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Gas Chromatography with Capillary Flow Technology

An effective analytical method for detecting pesticide residues in olive oil

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ABSTRACT: The detection of residual organophosphorous (OP) pesticides in processed olive oil is complicated by the chromatographically active nature of these compounds, which compromises chromatographic resolution. This study demonstrates a quick and effective analytical method for the determination of low ppm and trace-level OP pesticide residues in an olive oil extract. A J&W DB-35ms Ultra Inert (UI) 30 m × 0.25 mm, 0.25 µm column resolved the pesticides of interest in less than 16 minutes, yielding excellent peak shape for even the more problematic OP pesticides. The detection limits for most of the pesticides were 10–15 ng/mL. A simplified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method provided sufficient sample matrix clean-up while preserving low-level analyte detection. A capillary flow technology (CFT) device was installed post-column to split the effluent between the MSD and FPD and implement an automated backflush to diminish residual sample carryover and reduce instrument cycle times.

INTRODUCTION

The health benefits of a Mediterranean diet, and of olive oil in particular, are widely acknowledged (1, 2). However, as 4 kg of olives are needed to produce 1 kg of olive oil, residual pesticides can be concentrated in the final product and must be monitored to ensure toxic residues do not exceed safe levels (3). Many common insecticides used in olive tree pest protection belong to the organophosphorous (OP) class, and human toxicities for OP pesticides have shown acute as well as chronic effects from pesticide poisoning (4). OP pesticides present a challenge for analysis as they are chromatographically active compounds that can adsorb onto active sites in the sample flow path, particularly at trace levels, compromising the analytes' response.

Here, we report a sample preparation extraction to detect 16 different pesticides in olive oil samples, using a procedure based on the evaluation of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach for the analysis of pesticide residues in the high-lipid olive oil matrix (5). This approach simplifies the traditional, labor-intensive extraction and clean-up procedure, while providing just enough sample matrix clean-up for pesticide residues analysis. A gas chromatographic system capable of multisignal detection can provide complementary data for identification, confirmation, and quantitation of target analytes from a single injection. This method enables simultaneous detection of OP pesticides by gas chromatography with electron ionization mass spectrometry in selective ion monitoring mode (GC/MS-SIM) and flame photometric detection (FPD) in phosphorus mode by splitting the column effluent 1:1 between the mass selective detector (MSD) and FPD. The approach chosen here uses a GC/MSD/FPD system to identify and confirm the order of elution for peaks of interest. The GC/MS system was also equipped with backflush capability. This capability enables faster instrument cycle time by backflushing late-eluting matrix components back through the inlet purge valve.

An analyte protectant (AP) was included in the study methodology to help minimize the errors caused by matrix-induced signal enhancements–L-glulonic acid γ-lactone (gulonolactone), was chosen based on the results of a previous study examining APs (6).

EXPERIMENTAL

An Agilent 7890 GC/5975C MSD equipped with an FPD and 7683B autosampler was used for this series of experiments. A purged two-way capillary flow technology (CFT) device was used to split the effluent 1:1 to the MSD:FPD. The CFT device also enabled post-column backflush. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions

GC/MSD/FPD:	Agilent 7890/5975C
Sampler:	Agilent 7683B, 5.0 µl syringe
CFT device:	Purged 2-way splitter Split ratio 1:1 MSD:FPD
MSD restrictor:	1.43 m x 0.18 mm id deactivated fused silica tubing
FPD restrictor:	0.53 m x 0.18 mm id deactivated fused silica tubing
Aux EPC:	3.8 psi constant pressure
Column:	DB-35ms UI 30 m 0.25 mm 0.25 µm
Carrier:	Helium, constant pressure 28.85 psi at 95 °C
Inlet:	1 µL, Splitless, 250 °C Purge flow 60 mL/min at 0.15 min Gas saver 20 mL/min at 2 min
Oven:	95 °C (0.5 min) to 210 °C (25 °C/min), 10 °C/min to 250 °C (0.5 min), 20 °C/min to 290 °C, hold 4.5 min
Postrun backflush:	7.5 min @ 290 °C Aux EPC pressure 54 psi during backflush inlet pressure 2 psi during backflush
MSD:	300 °C transfer line, 300 °C source, 150 °C quad
FPD:	230 °C Hydrogen 75 mL/min, Air 100 mL/min Carrier + makeup (N_2) 60 mL/min

Table 2. Flow Path Supplies

Vials:	Amber crimp top glass vials (p/n 5183-4496)
Vial Caps:	Crimp caps (p/n 5181-1210)
Vial inserts:	250 µL glass/polymer feet (p/n 5181-8872)
Syringe:	5 µL (p/n 5181-1273)
Septum:	Advanced Green (p/n 5183-4759)
Inlet liner:	Ultra Inert single taper splitless liner with wool (p/n 5190-2293)
Ferrules:	0.4 mm id short; 85/15 vespel/graphite (p/n 5181-3323)
PCT fittings:	Internal nut (p/n G2855-20530)
PCT ferrules:	SilTite ferrules, 0.25 mm id (p/n 5188-5361)
20x magnifier:	20x Magnifier loop (p/n 430-1020)

REAGENTS AND CHEMICALS

All reagents and solvents were HPLC or Ultra Resi grade. Acetonitrile (ACN) from Honeywell (Muskegon, MI, USA), toluene from Burdick & Jackson, and acetone from JT Baker were purchased through VWR International (West Chester, PA, USA). The neat pesticide standards were purchased from Chem Service, Inc. (West Chester, PA, USA), gulonolactone from Aldrich (St. Louis, MO), and triphenyl phosphate from Alfa Aesar (Ward Hill, MA).

