycarbonate plastics and epoxy-based lacquers. These materials are associated with several types of food and beverage containers:

- Polycarbonate plastics are used to make re-useable rigid containers; commonly used for water bottles, baby bottles, plastic mugs, carboys and storage containers
- Epoxy-based lacquers are used to coat the inside of metal cans; to keep the foods or beverages from directly contacting the metal

Small amounts of BPA can migrate into the foods and beverages enclosed in these types of containers, particularly if the containers are exposed to elevated temperatures, such as when baby bottles are heated and when metal cans are filled while the food or beverage is still hot.

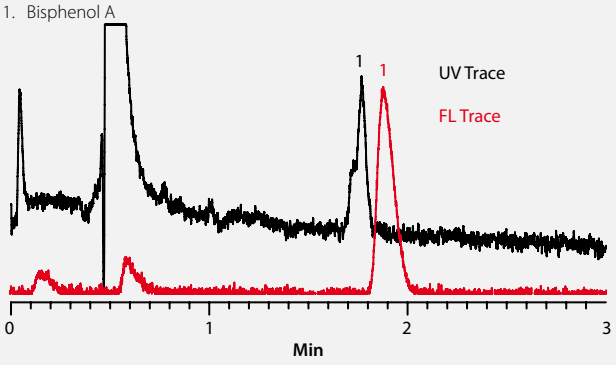
The extraction of BPA from drinking water samples using SPE is relatively simple. However, it is critical that the proper hardware (such as glass tubes with PTFE frits) is used. This hardware eliminates the possibility of introducing compounds which may be solvent-leached from alternative hardware (for example, polypropylene tubes with polyethylene frits). The use of SPE allows BPA to be extracted plus concentrated, which may result in greater method sensitivity compared to simple headspace or direct injection methods. The SPE procedure described in **Figure 44** works out to a 5-fold concentration.

HPLC is preferred for the analysis of BPA. This is because GC analysis of BPA requires derivatization. Figure 39 shows the HPLC analysis of a spiked water sample using two detectors in series, ultraviolet (UV) and fluorescence (FL). A better signal-to-noise ratio is obtained with the FL detector. Also of note is the slightly longer retention time and broader peak shape observed on the FL chromatogram. These are caused by the extra system volume contributed as the sample passes through the UV cell, as well as, the tubing connecting the detectors. The removal of the UV component and shortening the tubing connecting the column to the FL detector would eliminate these phenomena. A recovery of 88% was calculated by comparing results to an unspiked sample.

Figure 44. HPLC Analysis of BPA in Spiked Water

sample/matrix:	drinking water spiked with bisphenol A to a 0.2 µg/mL level
SPE tube:	Supelclean™ ENVI™-18, 500 mg, 6 mL glass tube, PTFE frit (54331-U)
condition:	1 mL 1% formic acid in acetonitrile; 1 mL DI water
sample addition:	5 mL spiked water sample
elution:	2 mL 1% formic acid in acetonitrile
eluate post-treatment:	1 mL evaporated; reconstituted to 0.5 mL with acetonitrile
column:	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm (53823-U)
mobile phase:	water:acetonitrile (60:40)
flow rate:	0.4 mL/min
pressure:	3268 psi (225 bar)
column temp.:	35 °C
detector:	UV (230 nm); FL (Ex 225 nm, Em 310 nm)
injection:	1 µL

1. Bisphenol A



Musty Odor in White Wine

Cork taint refers to a musty odor in wine caused by the presence of 2,4,6-trichloroanisole (TCA). The source of TCA is thought to be the fungal methylation of chlorophenols present in the wine. While TCA is relatively non-active, its precursors contain active hydroxyl functional groups and are best analyzed if derivatized. A quick procedure to monitor for the presence of TCA and its suspected precursors employs in-matrix derivatization followed by SPME then GC analysis. In summary:

- Chlorophenols are derivatized in-matrix using acetic anhydride
- Acylated derivatives are extracted from the headspace using SPME
- TCA, which is not derivatized, is simultaneously extracted with the chlorophenols

The resulting chromatogram from a spiked sample is shown in **Figure 45**. Percent recovery was calculated by comparing the results from the spiked sample to an unspike d sample. **Table 7** includes complete data.

