



ThermoFisher
S C I E N T I F I C

Chromatography Consumables Declassified:

the essential piece of the puzzle in your food safety analysis

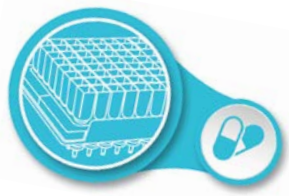
Xu Yanfang
Regional Product Specialist
Food Safety May – Jun 2017

The world leader in serving science

Essential piece of the puzzle in your food safety analysis



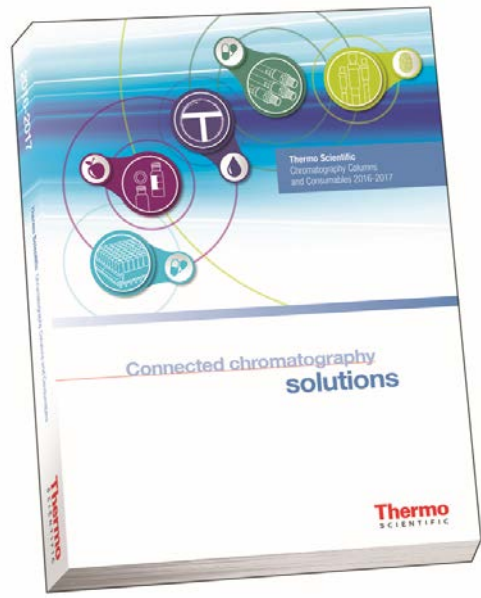
Thermo Chromatography Columns and Consumables



Sample Preparation



GC Columns & Accessories



BioLC Columns & Accessories



LC Columns & Accessories



Sample Handling

Food and Beverage



for food and beverage markets

Whether you are testing for contaminants or developing a new production process, we are here to help. Our innovative Thermo Scientific™ products and range of solutions allow you to deliver safe, high-quality food products that consumers expect.



Sample Preparation

Original Sample



Ready for Injection



Sample Preparation – QuEChERS

Qu

- Quick – More than 10 sampler per hour

E

- Easy – simple process with few steps

Ch

- Cheap – minimal solvent reagents and equipment

E

- Effective – wide scope, accurate, precise, compatibility with GC-MS & LC-MS

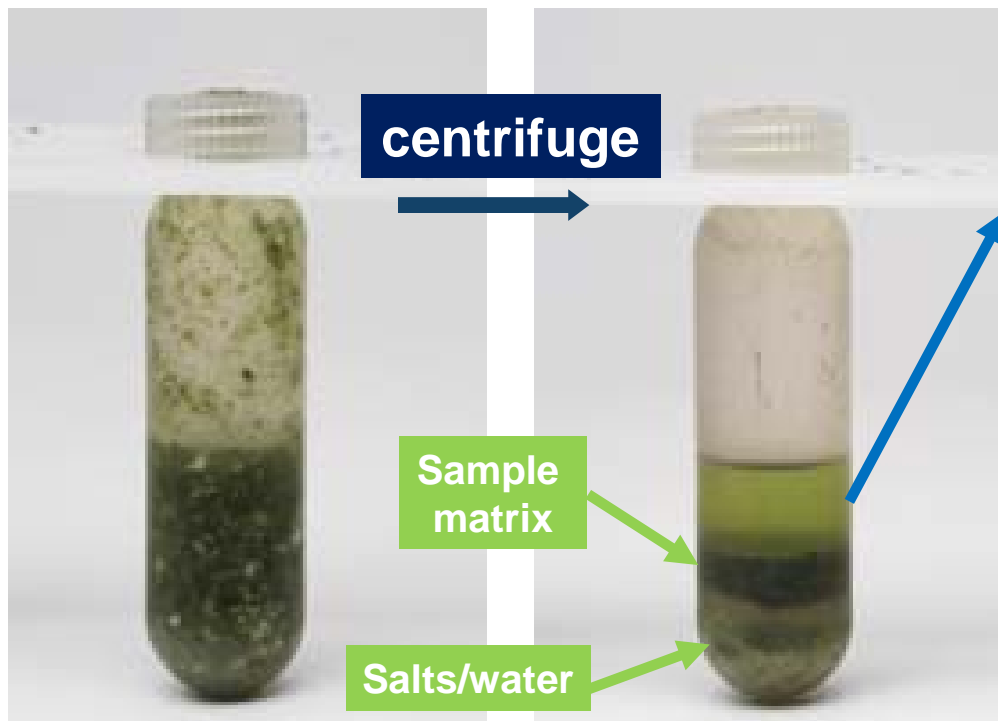
R

- Rugged – variations of method applicable to diverse sample types

S

- Safe – non chlorinated solvents contained in capped tube

1) Extraction



acetonitrile supernatant
containing extracted residues

2) Dispersive SPE







Note: Add sample to the tube, then solvent, then sorbent then mix, to avoid agglomeration

QuEChERS Method Variations

Method	Description
Original QuEChERS Method – introduced in 2003	Uses Sodium Chloride to enhance extraction
Dispersive AOAC 2007.01 Method	Uses Sodium Acetate as a buffer replacing Sodium Chloride
Dual Phase Variation	Uses PSA & GCB to remove high levels of chlorophyll and plant sterols
European Version	Similar to AOAC method – uses sodium chloride, sodium citrate dihydrate and disodium citrate sesquihydrate

How does QuEChERS work?

Matrix Type	Examples	Sorbent Requirements for Clean-Up
General Matrices	<ul style="list-style-type: none">• Apples• Cucumber• Melon 	MgSO ₄ , PSA Removal of excess water organic acids, fatty acids, sugars
Fatty Matrices	<ul style="list-style-type: none">• Milk• Cereals• Fish 	MgSO ₄ , PSA, C18 Additional removal of lipids & sterols
Pigmented Matrices	<ul style="list-style-type: none">• Lettuce• Carrot• Wine 	MgSO ₄ , PSA, C18, GCB Additional removal of pigments & sterols
High Pigmented Matrices	<ul style="list-style-type: none">• Spinach• Red Peppers 	MgSO ₄ , PSA, C18, GCB, Chlorofiltr™ Additional removal of chlorophyll

Application using QuEChERS – Breakfast Cereal

Modified European EN15662 – App Note 20639

Extraction

Add 5g of finely crushed cereal into a 50mL empty centrifuge tube, add 10mL Water.

Add Benzophenone standard
Sample spike: Add 3 µg of benzophenone to the sample followed by 10 mL of acetonitrile (0.3 µg/mL benzophenones spike)

Blank: No standards added, 10 mL of acetonitrile added. Mix thoroughly using a vortex and sonicate further for 30 minutes.

Add the contents of the QuEChERS kit (P/N 60105-337) to the centrifuge tube very slowly.

Shake vigorously using a vortex for 5 minutes and centrifuge for 10 minutes at 3500 rpm.

Dispersive SPE Clean-up (dSPE)

Transfer 5 mL of acetonitrile extract to the QuEChERS dSPE tube (P/N 60105-225).

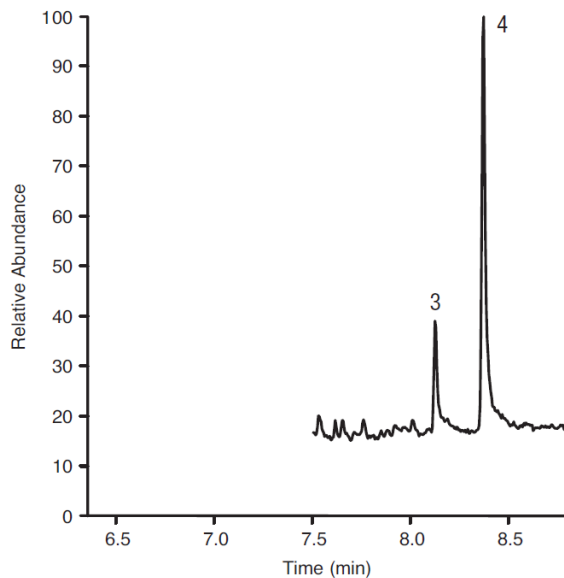
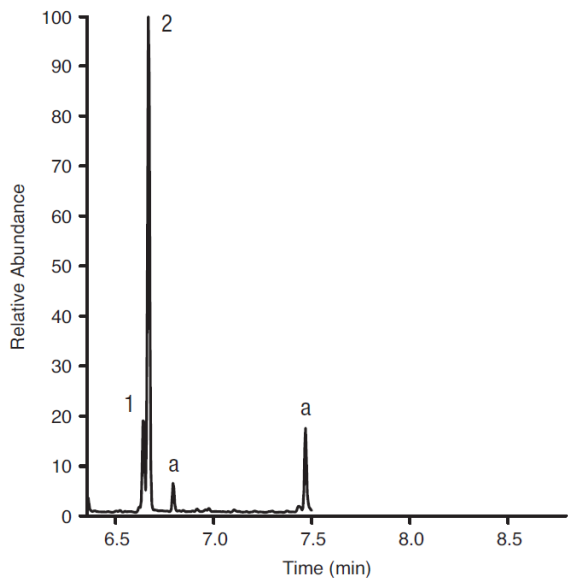
Shake vigorously for 5 minutes and centrifuge for 10 minutes at 3500 rpm.

Transfer 1 mL of sample extract to a GC vial.

Add 10 µL of 10 µg/mL internal standard to 1 mL of sample extract.



Application using QuEChERS – Breakfast Cereal



TraceGOLD TG-17MS

P/N 26089-1420 30 m x 0.25 mm x 0.25 μ m

Column flow: 1.2 mL/min

Oven temperature: 80°C (1.0 min)

30°C/min, 280°C (5 min)

GCMS-SIM Mode

Peak	Compound	t_R (min)	Linearity	% Recovery	%RSD (n=6)
1	Benzophenone-d10 (IS)	6.64	–	–	–
2	Benzophenone	6.67	0.9996	101.7	2.3
3	4-fluoro-4'-hydroxybenzophenone (IS)	8.13	–	–	–
4	4-hydroxybenzophenone	8.37	0.9996	82.3	4.6
a	Matrix impurities	6.79, 7.47	–	–	–

Application using QuEChERS – Red Wine

AOAC acetate buffered procedure – App Note 20830

Extraction

Transfer 15 mL red wine sample into a 50 mL centrifuge tube.