SOLUTIONS AND STANDARDS

The 16 target OP pesticides tested for detection were:

- Acephate
- Azinphos-ethyl
- Azinphos-methyl
- Carbophenthion
- Chlorpyrifos
- Diazinon
- Dimethoate
- Fenitrothion
- Fenthion
- Malathion
- Methamidophos
- Methidathion
- Omethoate
- Parathion
- Parathion-methyl
- Pirimiphos-methyl

Triphenyl-phosphate (TPP) was used as a surrogate standard.

1 µg/mL and 5 µg/mL spiking solutions were prepared of each of the test pesticides. TPP was prepared at concentrations of 1, 15, and 100 µg/mL in toluene. An analyte protectant solution was prepared by dissolving the neat gulonolactone in a minimum amount of water and appropriate amount of ACN to yield a 50 mg/mL concentration. The appropriate amount of gulonolactone solution was added to the calibration standards to yield a 0.5 mg/mL concentration in each standard.

QuEChERS Sample Preparation Workflow

Weigh 3 g sample (± 0.05 g) 50 mL centrifuge tube

↓

Add surrogate standard and QC spike solution if necessary, vortex 1 min

↓

Add 7 mL of of cold DI water and 2 ceramic bars (p/n 5982-9313), vortex 1 min

↓

Add 10 mL of ACN, vortex 1 min

↓

Add Agilent original QuEChERS extraction salt packet for 10 g samples (p/n 5982-5550)

↓

Shake vigorously for 1 min on a mechanical shaker at 1500 rpm

↓

Centrifuge at 4000 rpm for 5 min

↓

Transfer 8 mL of upper ACN layer to Agilent AOAC dispersive SPE 15 mL tube for fatty samples (p/n 5982-5158)

↓

Vortex 1 min, centrifuge at 4000 rpm for 5 min

↓

Transfer upper ACN layer to Agilent AOAC dispersive SPE 15 mL tube for fatty samples (Agilent p/n 5982-5158)

↓

Vortex 1 min, centrifuge at 4000 rpm for 5 min

↓

Transfer extract to vial and add 0.5 mg/mL analyte protectant

↓

Analyze extract by GC/MS/FPD

Figure 1. Flow chart for the QuEChERS sample preparation procedure for pesticides in olive oil.

SAMPLE PREPARATION

A sample of extra virgin olive oil was purchased from a local grocery store. The sample extraction method used a modified QuEChERS approach, as illustrated in Figure 1. Once the samples were prepared in this way, the extract was analyzed by GC/MS/FPD using the chromatographic conditions in Table 1. Extractions of water and acetonitrile aliquots were prepared in the same manner as the samples and served as reagent blanks.

RESULTS AND DISCUSION

The 16 targeted OP pesticides were resolved on the Agilent J&W DB-35ms UI 30 m × 0.25 mm, 0.25 µm analysis column in less than 16 minutes.

The pesticide matrix-matched standard in the Figure 2 chromatogram exhibits good separation and peak shape for all of the pesticides.

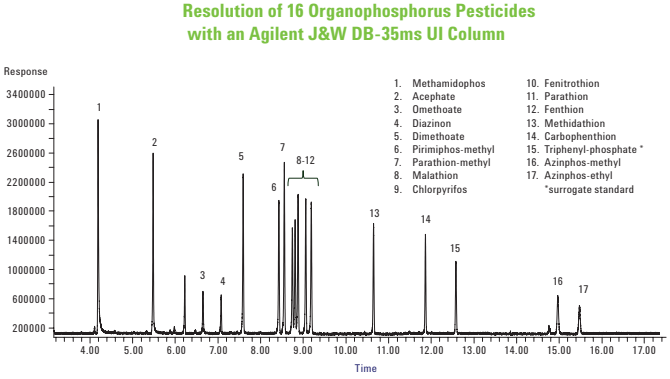

Figure 2. GC/FPD chromatogram of a 100 ng/mL matrix-matched OP pesticide standard with analyte protectant analyzed on an J&W DB-35ms UI 30 m × 0.25 mm, 0.25 µm capillary GC column. Chromatographic conditions are listed in Table 1.

Chromatography of OP pesticides can be problematic, especially for polar pesticides, often yielding broad peak shapes or excessive tailing, making reliable quantitation at low levels difficult. The high level of inertness of the DB-35ms UI results in better peak shape and decreased sample adsorption on active sites within the column, enabling lower detection limits. Figure 3 depicts the excellent peak shape at 15 ppb for the four polar OP pesticides with the DB-35ms UI column.

The analyte protectant used in this analysis, gulonolactone, effectively reduced matrix-related effects and improved the analyte response. Since FPD in phosphorus mode is selective only to analytes containing phosphorus, it is able to detect low levels of OP pesticides in complex matrices such as olive oil with minimal matrix interferences. Excellent signal-to-noise ratios were seen at trace levels, indicating a high level of sensitivity.

The FPD was able to detect OP pesticides down to 10 ng/mL with the exception of omethoate, diazinon, azinphos-methyl, and azinphos-ethyl, which were detected at a slightly higher limit of detection of 15 ng/mL. The detection levels for the targeted OP pesticides were within the maximum residue levels (MRLs) range of 0.01–2 mg/kg established by the US, EU, and Codex Alimentarius for pesticide residues in olives (7-9).

Sample preparation using the QuEChERS approach was effective in retaining the OP pesticides in the spiked oil sample and providing sufficient clean-up of the sample matrix for GC analysis. Figure 4 shows an olive oil sample which was fortified with the OP pesticide mix and prepared using QuEChERS. A blank matrix trace is shown below the analyte trace to indicate the level of potential matrix interference with the analytes of interest. Peak shapes for the organophosphorus pesticides are still quite sharp and well-resolved, indicating excellent performance on the DB-35ms UI column in an olive oil

matrix. The performance of the DB-35ms UI column yielded excellent linearity over the calibration range of this study. The linearity of the column as defined by the R2 values of the calibration standard curve was ≥ 0.999 for all the pesticides studied.