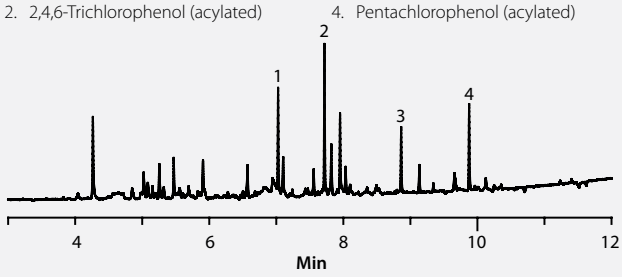
Table 7. Results from Red Wine (Spiked Sample at 100 ng/L)

Analyte	Unspiked (ng/L)	Spiked (ng/L)	Recovery (%)
2,4,6-Trichloroanisole	not detected	60.7	61
2,4,6-Trichlorophenol	22.7	96.3	74
2,3,4,6-Tetrachlorophenol	not detected	55.7	56
Pentachlorophenol	3.3	33.5	30

Figure 45. GC Analysis of TCA and Precursors in Spiked Red Wine

sample/matrix:	1.5 mL red wine spiked with each analyte at 100 ng/L + 600 µL 5% potassium carbonate (K_2CO_3) + 240 mg sodium chloride (NaCl) + 60 µL acetic anhydride
SPME fiber:	metal fiber coated with 100 µm PDMS (57928-U)
extraction:	headspace, 50 °C for 30 min with stirring
desorption process:	250 °C for 3 min
column:	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
oven:	50 °C (1 min), 25 °C/min to 280 °C
detector:	ECD, 290 °C
carrier gas:	helium, 1.5 mL/min
liner:	0.75 mm I.D., direct (SPME) type, straight design (2637501)

1. 2,4,6-Trichloroanisole
2. 2,4,6-Trichlorophenol (acylated)
3. 2,3,4,6-Tetrachlorophenol (acylated)
4. Pentachlorophenol (acylated)



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Cat. No.	Product Description
SPME Fiber Assemblies	
57304	85 µm polyacrylate, for manual holder, 24 ga, 3 ea
57305	85 µm polyacrylate, for autosampler holder, 24 ga, 3 ea
57294-U	85 µm polyacrylate, for autosampler holder, 23 ga, 3 ea
57928-U	100 µm PDMS metal, for autosampler holder, 23 ga, 3 ea
SPE Tube	
54331-U	Supelclean ENVI-18, 500 mg, 6 mL glass tube, PTFE frit, 30 ea
GC Columns	
28471-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm
28473-U	SLB-5ms, 30 m x 0.25 mm I.D., 0.50 µm
Ascentis Express HPLC Columns (2.7 µm particles)	
53823-U	C18, 10 cm x 2.1 mm I.D.
53913-U	RP-Amide, 10 cm x 2.1 mm I.D.
53939-U	HILIC, 10 cm x 2.1 mm I.D.
53569-U	F5, 10 cm x 2.1 mm I.D.
Ascentis Express HPLC Columns (5 µm particles)	
50537-U	C18, 15 cm x 4.6 mm I.D.
50289-U	HILIC, 15 cm x 4.6 mm I.D.
50631-U	F5, 15 cm x 4.6 mm I.D.
Analytical Standards	
R5010	Resveratrol, 100 mg
239658-50G	Bisphenol A, >99 %, 50 g
47526-U	2,4,6-Trichloroanisole, 100 µg/mL in methanol, 1 mL
40019	2,4,6-Trichlorophenol, 5000 µg/mL in methanol, 1 mL
48264	2,3,4,6-Tetrachlorophenol, 5000 µg/mL in methanol, 1 mL
40062	Pentachlorophenol, 5000 µg/mL in methanol, 1 mL
Analytical Reagents and Solvents	
33148	BSTFA + TMCS, 99:1 (Sylon BFT), 20 x 1 mL
33085	Acetic anhydride, 10 x 2 mL
34967	Acetonitrile, LC-MS CHROMASOLV®, >99.9%

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