Spike with 50 μ L of the 30 μ g/mL triphenyl phosphate solution (corresponding to 100 ng/mL).

Add 15 mL of acetonitrile containing 1% acetic acid and vortex for 1 min.

Add contents of the Mylar pouch containing 6 g MgSO_4 and 1.5 g sodium acetate, and shake vigorously on a horizontal shaker or vortex for 1 min.

Centrifuge at $\geq 3,750$ rcf for 5 min.

The supernatant is now ready for dSPE clean-up.

Dispersive SPE Clean-up (dSPE)

Transfer 1 mL of the supernatant into a 2 mL dSPE tube containing 150 mg MgSO_4 and 150 mg PSA and vortex for 30 s.

Centrifuge at $\geq 15,000$ rcf for 5 min.

Transfer 0.3 mL of the purified extract into an autosampler vial, add 0.3 mL of reagent water, vortex, and filter with a 0.2 μ m syringe filter.

The sample extract is now ready for LC-MS/MS analysis.



Red wine extract before and after dSPE clean-up

Application using QuEChERS – Red Wine

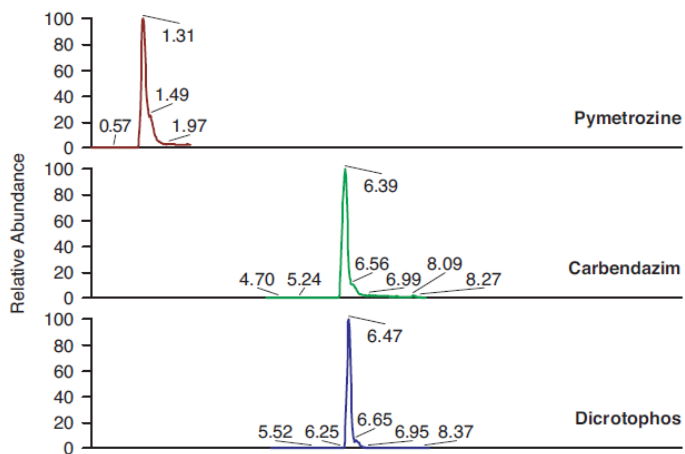
Accucore aQ

P/N 17326-102130 2.6 μ m, 100 \times 2.1 mm

Gradient Method

- 0.3 % formic acid and 0.1 % ammonia formate in ultrapure water
- 0.1 % formic acid in methanol

LCMS/MS

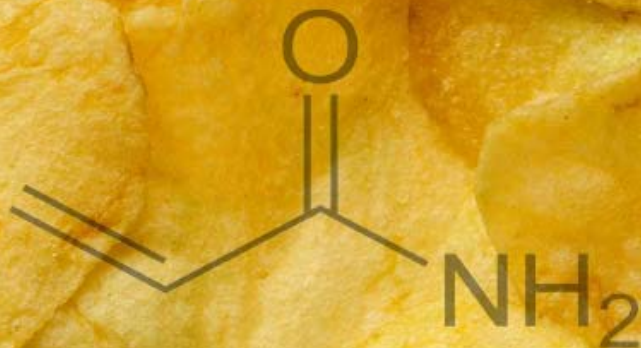


Good Resolution and Peak Shapes

Pesticide	10 ng/mL (n=6)		50 ng/mL (n=6)		100 ng/mL (n=6)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Methamidophos	78.5	6.1	84.2	2.0	91.0	11.4
Pymetrozine	64.5	5.5	61.9	2.4	63.3	12.1
Carbendazim	66.3	4.1	66.2	4.1	53.4	19.6
Dicrotophos	82.0	2.4	80.2	1.0	81.4	13.6
Acetachlor	85.3	3.2	88.9	2.4	84.5	13.5
Thiabendazole	78.8	4.6	75.4	5.9	62.9	19.6
DIMP	95.8	2.9	94.0	4.3	91.4	13.2
Tebuthiuron	87.3	2.1	87.3	2.1	89.6	12.0
Simazine	97.7	2.5	99.3	2.5	92.2	11.4
Carbaryl	95.5	3.3	91.6	1.5	90.0	10.5
Atrazine	91.0	1.8	90.1	1.9	89.1	5.9
DEET	93.7	1.9	93.9	2.6	90.7	8.1
Pyrimethanil	94.2	3.1	91.0	2.1	82.7	13.7
Malathion	99.0	2.4	96.7	2.7	89.1	11.4
Bifenazate	103.3	3.4	97.5	3.0	84.5	11.3
Tebuconazole	95.0	3.0	94.1	3.1	83.6	8.4
Cyprodinil	98.7	2.3	96.6	2.3	90.4	5.2
Diazinone	98.5	2.5	100.1	3.5	80.2	17.6
Zoxamide	101.7	1.7	101.1	2.5	91.8	6.5
Pyrazophos	95.5	2.5	96.3	3.3	79.9	18.5
Profenofos	91.8	4.8	88.4	2.3	91.8	7.9
Chlorpyrifos	95.5	7.2	95.1	3.3	75.8	20.8
Abamectin	92.5	2.6	88.7	3.7	79.3	14.5
Bifenthrin	93.2	4.2	93.3	5.9	87.8	12.5
Overall average	90.6	3.3	89.7	2.9	83.2	12.5

Acrylamide

– **potential human carcinogen**



The World Health

Organization (WHO) has set

a safe limit of 500 ng/mL

acrylamide in drinking water.

Higher levels of 100–1000

ng/g are determined in some

foods such as potato chips

or french fries.

Application using Hypercarb SPE – Potato Chips

HyperSep Hypercarb SPE cartridge
500 mg/6 mL

Sample Pretreatment

- Crush chips with mortar and pestle and 1 g was weighed into a vial.
- A 1 g portion of the sample was spiked with acrylamide standard in 2% formic acid / water.
- The sample was then filtered through a filter membrane.

Conditioning

- Add 4 mL methanol, 4 mL water, and 4 mL 2% formic acid / water to the SPE cartridge.

Application

- Apply 1 mL of extract in 2% formic acid / water under vacuum at 1 mL/min to the SPE cartridge.

Washing

- Add 1 mL water to the SPE cartridge and dry for 20 min under vacuum.

Elution

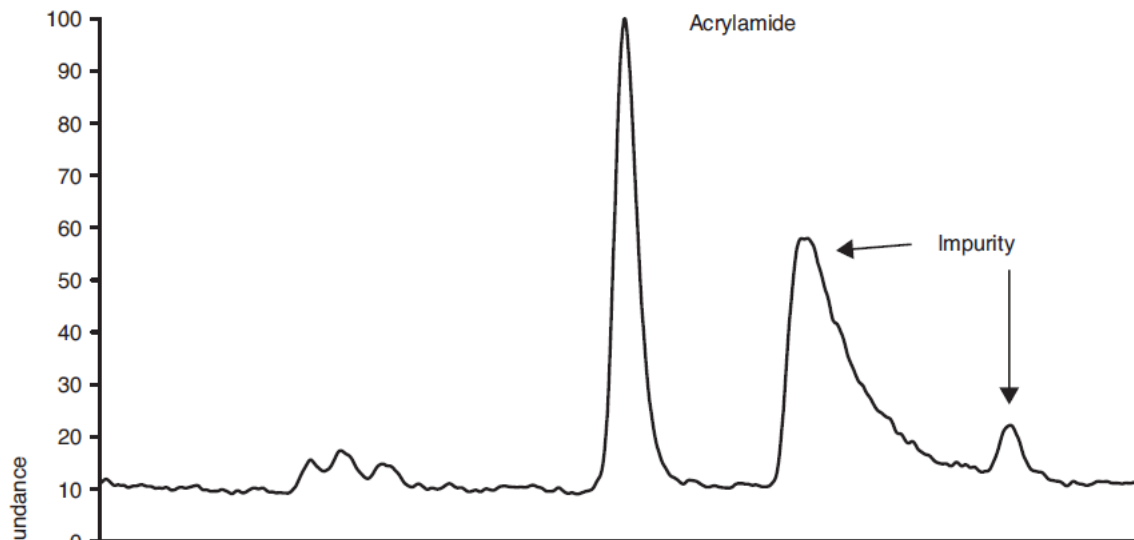
- Apply 4 mL methanol to the SPE cartridge.

Additional

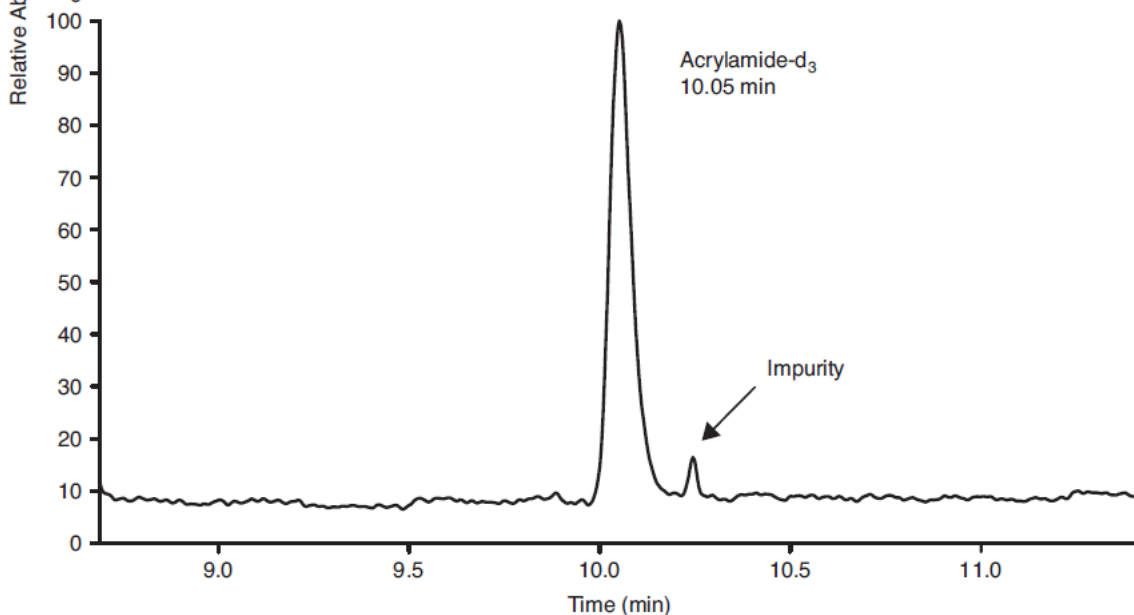
- Evaporate methanolic extract and reconstitute with 1 mL of 1 µg/mL of internal standard in methanol to the SPE cartridge.