Recoveries were determined by GC/FPD at the 20, 100, and 500 ng/mL levels. The recoveries of the pesticides were greater than 70 percent with RSDs below 10 percent except in the case of acephate, which was slightly lower with an average recovery of 66 percent.

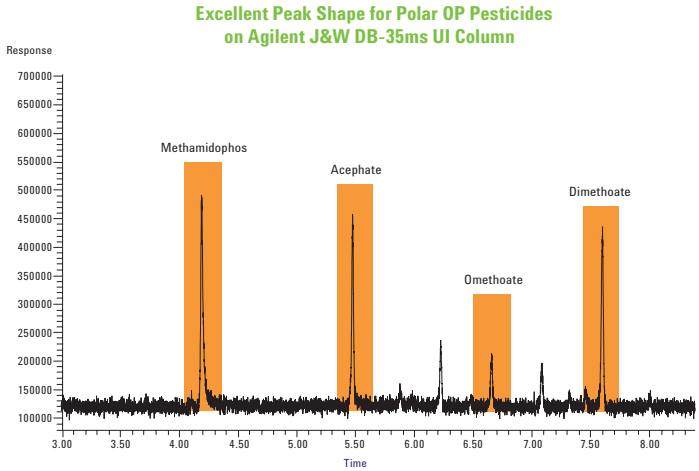

Figure 3. Enlarged section of the GC/FPD chromatogram of a 15 ng/mL matrix-matched pesticide standard with analyte protectant analyzed on an J&W DB-35ms UI capillary column. Chromatographic conditions are listed in Table 1.

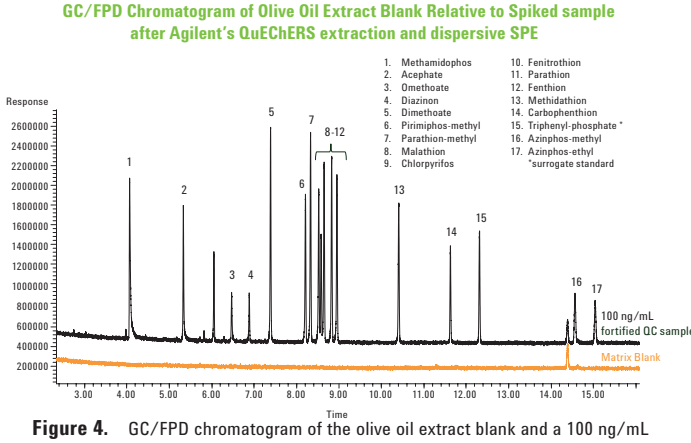

Figure 4. GC/FPD chromatogram of the olive oil extract blank and a 100 ng/mL fortified olive oil extract both with analyte protectant analyzed on an J&W DB-35ms UI capillary column. Chromatographic conditions are listed in Table 1.

CONCLUSIONS

The Agilent J&W DB-35ms UI capillary column resolved the targeted OP pesticides and provided excellent peak shapes for the polar pesticides, allowing for more reliable quantitation at low levels. Detection levels for the OP pesticides in olive oil were at or below the EU, Codex, and US maximum residue levels for olives. Matrix-matched calibration standards yielded regression coefficients R2 ≥ 0.999 and recoveries of fortification studies were 63 percent to 107 percent with an average RSD < 9 percent, further demonstrating the effectiveness of using the J&W DB-35ms UI columns for residual pesticide determination.

By splitting the column effluent between the MSD and FPD, selectivity, identification, and confirmation of OP pesticides from a single injection are achieved, thereby increasing laboratory productivity. GC/MS-SIM provides selectivity and confirmation, while further specificity and quantitation is achieved by FPD in phosphorus mode. The QuEChERS approach was successful at providing just enough sample clean-up to minimize matrix interferences while still maintaining low-level analyte detection. The simple QuEChERS extraction method allows for faster sample prep facilitating higher sample throughput. Residual sample matrix carryover is removed through use of backflush, which eliminates the need for a bakeout cycle, significantly reducing analytical run times.

This trial successfully demonstrates a quick and efficient analytical method to monitor low- and trace-level OP pesticide residues in olive oil samples.

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Cost-Effective Analysis of Major, Minor, and Trace Elements in Foodstuffs Using the 4100 MP-AES

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INTRODUCTION

Whether the goal is food safety, ensuring quality or establishing provenance, measuring the trace element content of foods and beverages that we all consume is of paramount importance. While some elements are essential for our well-being at low concentrations, others like lead and chromium are highly toxic and more still are being linked to viral, neurological and other diseases. Food scares related to contamination or poor quality not only constitute a health risk, they also undermine consumer confidence. This can lead to lost earnings through reduced sales and loss of credibility through adverse publicity.

Atomic spectroscopy is well-established for the analysis of metals in foods. The technique employed often depends on the requirements of the application in terms of elements of interest, expected concentrations, and number and type of samples. Other important procurement factors that influence instrument selection include purchase and operational budget for consumables, gases, power and labor, as well as service and maintenance costs.

With lab budgets coming under increasing pressure, Agilent has expanded its atomic spectroscopy portfolio to include the 4100 Microwave Plasma-Atomic Emission Spectrometer (MP-AES). MP-AES is a new analytical technique that uses a microwave-induced nitrogen plasma to provide elemental analysis, with significantly reduced running costs through the use of nitrogen as its plasma gas.