App Note 20734

Application using Hypercarb SPE – Potato Chips



Specified Concentration (µg/mL)	Calculated Concentration	% Difference
0.25	0.225	-9.83
0.50	0.469	-6.22
1.00	0.983	-1.70
2.50	2.520	0.79
5.00	4.979	-0.41
10.0	10.037	0.37



TraceGOLD TG-WaxMS

P/N 26088-1420 30 m x 0.25 mm x 0.25 µm

Column flow: 1.2 mL/min

Oven temperature: 80°C, 10°C/min, 250°C

GCMS



2 mL Vials and Caps



Lower detection limits ↙
what kind of vial and caps are suitable?

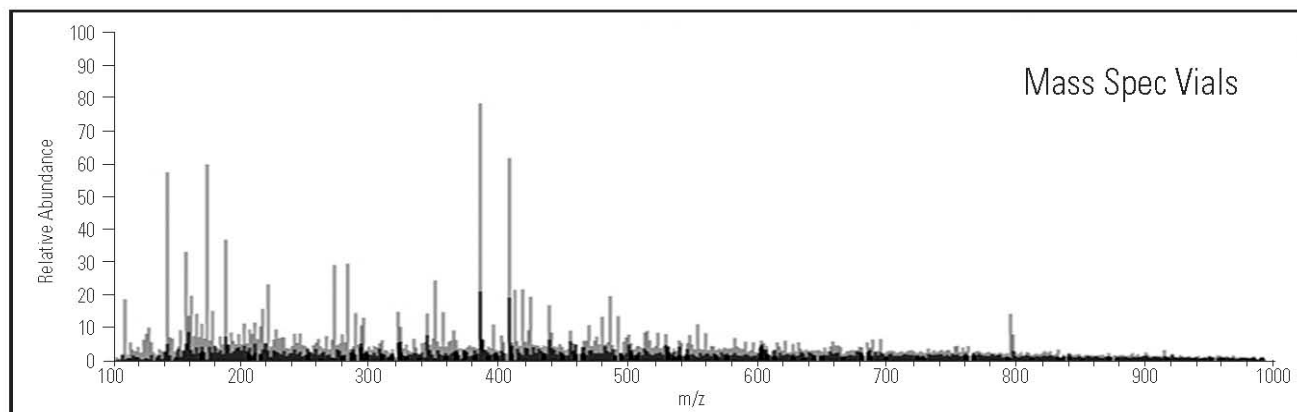
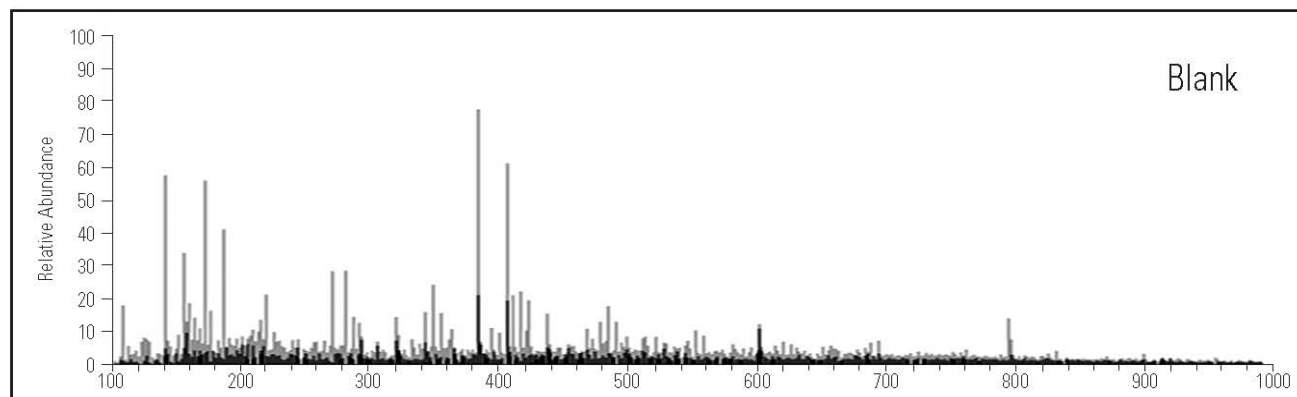
Mass Spec Vial Kits – Pre-Cleaned

- Vials are cleaned by our unique, proprietary processing method to assure the lowest possible background of any product
- Packaging is cleaned to the same high standard
- Mass Spec Vials are the only pre-cleaned chromatography vials on the market



Mass Spec Vial Kits – Tested for LC/MS Residues

LC/MS Background Scan

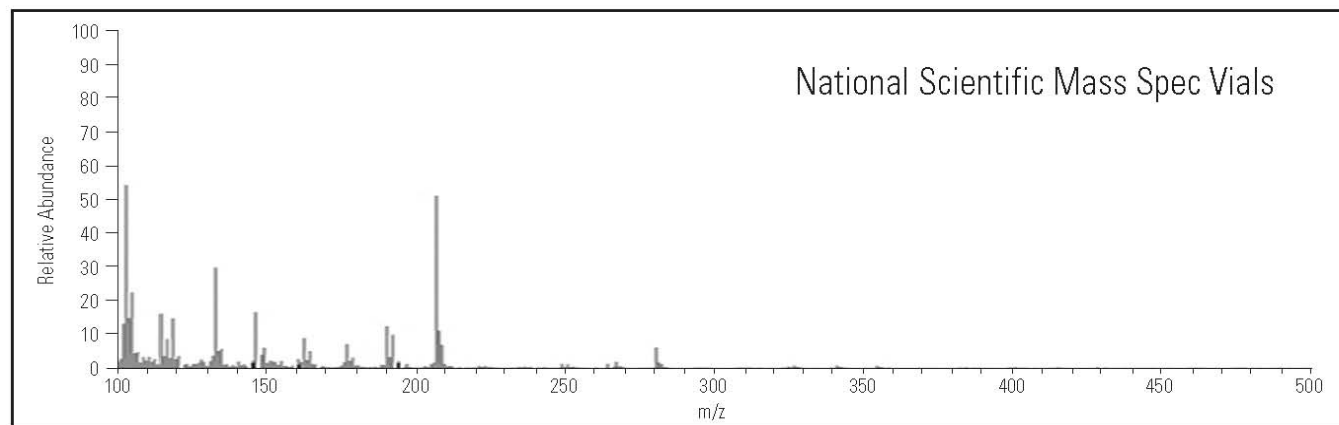
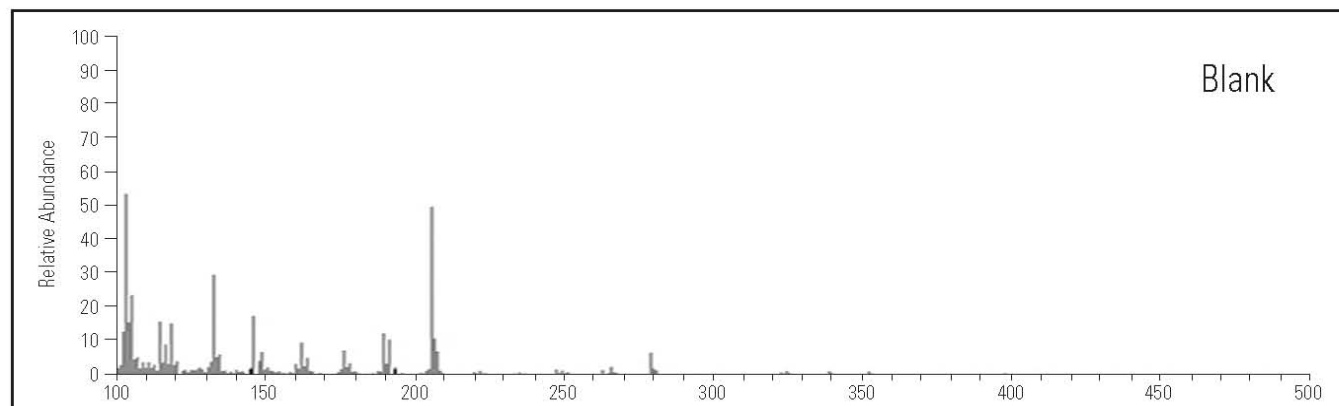


Conditions:
Positive ESI
100 to 1000m/z
1.51e5

Vials and closures are tested for background residue by positive electrospray LC/MS

Mass Spec Vial Kits – Tested for GC/MS Residues

GC/MS Background Scan



Conditions:

Positive EI
Oven Temp 40 to 290°C
50 to 500m/z
2.00e5

Vials and closures are tested for background residues by GC/MS

Mass Spec Vial and Caps Configurations

Vial profiles

(selected by the most frequently purchased products)

- 2mL Clear and Amber
- 2mL Silanized Clear and Amber
- 200 μ L Fused Insert
- 350 μ L Fused Insert
- 1mL Total Recovery
- 1.5mL High Recovery



Bonded Natural PTFE/ Ultra High Purity, Color-free Silicone


- Each vial is offered with two cap configurations
 - **Blue** caps feature solid PTFE/silicone bonded septa
 - **Gray** caps feature pre-slit PTFE/silicone bonded septa
- Bonded septa for resistance to push-through
- PTFE and silicone layers are free from additives including colorants
- Closures are processed to give the lowest possible levels of residual siloxanes and other trace contaminants



Mass Spec Vial Kits – Certified

- Certificate of Conformance included with every convenience kit
- Positive ESI LC/MS and GC/MS background scans included
- Physical testing, LC/MS, GC/MS and particulate testing for a completely characterized vial




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**National Scientific Certified Mass Spec Vial Kit
Certificate of Conformance**

Part Number: MSCERT4000-39TR

Description: MS Certified 12X32mm Target DP, 1mL Clear glass, Total recovery vial, Polypropylene open top cap, Bonded Pre-slit, PTFE/Silicone septum – 100/pack

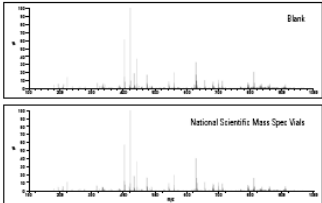
Lot Number: _____

Test Parameter	Description
Glass	Type 1, Class A, 33 Expansion Clear Borosilicate Glass
Dimension	Height, Diameter, Bottom Thickness, Neck Length, Thread Profile, Annealing
Cap/Septum	Diameter, Height, Thread Integrity, Center Flash, Septum Insertion, Sealing/Resealing

Particle Counts

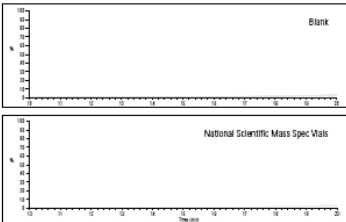
Particle Size (µm)	≥ 0.1	≥ 0.15	≥ 0.2	≥ 0.3	≥ 0.5
Particles/mL/Vial	<0.1	<0.1	0.3	0.4	0.2

LC/MS Background, Positive ESI



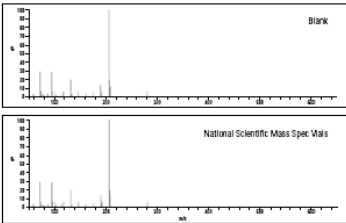
Conditions:
 RT: 0.01-25.01
 AC: 1518
 NL: 3.9664
 T: ac ESI full MS
 (100.00-1000.00)

GC/MS Background Chromatogram




Conditions:
 RT: 10.00-20.00
 NL: 1.00E7
 TIC MS

GC/MS Background, Positive ESI



Conditions:
 RT: 10.00-20.00
 AV: 2416
 NL: 1.20E5
 T: ac ESI MS
 (50.00-650.00)

QC Certification: _____ Date: _____


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Accucore



Hypersil GOLD



Acclaim



Hypercarb



Viper



MABPac SEC-1

MABPac SCX

MABPac Rp

EASY-Spray



Cola Colouring Linked to Cancer

Laboratory Equipment - Cola Coloring Linked to Cancer - Windows Internet Explorer

http://www.laboratoryequipment.com/News-cola-coloring-linked-to-cancer-022511.aspx?et_cid=1185853&et rid=54642045&linkid=http%3A%2F%2Fwww.laboratoryequipment.com

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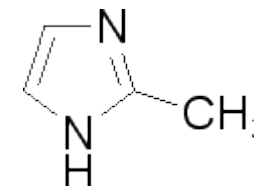
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Cola Coloring Linked to Cancer

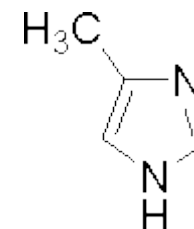
February 25, 2011

The "caramel coloring" used in Coca-Cola, Pepsi, and other foods is contaminated with two cancer-causing chemicals and should be banned, according to a regulatory petition filed by the Center for Science in the Public Interest.

In contrast to the caramel one might make at home by melting sugar in a saucepan, the artificial brown coloring in colas and some other products is made by reacting sugars with ammonia and sulfites under high pressure and temperatures. Chemical reactions result in the formation of 2-methylimidazole and 4-methylimidazole, which in government-conducted studies caused lung, liver, and thyroid cancer.



2-Methylimidazole

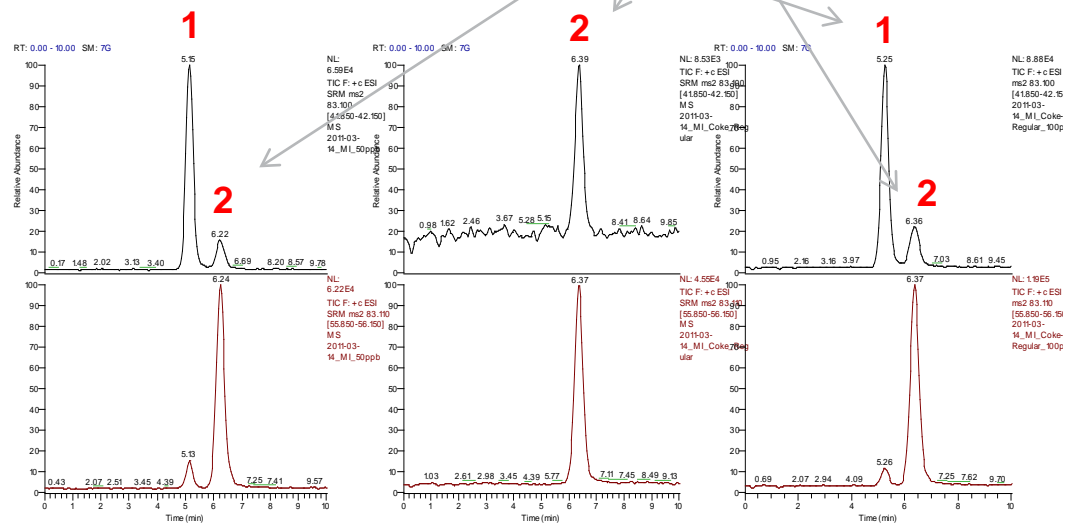
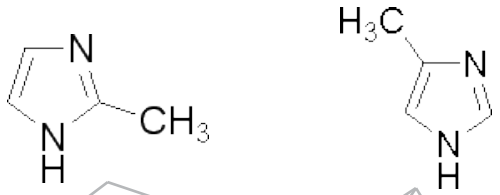


4-Methylimidazole

2-Methylimidazole & 4-Methylimidazole in Cola

2-Methylimidazole

4-Methylimidazole

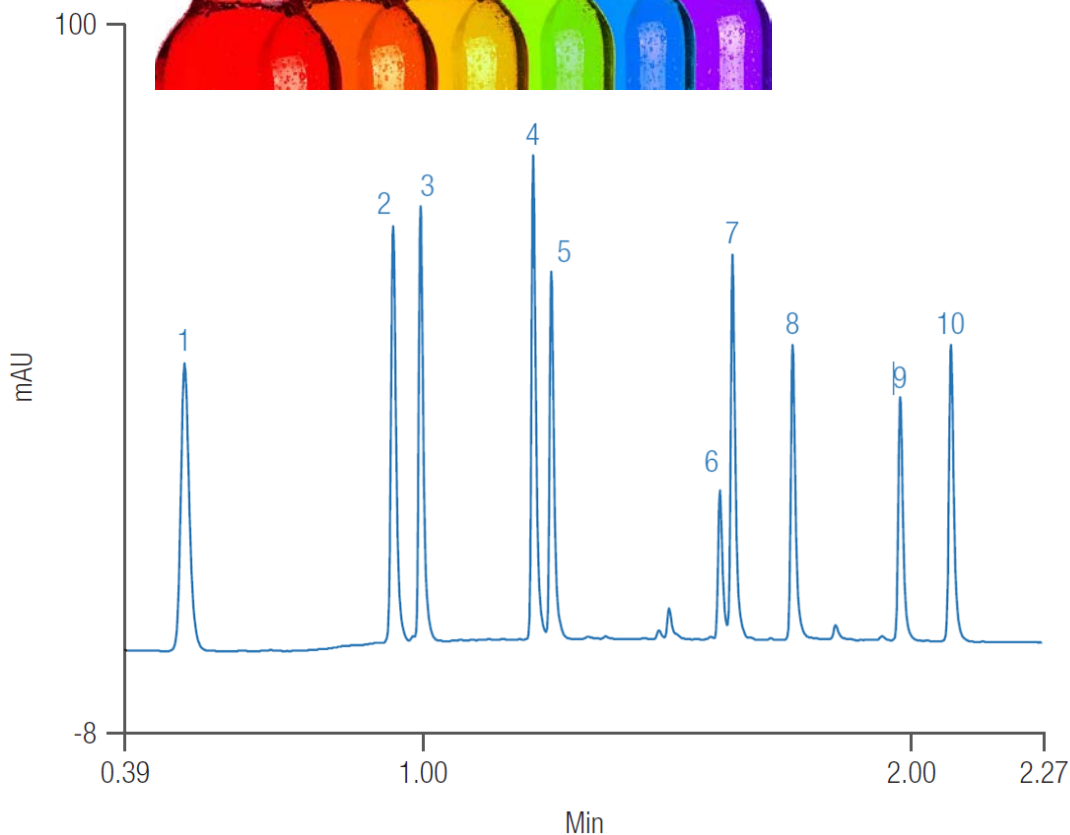


Column: **Acclaim Trinity P1**, 3µm
 Dimension: 2.1x50 mm
 Mobile Phase: 10/90 v/v MeOH/ NH₄OAc, (5 mM, pH5.7)
 Temperature: 30°C
 Flow Rate: 0.5 mL/min
 Inj. Volume: 5 µL
 Detection: MS-MS

Peaks:
 1. 2-Methylimidazole
 2. 4-Methylimidazole

Coke
 with 100ppb spike

Food dyes in carbonated beverage



HPLC column: **Hypersil Gold VANQUISH C18**
 50 mm x 2.1 mm x 1.9 μm

Mobile phase A: 20 mM (NH₄)₂HPO₄, pH 8.8 in water adjusted with 8N NaOH

Mobile phase B: 20 mM (NH₄)₂HPO₄, pH 8.8 / acetonitrile (50 50 v/v)

Gradient conditions: Time (min) A% B% -3.0 95.0 5.0 0.0 95.0 5.0 1.5 0.0 100.0 2.2 0.0 100.0 2.3 95.0 5.0

Flow rate: 0.55 mL/min

Column Temp: 40 °C (still air mode)