EXPERIMENTAL

This work describes the analysis of various certified and standard reference materials per the sample descriptions below:

- NIES CRM No.7 Tea Leaves: from National Institute of Environmental Studies (NIES), Japan.
- NIES CRM No.10c Rice Flour: from National Institute of Environmental Studies (NIES), Japan.
- NIST SRM 1577 Bovine Liver: from National Institute of Standards and Testing, USA.
- CRM-Wheat Flour: from High Purity Standards, USA
- CRM-Milk Powder: from High Purity Standards, USA
- CRM-Oyster Tissue: from High Purity Standards, USA

SAMPLE PREPARATION

A simple acid digestion method was used to prepare three of the samples. Initially, 0.25 g of the tea leaves CRM, 0.5 g of bovine liver SRM, and 1 g of rice flour CRM were weighed into separate 250 mL beakers. This was then followed by the addition of 10 mL of HNO_3 and each beaker was covered with a watch glass. The samples were heated on a hot plate until completely dissolved. After cooling to room temperature, each digest was transferred to a 100 mL volumetric flask and made up to the required volume by adding Milli-Q water.

Pre-prepared sample solutions of CRM-Wheat Flour, CRM-Milk Powder, and CRM-Oyster Tissue in 4% HNO_3 were purchased from High Purity Standards, USA.

Working standards and a blank were matrix-matched with the samples.

INSTRUMENTATION

The innovative 4100 MP-AES with its proprietary Microwave Excitation Assembly is a sequential atomic emission spectroscopic technique capable of fast, unattended multi-element analysis at varying concentration levels using a nitrogen plasma. The unique Microwave Excitation Assembly focuses and contains the microwave energy that is created via a concentrated axial magnetic field around the torch. This creates a robust toroidal plasma that allows the stable introduction of liquid samples. With a central channel temperature of ~5,000 K, MP-AES is highly suited to spectroscopic analysis, as it creates high intensity atomization emission lines. In addition to simplified spectra, nitrogen-MP-AES offers reduced operating costs and increased lab safety compared to flame AA, through the avoidance of costly and highly flammable gases such as acetylene.

The analysis was carried out using an Agilent 4100 MP-AES equipped with a standard MP-AES torch, concentric nebulizer, and glass cyclonic spray chamber.

Operating parameters are shown in Table 1.

Table 1. Agilent 4100 MP-AES operating parameters

Instrument parameter	Setting
Nebulizer pressure	160–180 kPa
Read time	3 s (10 s for MDL)
Number of replicates	3 (10 for MDL)
Stabilization time	15 s
Background correction	Auto

METHOD DETECTION LIMITS

The Method Detection Limits were determined from the analysis of digested blank samples. The selected analytical wavelengths and method detection limits (3σ) are listed in Table 2.

Table 2. Agilent 4100 MP-AES element wavelength and method detection limits (ppb)

Element	Wavelength (nm)	MDL (ppb)
Al	396.152	0.5
Ba	455.403	0.02
Ca	445.478	14
Cd	228.802	1.2
Co	340.511	4
Cr	425.433	0.5
Cu	327.396	0.4
Fe	371.993	3
K	769.897	3
K	404.414	280
P	213.618	100
Pb	405.781	5
Pb	368.343	12
Mg	518.361	4
Mn	403.076	0.5
Mo	379.825	1.5
Na	589.592	3
Na	568.821	140
Ni	341.476	2
Ni	352.453	2
Sr	407.771	0.01
Zn	213.857	4

ANALYSIS OF FOODSTUFFS

Results of the analysis of major, minor and trace extractable elements in six different foodstuffs are listed in Tables 3 to 8. The measured values (carried out in triplicate) are in good agreement with the certified values for all CRM and SRM samples.

Table 3. Results of NIES No.7 Tea Leaves

Element	Measured values	Certified values
	wt%	**wt%**
Ca	0.314 ± 0.013	0.320 ± 0.012
Mg	0.150 ± 0.004	0.153 ± 0.006
K	1.861 ± 0.074	1.86 ± 0.07
	mg/kg	**mg/kg**
Ba	5.76 ± 0.57	5.7*
Cd	nd	0.03 ± 0.03
Co	nd	0.12*
Cr	nd	0.15*
Cu	7.13 ± 0.81	7 ± 0.3
Pb	nd	0.8 ± 0.03
Ni	6.03 ± 0.63	6.5 ± 0.3
Sr	3.63 ± 0.43	3.7*
Zn	34 ± 3	33 ± 3

* Reference values only

Table 4. Results of NIES No.10c Rice Flour

Element	Measured values	Certified values
	wt%	**wt%**
Mg	0.127 ± 0.006	0.125 ± 0.008
K	0.279 ± 0.012	0.275 ± 0.010
P	0.300 ± 0.010	0.335 ± 0.008
	mg/kg	**mg/kg**
Al	1.49 ± 0.13	1.5*
Ca	95.4 ± 7.0	95 ± 2
Cd	1.83 ± 0.14	1.82 ± 0.06
Co	nd	0.007*
Cr	nd	0.08*
Cu	4.03 ± 0.32	4.1 ± 0.3
Fe	10..6 ± 0.15	11.4 ± 0.8
Mo	nd	1.6 ± 0.1
Ni	nd	0.30 ± 0.03
Sr	0.2	0.2*
Zn	21.8 ± 1.0	23.1 ± 0.8