Injection volume: 1 μL

UV detection: 254 nm and 3D Field

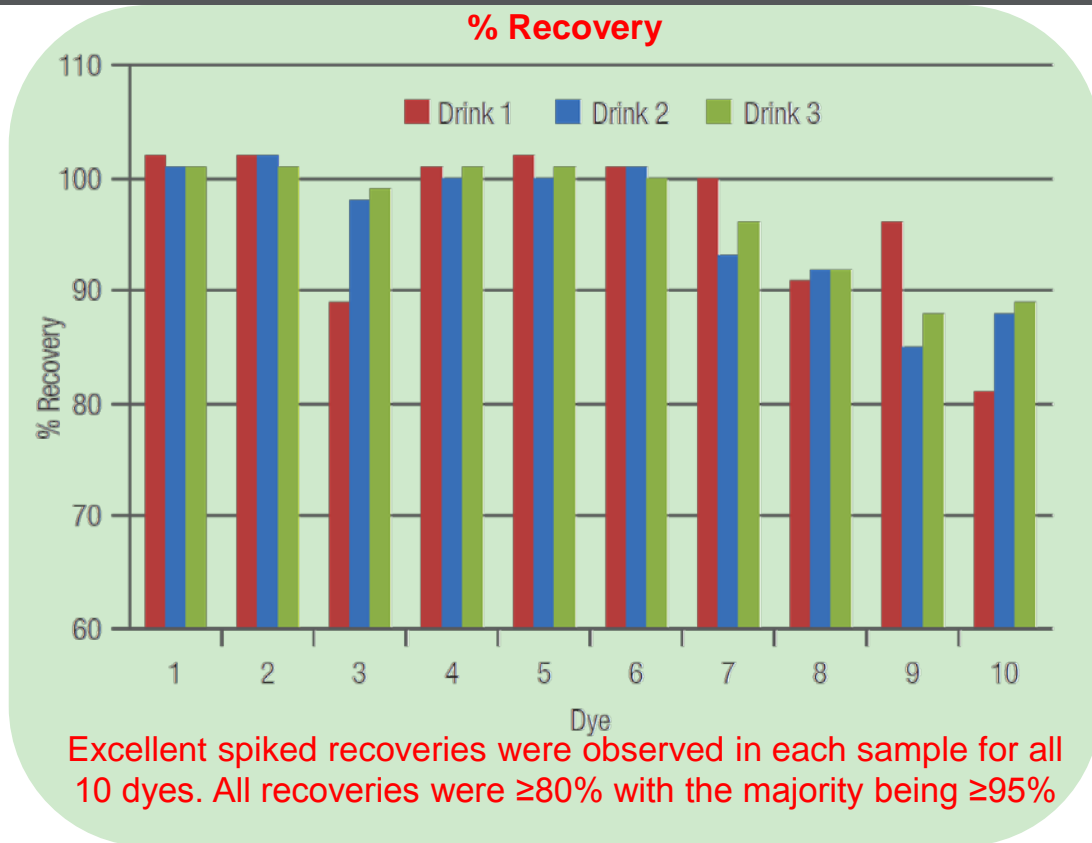
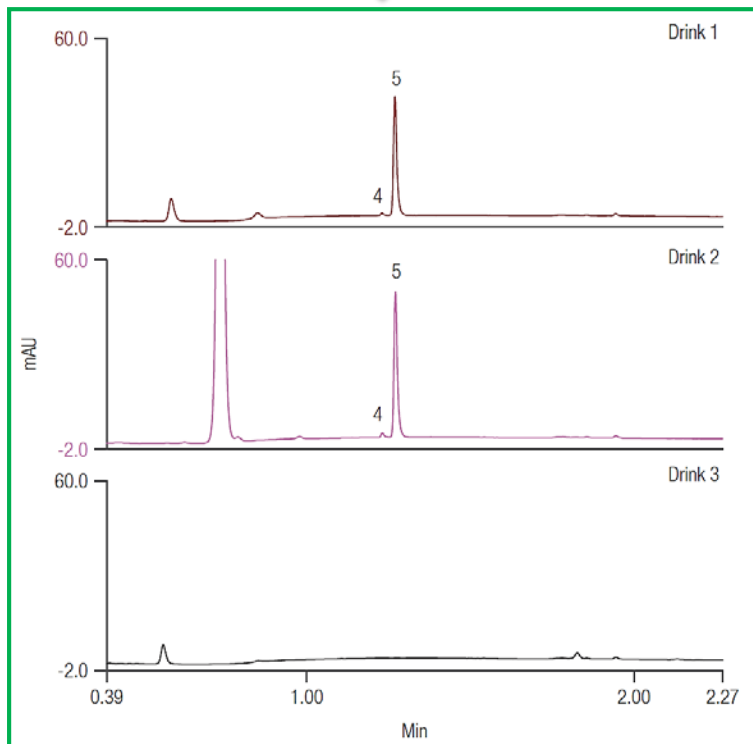
Gradient mixer: 200 μL

Peak	Compounds	RT	Area	% RSD (n=6)
1	Tartrazine	0.240	0.126	
2	Amaranth	0.084	0.307	
3	Indigo carmine	0.044	1.958	
4	New coccine	0.033	0.197	
5	Sunset yellow	0.032	0.170	
6	Fast green FCF	0.022	0.143	
7	Eosin Y	0.030	0.237	
8	Erythrosine	0.020	0.311	
9	Phloxine B	0.023	0.170	
10	Bengal rose	0.030	0.313	

Full resolution of all 10 dyes (>1.5 EP resolution) was achieved in approximately two minutes on the Vanquish Flex Binary UHPLC system using a Hypersil GOLD VANQUISH C18 column

Food dyes in carbonated beverage

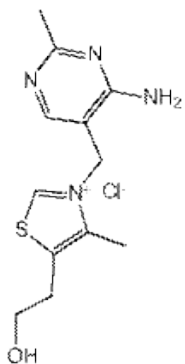
Sample	Drink	Colour
Carbonated drinks	1	Bright Yellow
	2	Bright Orange
	3	Bright Red



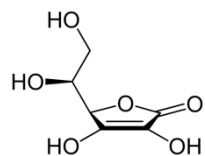
Drink	Colour	Dye Concentration $\mu\text{g/mL}$	
		New Coccine	Sunset Yellow FCF
1	Bright Yellow	1.9	79.8
2	Bright Orange	2.8	97.7



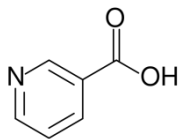
Water-Soluble Vitamins



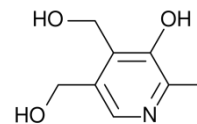
Thiamine (B1)



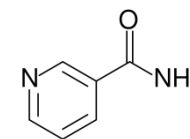
Ascorbic Acid (C)



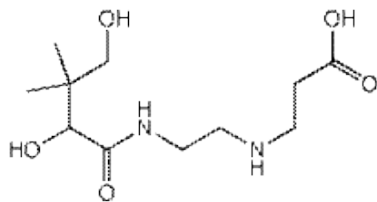
Niacin (B3)



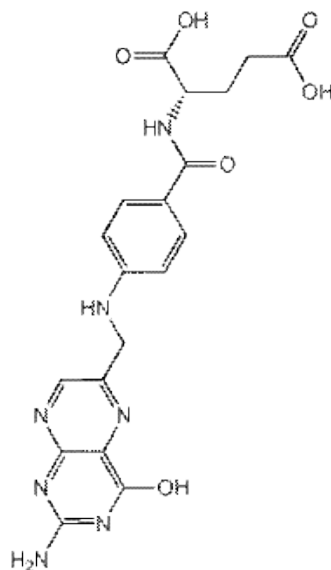
Pyridoxine (B6)



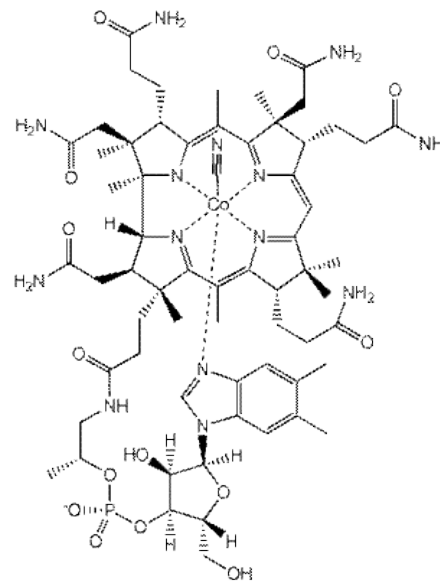
Niacinamide (B3)



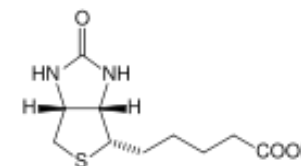
Pantothenic Acid



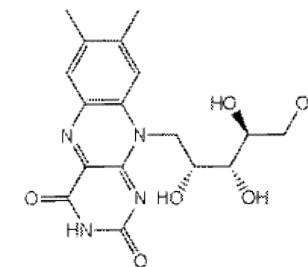
Folic Acid (Bc)



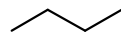
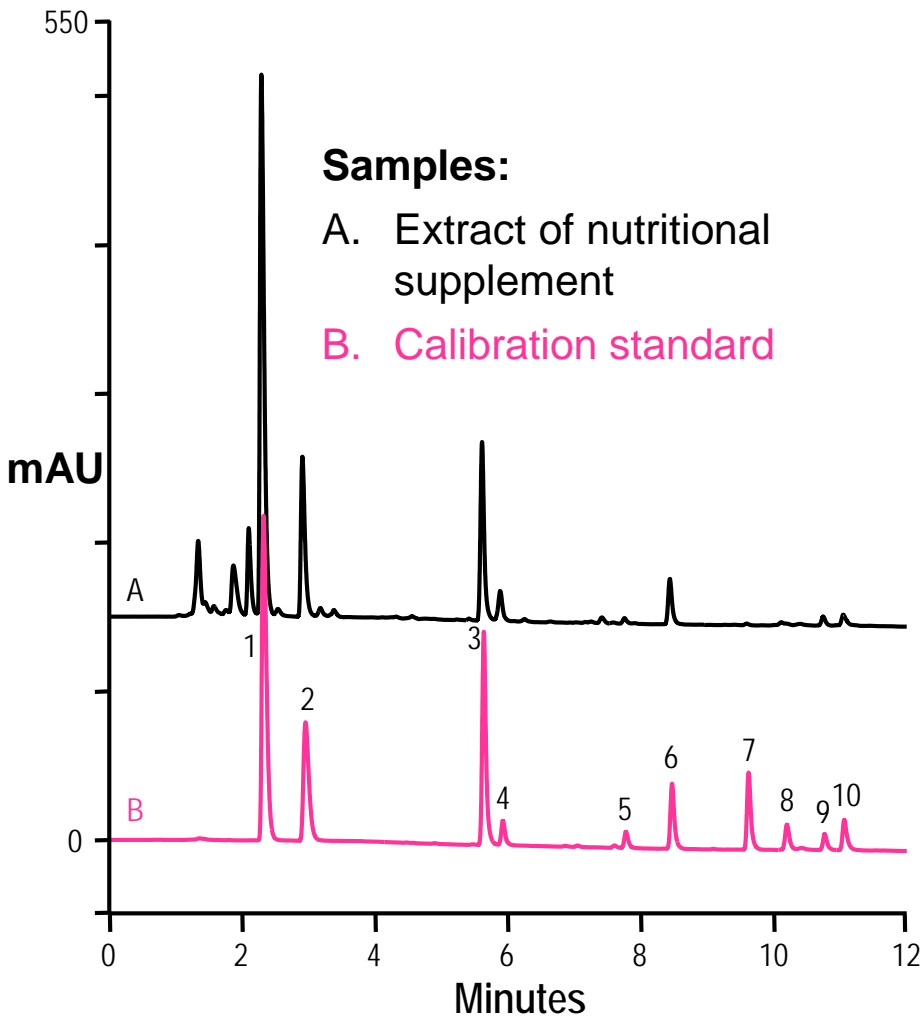
Cyanocobalamin (B12)



Biotin (H)



Riboflavin (B2)



Column: **Acclaim PolarAdvantage**, 3 μm , 150x4.6 mm

Mobile Phases:

A: acetonitrile

B: water

C: 30 mM sodium hexanesulfonate, 30 mM H_3PO_4 , adjusted to pH 3.08 with NH_4OH

Gradient times:	-7	0	14	16
%A	0	0	30	30
%B	65	65	35	35
%C	35	35	35	35

Flow: 1.0 mL/min

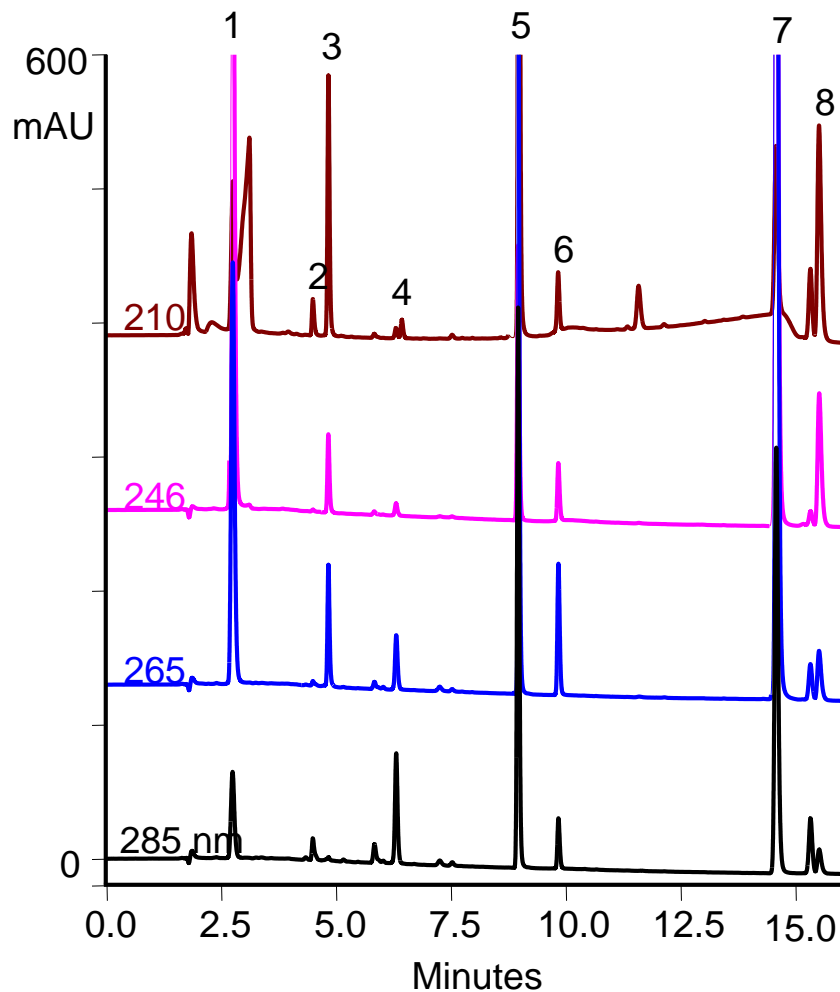
Temperature: 30 ° C

Injection: 20 μL

Detector: 210, 246, 265, and 285 nm

Peaks:	($\mu\text{g/mL}$)	($\mu\text{g/mL}$)
1. Ascorbic acid	120	6. Pyridoxine 2.5
2. Nicotinic acid	10	7. Folic acid 5.0
3. Niacinamide	10	8. Vitamin B12 1.5
4. Pantothenic acid	20	9. Thiamine 2.5
5. Riboflavin 5'-phosphate	2.5	10. Riboflavin 2.5

Ingredients in an Energy Drink



Column: **Acclaim PA2**, 3 μ m

Dimension: 4.6 \times 150 mm

Mobile Phases

A: Acetonitrile

B: 30 mM phosphate buffer, pH 3.28

C: 30 mM phosphate buffer, pH 2.54

Gradient:

Times (min): 0 14 16

%A: 0 40 40

%B: 100 0 0

%C: 0 60 60

Flow Rate: 1.0 mL/min

Temperature: 30 $^{\circ}$ C

Injection: ASI-100 autosampler, 5 μ L

Detection: UV 210, 246, 265, 285 nm

Peaks:

1. Ascorbic acid

2. Pyridoxine

3. Niacinamide

4. Pantothenic acid

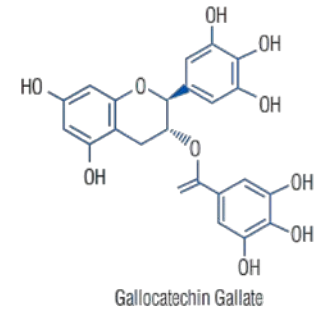
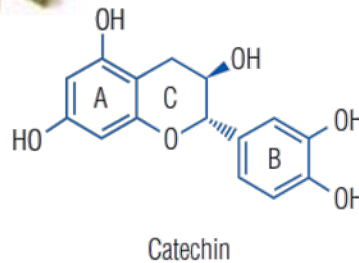
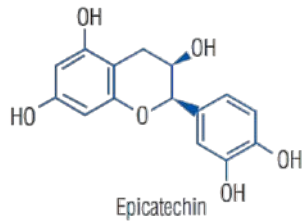
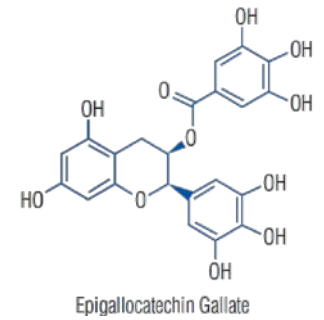
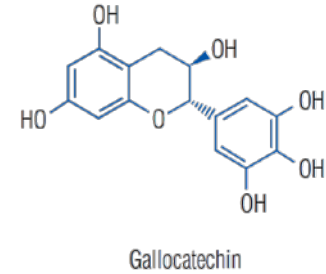
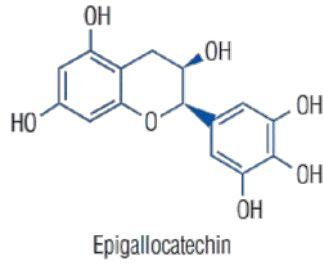
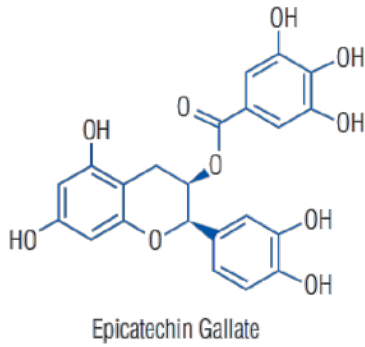
5. Caffeine

6. Riboflavin

7. Sorbic acid

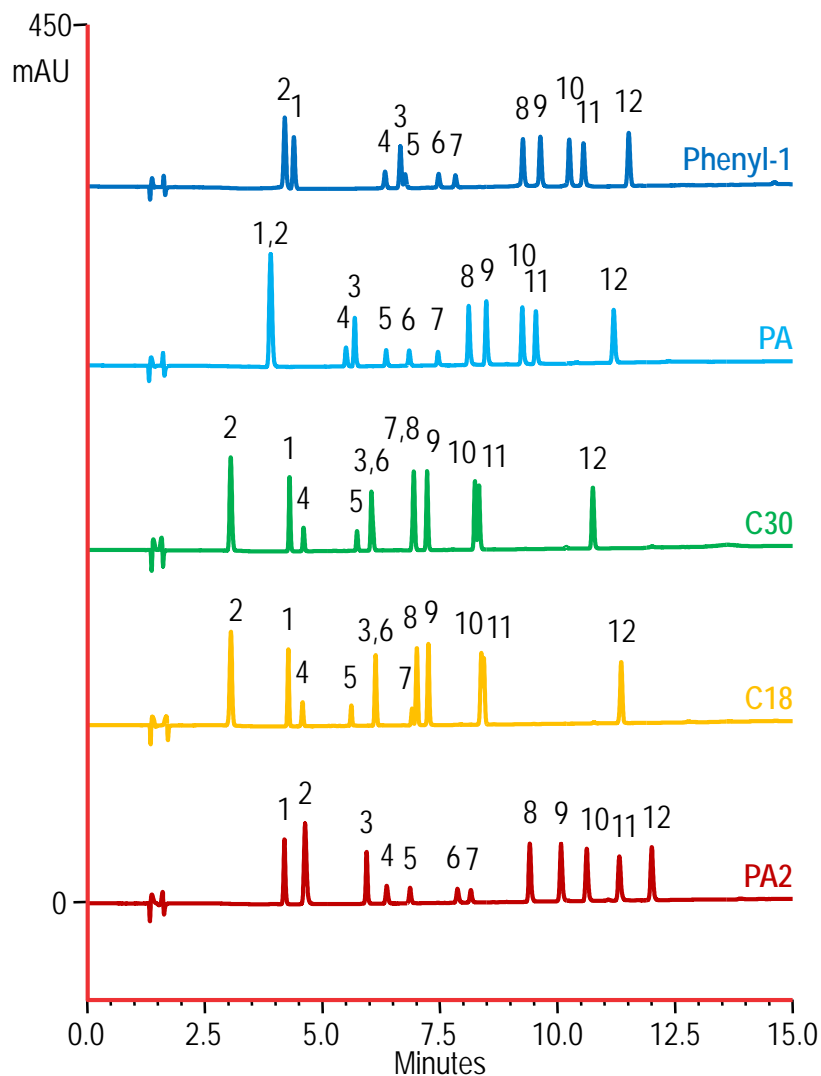
8. Benzoic acid

Analysis of Catechins



Catechins are powerful antioxidants found in tea that are thought to provide several of these health benefits.