* Reference values only

Table 5. Results of NIST 1577 Bovine Liver

Element	Measured values	Certified values
	wt%	**wt%**
Na	0.247 ± 0.006	0.243 ± 0.013
K	1.00 ± 0.08	0.97 ± 0.06
	mg/kg	**mg/kg**
Ca	131	123*
Cd	nd	0.27 ± 0.04
Co	nd	0.18*
Cu	185 ± 6	193 ± 10
Fe	266 ± 5	270 ± 20
Pb	nd	0.34 ± 0.08
Mg	625 ± 45	605*
Mn	10.4 ± 1.41	10.3 ± 1
Mo	nd	3.2*
Sr	0.15 ± 0.07	0.14*
Zn	125 ± 4	130 ± 10

* Reference values only

Table 6. Results of CRM-Wheat Flour

Element	Measured values	Certified values
	(mg/kg)	**(mg/kg)**
Al	0.83 ± 0.02	0.85 ± 0.01
Ca	9.64 ± 0.97	9.5 ± 0.1
Cd	nd	0.0015*
Co	nd	0.001*
Cr	0.013 ± 0.001	0.014*
Cu	0.09 ± 0.008	0.1 ± 0.002
Fe	0.81 ± 0.04	0.90 ± 0.01
K	62.5 ± 0.5	65 ± 0.7
P	61.1 ± 1.7	65 ± 0.7
Pb	0.05 ± 0.001	0.050 ± 0.003
Mg	20.8 ± 0.1	20.0 ± 0.2
Mn	0.36 ± 0.02	0.4 ± 0.008
Ni	nd	0.009 ± 0.001
Zn	0.47 ± 0.05	0.50 ± 0.01

* Reference values only

Table 7. Results of CRM-Milk Powder

Element	Measured values (mg/kg)	Certified values (mg/kg)
Al	nd	0.020 ± 0.002
Ca	131 ± 9	130 ± 1
Co	nd	0.0004*
Cr	nd	0.0003*
Cu	0.006 ± 0.001	0.007 ± 0.001
Fe	0.018 ± 0.002	0.020 ± 0.001
K	178 ± 6	170 ± 2
P	98.7 ± 1.3	100 ± 1
Pb	nd	0.002*
Mg	11.9 ± 0.2	12 ± 0.1
Mn	0.003 ± 0.002	0.003*
Na	48.7 ± 2.6	50 ± 1
Zn	0.48 ± 0.05	0.50 ± 0.01

* Reference values only

Table 8. Results of CRM-Oyster Tissue

Element	Measured values (mg/kg)	Certified values (mg/kg)
Al	2.92 ± 0.07	3*
Ca	15.0 ± 0.49	15*
Cd	nd	0.03*
Co	nd	0.004*
Cr	nd	0.007*
Cu	0.56 ± 0.05	0.6*
K	100 ± 0.96	100*
P	79.1 ± 0.9	80*
Pb	nd	0.005*
Mg	12.1 ± 0.2	12*
Mn	0.18 ± 0.01	0.2*
Na	48.9 ± 0.8	50*
Ni	nd	0.01*
Zn	8.3 ± 0.4	9*

* Reference values only

CONCLUSIONS

MP-AES offers any food testing facilities dependent on acetylene-based instrumentation a real alternative in terms of sensitivity, multi-element capability, and speed of analysis, while cutting operating costs and improving the safety of the lab environment through the use of non-flammable nitrogen.

This study shows that following a quick and simple acid digestion sample preparation procedure (required for three of the six diverse food samples), all six certified and standard reference materials can be analyzed for trace and major element concentrations with good accuracy by MP-AES. The addition of the Agilent 4107 Nitrogen Generator is also possible in order to perform this analysis with significantly lower gas costs or for analysis in remote locations where sourcing of gases is costly or difficult.

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Determination of Fatty Acid Methyl Esters (FAMEs) in Salmon Oil Using Automated Sample Preparation

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INTRODUCTION

The automated derivatization of fatty acids (FAs) was performed with the Agilent 7696A Sample Prep WorkBench. Since free fatty acids show tailing in gas chromatography, transformation of fatty acids into fatty acid methyl esters (FAMEs) is widely used. Manual sample derivatization is time-consuming and may lead to poor repeatibility. Automated derivatization shows significant enhancement of reproducibility and saves time. Especially for highly unsaturated fatty acids, slight variations in reaction temperature and time can negatively affect repeatability when using manual procedures.

Salmon oil is an excellent source of polyunsaturated omega-3 fatty acids. The two main fatty acids—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—have been identified as important health factors and are correlated with a normal function of the heart. The concentration of EPA and DHA is the crucial quality factor for salmon oil capsules. This application note demonstrates the use of the Agilent 7696A Sample Prep WorkBench for derivatization and subsequent determination of both EPA and DHA from salmon oil capsules.

MATERIALS AND METHODS

For sample preparation, 10 mg of salmon oil was weighed into a 2-mL autosampler vial. The sample was diluted in 500 µL of tert-butyl methyl ether (TBME), using the liquid-dispensing module of the Agilent 7696A Sample Prep WorkBench and mixed for 90 seconds with the onboard vortex mixer. A 250-µL aliquot of the prepared sample was transferred to an empty vial and 125 µL of a Trimethylsulfoniumhydroxide (TMSH) derivatization solution [MachereyNagel, Düren] was added and the mixture was again mixed using the vortex mixer of the WorkBench. The mixture was heated for 5 minutes at 80 °C in the single vial heater. The flow diagram for the automated procedure on the Agilent 7696A Sample Prep WorkBench is in shown in Figure 1.

The gas chromatographic conditions were chosen as shown in Table 1.