Selectivity Comparison: Catechins and Caffeine in Tea



Columns: **Acclaim C18, C30, PA, PA2 and Phenyl-1**, 3 μ m
 Dimension: 3 x 150 mm
 HPLC System: UltiMate[®] 3000-RS
 Mobile Phases:
 A: Acetonitrile
 B: 100 mM Formic acid + 20 mM ammonium formate
 C: water

Gradient times, min:	-6	0	12	15
%A	5	5	50	50
%B	10	10	10	10
%C	85	85	40	40

Flow rate: 0.60 mL/min
 Injection: 5 μ L
 Temperature: 30°C
 Detection: UV at 254 nm, 5 Hz, 1 s resp. time

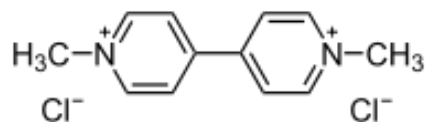
Peaks:

1. theobromine	7. epicatechin
2. gallic acid	8. epigallocatechin gallate
3. caffeine	9. galocatechin gallate
4. galocatechin	10. epicatechin gallate
5. epigallocatechin	11. catechin gallate
6. catechin	12. 3,4,5-trihydroxy cinnamic acid

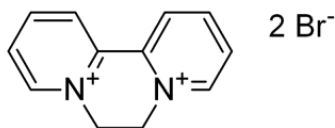
Paraquat (Pq) and Diquat (Dq)



- **Non-selective, nonsystematic contact herbicides**
- **Environmental & safety concerns**
 - Toxic to humans through contact (e.g. oral, respiratory, dermal)
 - Moderately hazardous: LD50 ~35 mg/kg for human
 - Banned or restricted in several European countries and in Japan



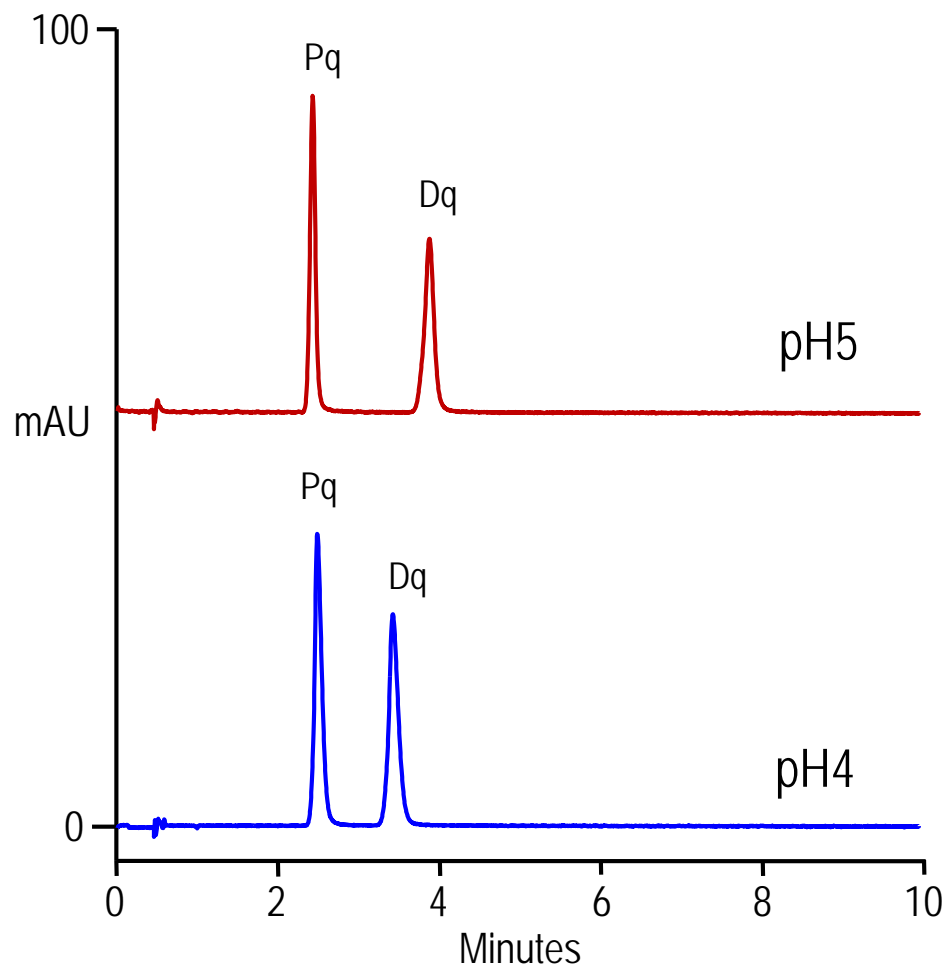
Paraquat (Pq)



Diquat (Dq)

- **Regulation**
 - The U.S. EPA regulation: < 20 µg/L for Dq in drinking water
 - European Union (EU)'s general rule for pesticides in drinking water (98/83/EC):
 - < 0.1 µg/L of each individual pesticide
 - < 0.5 µg/L for the total concentration
- ***Food safety concerns in developing countries***

Separation of Paraquat and Diquat



Column: **Acclaim Trinity Q1**, 3 μ m
Dimensions: 3.0 x 50 mm
Mobile Phase: 75/25 v/v CH₃CN/ 100 mM NH₄OAc, pH5
Temperature: 30 ° C
Flow Rate: 0.60 mL/min
Inj. Volume: 2 μ L
Detection: UV, 290 nm
Sample: Dq and Pq (0.1 mg/mL each)

Pq/Dq	pH4	pH5
Resolution (Rs)	5.1	8.8
Retention (k)	4.7/6.8	4.5/7.9
Asymmetry (As)	1.31/1.18	1.08/0.96
Efficiency	3900/4800	6200/5600

Heart of Separation – GC Columns



Supplies

- Carrier Gas Filters
- Syringes
- Liners
- Inlet seals
- Ferrules



TraceGOLD & Trace GC Columns



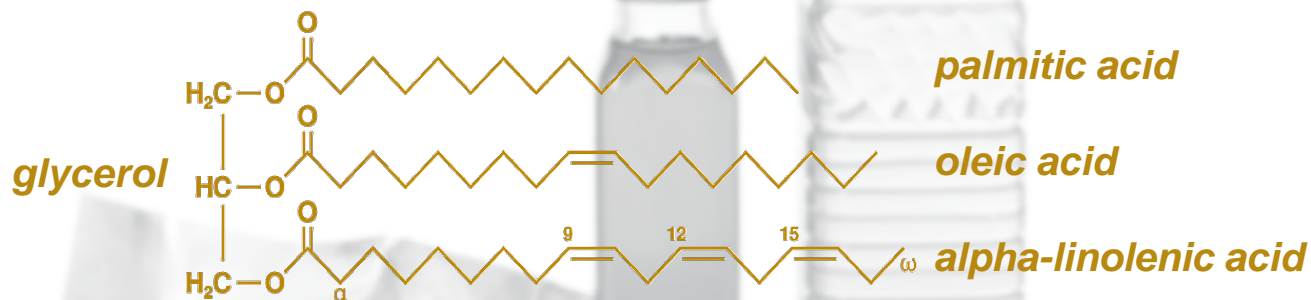
Derivatization Reagents



Reacti-Therm

FAMES Analysis in Edible Oils

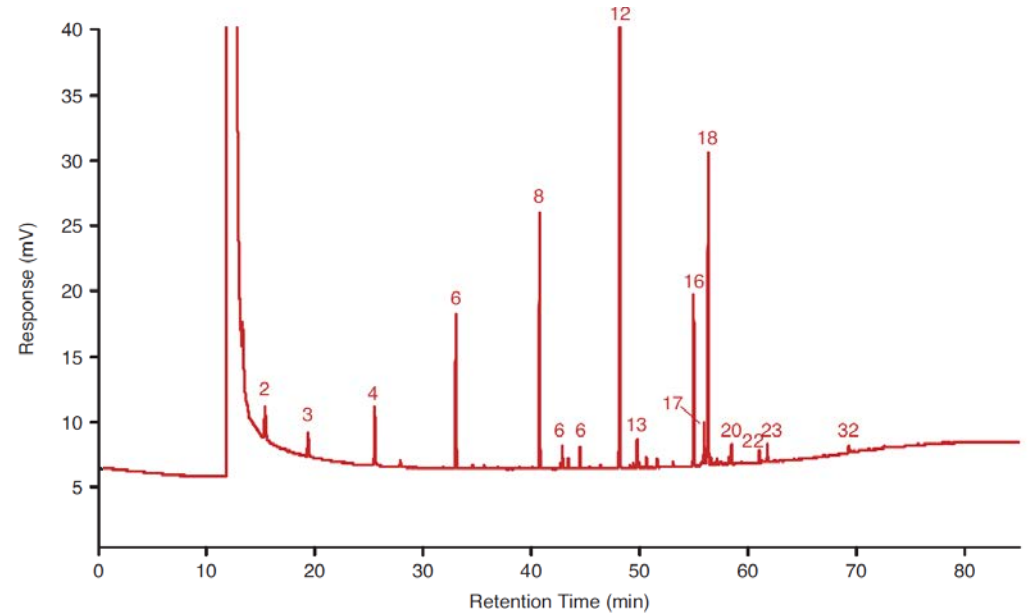
- FAMES analysis is an important tool both for **characterizing fats and oils** and for **determining the total fat content in foods**
- Fats consist of triglycerides: glycerol esters and long chain aliphatic acids (fatty acids)



A general triglycerides

- Derivatization of fatty acids to FAMES for more amendable analysis
 - Direct analysis of free fatty acids will lead to reproducibility issue, as these polar acids will form hydrogen bonds and adsorb on the surface of contact

HOW?