Table 1. GC/FID Conditions

Peak identification	
C14:0	Myristic acid
C16:0	Palmitic acid
C16:1	Palmitoleic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C20:0	Arachidic acid
C18:3	γ-Linolenic acid
C20:1	Gadoleic acid
C18:3	Linolenic acid
C22:1	Erucic acid
C20:4	Arachidonic acid
C20:5	Eicosapentaenoic acid
C24:1	Nervonic acid
C22:6	Docosahexaenoic acid

GC conditions

Instrument	Agilent 6890 Series GC
Column	HP 88, 100 m × 250 µm, 0.20 µm
Injection volume	2 µL
Injector	Split/Splitless, Split 50:1
Carrier gas	H_2
Temperature-program	70 °C–260 °C
Flow	1.4 mL/min
Detector	250 °C, FID
	H_2 flow: 40 mL/min
	Air flow: 450 mL/min
	Makeup flow, N_2: 45 mL/min

AGILENT WORKBENCH PROGRAM

Figure 1. Flow diagram of FAME sample preparation with the Agilent 7696A Sample Prep WorkBench

RESULTS AND DISCUSSION

Figure 2 shows the separation of FAMEs from salmon oil on an Agilent 7696A WorkBench. The separation allows the unequivocal identification of all FAMEs. The two compounds of main interest show retention times of 35.07 minutes (EPA) and 40.55 minutes (DHA). Besides EPA (23.7%) and DHA (20.0%), salmon oil further consists of unsaturated fatty acids oleic (12%), linoic (11%) and palmitoleic (8%) acid. The content of saturated fatty acids, palmitic and stearic acid, is low, 4% and 5%, respectively.

For the repeatability test, 10 individual salmon oil samples were derivatized and analyzed to determinate the reproducibility of the automatic sample preparation and chromatography. As shown in Figure 3, excellent repeatability was obtained. The absolute areas of the EPA and DHA signals showed standard deviations of less than 1% (EPA 0.51%, DHA 0.78%). Moreover, variations of the EPA and DHA relative concentrations were stable. Relative standard deviations of 0.85% for EPA and 1.22% for DHA were achieved. No outliers were observed over the 10 samples.

The total runtime for sample preparation on the Agilent 7696A Sample Prep WorkBench was only 20 minutes per sample, whereas the time for the manual derivatization depends on the skills of the laboratory technician and can take up to 2 hours.

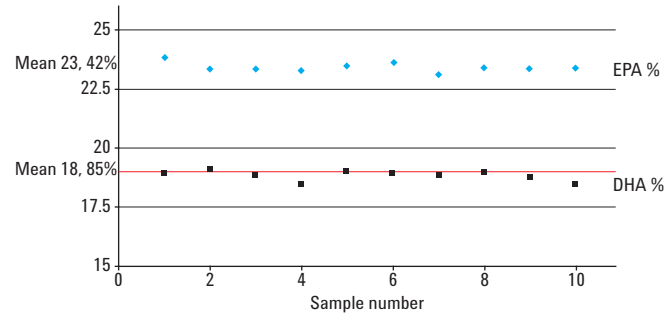

Figure 3. Repeatability data of of automated FAME determination in salmon oil

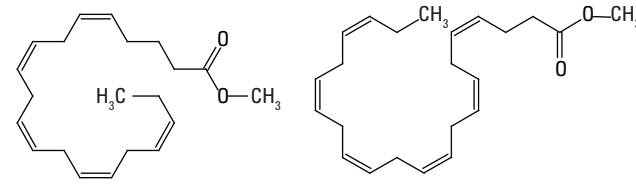

Figure 4. Structure of EPA methyl ester (left) and DHA methyl ester (right)

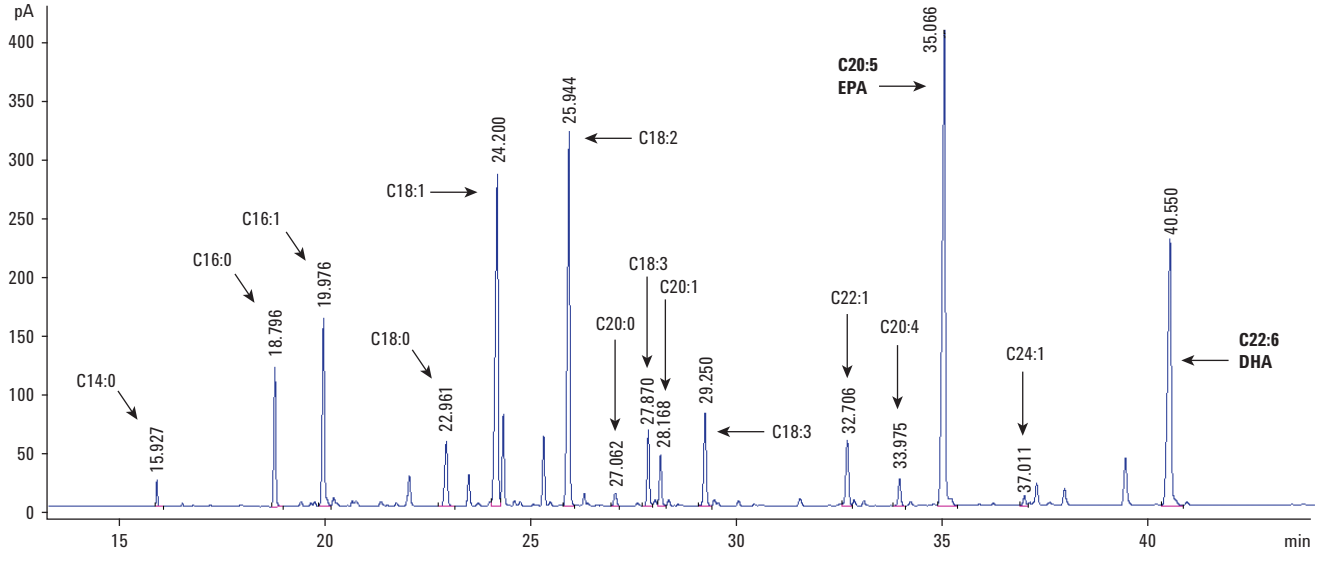

Figure 2. GC/FID chromatogram of a salmon oil sample, prepared using Agilent WorkBench 7696A

Table 2. Percent distribution of fatty acids in salmon oil sample

C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C18:3	C22:1	C20:4	C20:5	C24:1	C22:6
0.71	4.68	7.95	3.54	12.95	13.86	0.36	2.61	1.71	3.33	3.35	0.87	23.79	0.36	19.93

CONCLUSION

The automated sample derivatization is easy, fast, and reliable. For samples with high relative concentrations of polyunsaturated fatty acids especially, the automation is significantly more reliable than manual procedures.

REFERENCE

(1) Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH) (ISO 12966-3:2009)

www.agilent.com/chem

Published in USA

Method for the Determination of Chemical Contaminants in Marine Shellfish

Agilent Technologies, Inc.

INTRODUCTION

Chemical contaminants that are released into the marine environment may be ingested (absorbed) by fish and shellfish and thus become introduced into the human food chain. Lipophillic chemicals such as OCPs and PCBs can bioaccumulate in the fatty tissues of marine fish and shellfish. The longer an organism is exposed to a contaminated environment, the higher the likely levels of contaminants.

The Clean Seas Environmental Monitoring Program (CSEMP) is an initiative designed to monitor the levels of chemical contamination in the United Kingdom's coastal and estuarine areas. The major drivers for this program are:

- To meet the mandatory monitoring requirements under Oslo and Paris Convention (OSPAR) Joint Assessment and Monitoring Program (JAMP)
- To comply with EC Directives.

Agilent Technologies has partnered with a leading European Analytical Laboratory to develop a sample preparation method based on a modified QuEChERS extraction along with a GC/MS/MS method for the determination of selected OCPs, PAHs, and PCBs in marine shellfish (Mussel) tissue. The GC/MS/MS method provides reproducible and sensitive determination of OCPs, PCBs, and PAHs that employs large-volume (solvent vent) injection using a Multimode inlet (MMI) and post-column, post-run backflush in order to remove high-boiling matrix components that would otherwise remain in the column between analyses and subsequently cause degradation of chromatographic performance and contamination of the mass spectrometer ion source.

The analytical method meets the detection limit requirements of 0.1 µg/Kg for OCPs and PCBs, and 0.5–1.0 µg/Kg for PAHs.

COMPOUNDS

- 16 OCPs
- 19 PAHs
- 7 PCBs

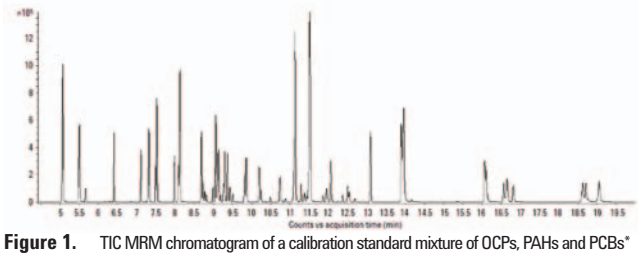

Figure 1. TIC MRM chromatogram of a calibration standard mixture of OCPs, PAHs and PCBs*

KEY BENEFITS

- Sample extraction based on a modified QuEChERS method
- Recoveries for all analytes in the range of 85.4% to 123.9%
- Large-volume (solvent vent mode) injection using a multimode inlet ensuring required detection limits are met
- Retention time locked chromatographic method for ease of set-up and on-going maintenance
- Capillary flow technology for post-column, post-run backflush to ensure chromatographic method robustness and prevent contamination of the MS ion source with high-boiling matrix
- Mass Hunter software that is very powerful yet easy to master, providing excellent data review capabilities and easy, flexible reporting.

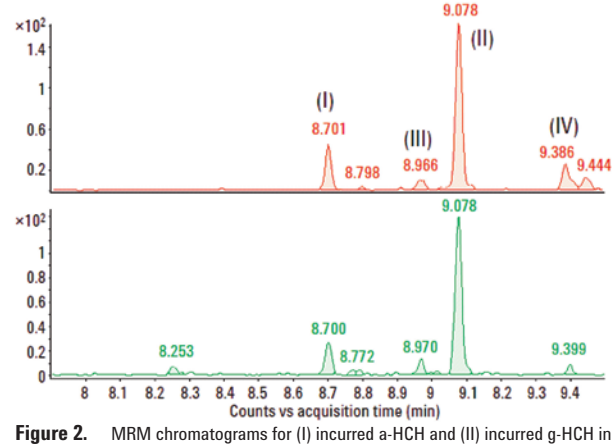

Figure 2. MRM chromatograms for (I) incurred a-HCH and (II) incurred g-HCH in mussel sample, concentrations 0.06 and 0.30 µg/Kg, respectively. Peaks (III) and (IV) are traces of incurred b-HCH and d-HCH, respectively

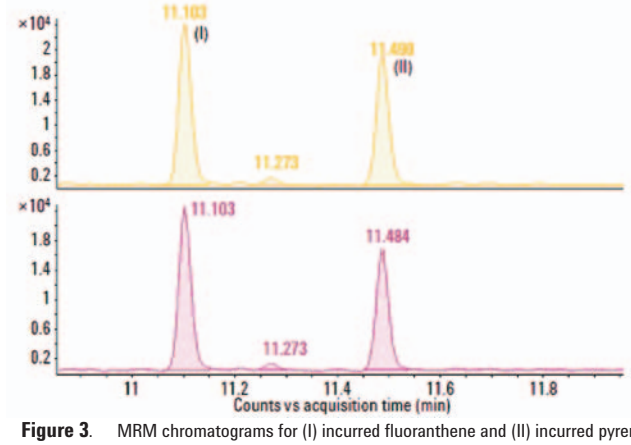

Figure 3. MRM chromatograms for (I) incurred fluoranthene and (II) incurred pyrene in mussel sample, Concentrations 8.64 and 5.83 µg/Kg, respectively

* Full analytical details are available in Agilent Technologies publication 5990-7714EN.