Margarine

Presence of FAMES

Sample Preparation

1

- Magnetic stirrer
- 1 mL Hexane
- 0.5 mL 14% BF₃-methanol



Reacti-Vial

2

- 50mg liquid fat sample



Magnetic stirrer
(Teflon-coated)

3

- Cap the vial
- Place in the Reacti-Therm for 30mins at 50°C



Reacti-Therm



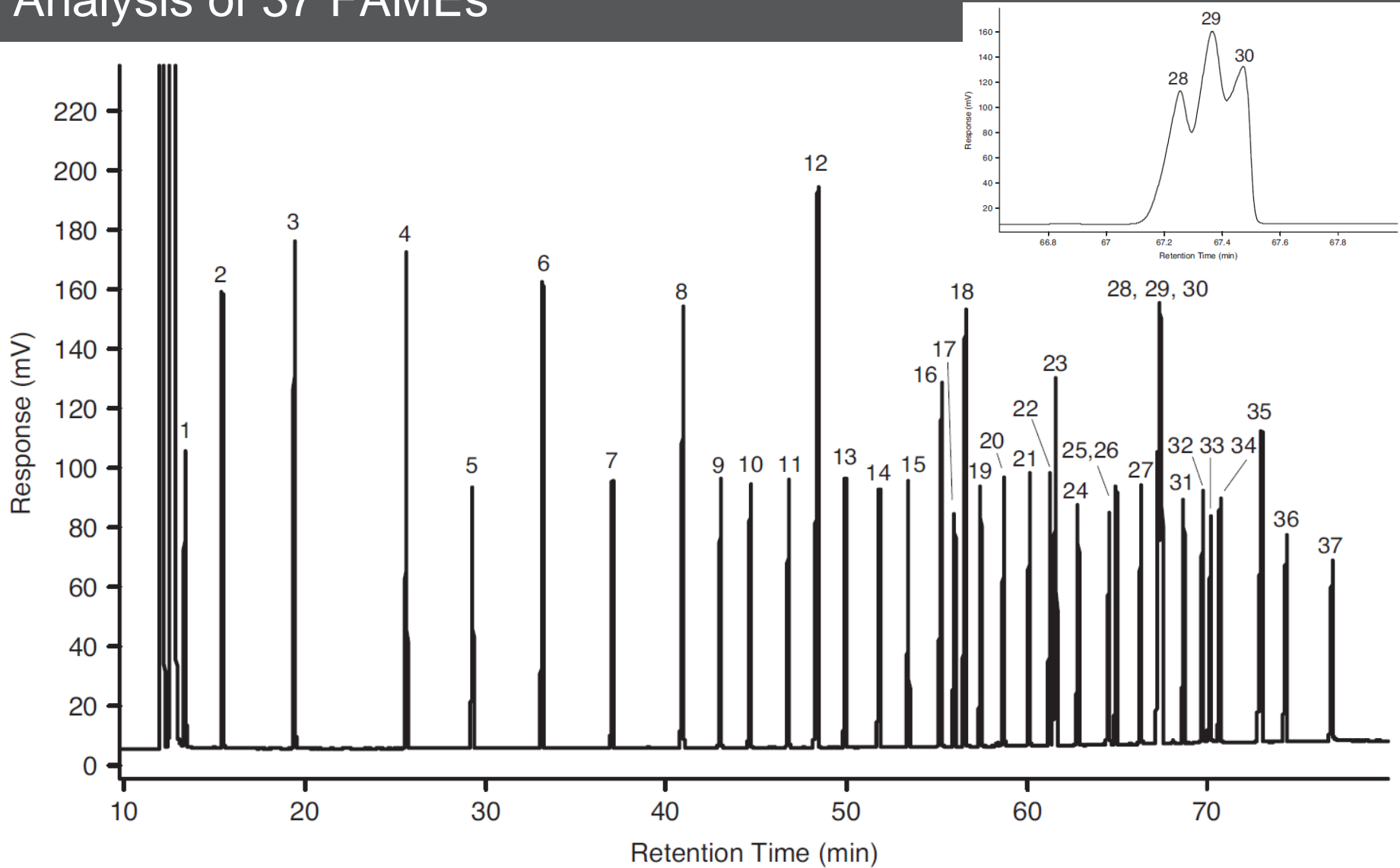
4

- Cool mixture to room temperature
- Add 1 mL water

5

- Extract the organic layer after phase separation
- Transfer the solution to GC vial for analysis

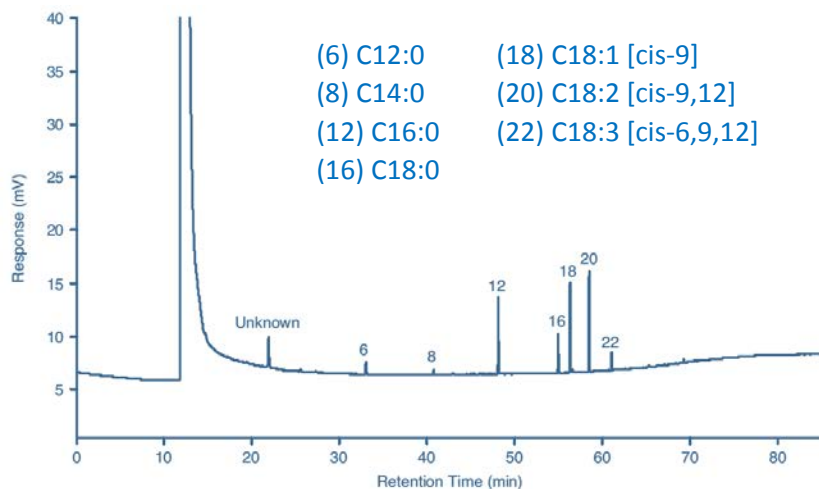
Analysis of 37 FAMES



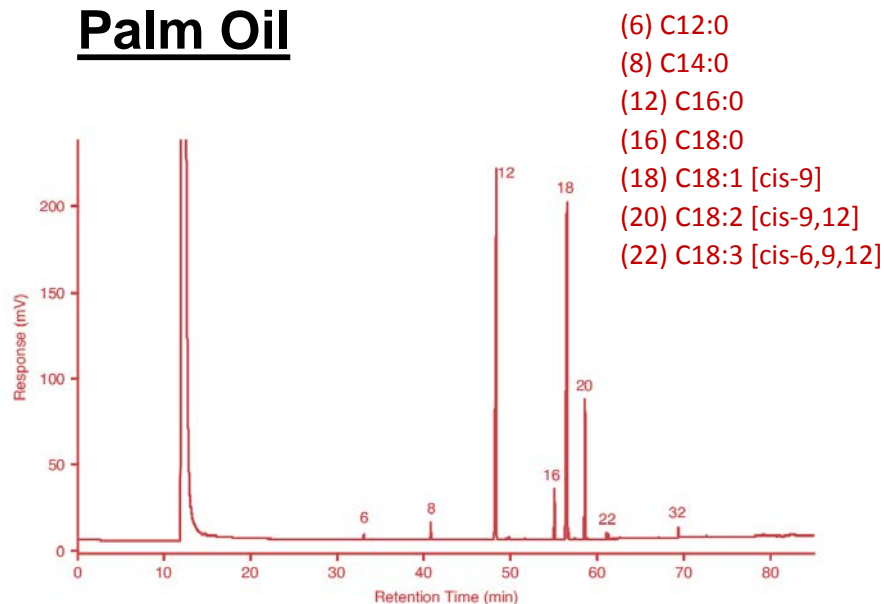
TRACE TR-FAME GC column 100 m × 0.25 mm × 0.20 μm

FAMES present in 3 edible oils

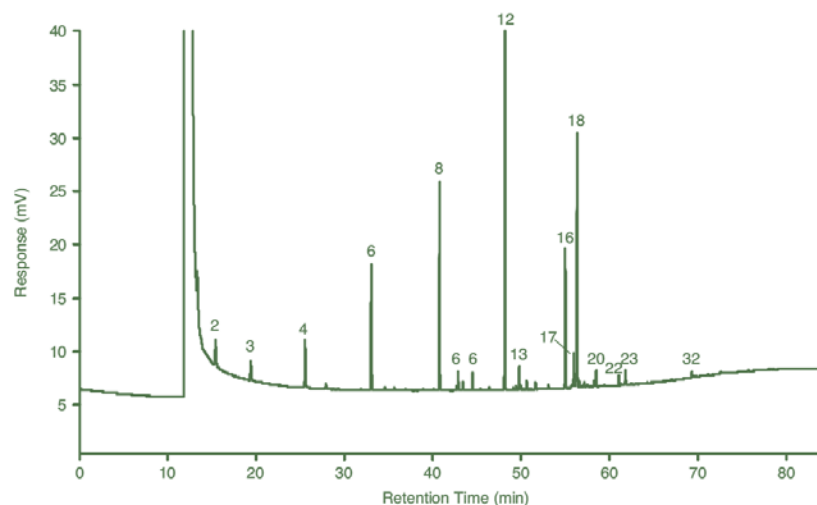
Butter



Palm Oil



Margarine



App Note 20733



Q & A