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Published in USA

The Measure of Confidence

Agilent Technologies

Analysis of Medium Volatility Sulfur Compounds in Coffee Using Agilent GC/Q-TOF

Agilent Technologies, Inc.

INTRODUCTION

The resolution, sensitivity, and mass accuracy of the Agilent 7200 GC/Q-TOF system provide rapid, simple, and reliable analysis of trace levels of sulfur compounds in coffee.

Volatile sulfur, containing compounds present in coffee, plays an important role in aroma and flavor. Characterization of desirable coffee aroma can be a challenging task, since many of these compounds are present in trace amounts. Identification and quantitation of sulfur compounds present in complex food matrices at trace levels (low ng/mL in matrix) often requires time-consuming sample preparation as well as elaborate techniques with high separation power, such as 2D GC combined with mass spectrometry. Using the resolution, sensitivity, and speed of an Agilent GC/Q-TOF system, consistent product quality can be monitored with minimal sample preparation and a standard 1D GC method.

For the GC/Q-TOF method, a simple liquid-liquid extraction is adequate sample preparation before the analysis of medium volatility sulfur compounds in coffee. High-resolution mass spectra with low mass error help to resolve compounds of interest from severe matrix interferences. The 7200 GC/Q-TOF provides low pg method detection limits with less than 5 ppm error in mass accuracy. Linearity up to three orders of magnitude in matrix is achieved with a correlation coefficient > 0.995. Standard addition method was successfully applied for determination of 2-formyl thiophene and 2-acetyl thiazole concentrations at ng/mL levels naturally occurring in coffee extract.

In summary, an Agilent 7200 GC/Q-TOF system is able to provide trace-level compound profiling in complex food matrices, without the need for complex and tedious sample preparation and separation methods.

The method was developed by Nobuo Ochiai and Kikuo Sasamoto of Gerstel K.K. and Ryo Ogasawara and Sofia Aronova of Agilent Technologies, Inc.

KEY BENEFITS

- The Agilent 7200 GC/Q-TOF enables a fast and simple method for routine analysis of sulfur compounds in complex food matrices.
- High sensitivity allows qualitative and quantitative analysis of volatile sulfur compounds in coffee down to 1 pg on column.
- The resolution and mass accuracy of the Agilent 7200 Series GC/Q-TOF provide sufficient selectivity for analyte quantitation in complex food matrices.
- Linearity up to three orders of magnitude facilitates quantitation over a large concentration range.

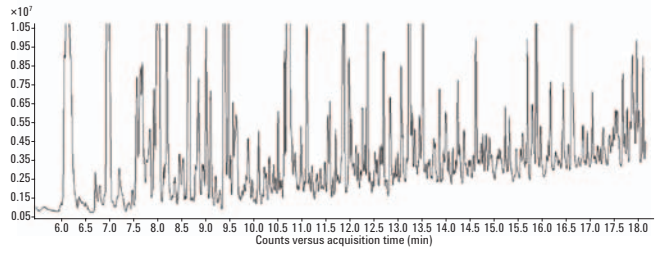

Figure 1. TIC of coffee extract (not spiked with sulfur compound standards), illustrating the complexity ot the matrix

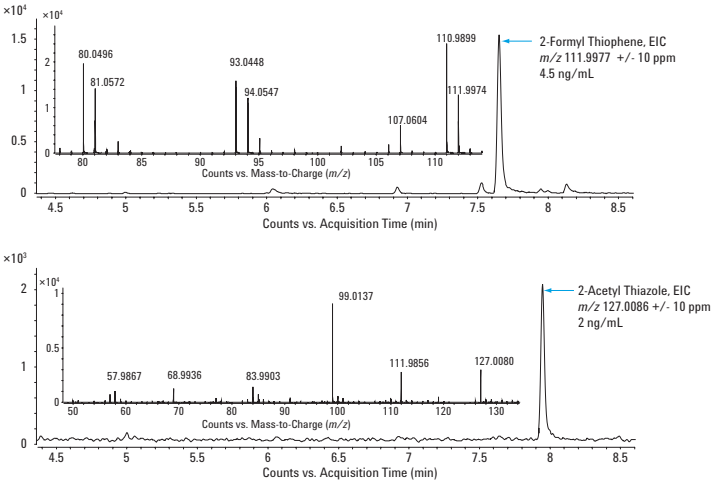

Figure 2. EIC and mass spectra of 2-formyl thiophene and 2-acetyl thiazone at their natural levels extracted from a coffee matrix

Table 1. Mass error for 2-formyl thiophene and 2-acetyl thiazole measured in spiked coffee extract

pg on column	Mass error, ppm 2-formyl thiophene	2-acetyl thiazole
1	-3.57150	-0.78735
2	-4.46438	-0.78735
5	-2.67863	-0.78735
10	-2.67863	0.78735
20	-2.67863	0.00000
50	-0.89288	1.57470
100	0.00000	1.57470
200	-1.78575	1.57470
500	2.67863	-1.57470
1000	1.78575	-1.57470
Average	2.32148	1.10229

www.agilent.com/chem

Published in USA

Simple, Rapid Analysis of Trace Metals in Foods Using the Agilent 7700x ICP-MS

Steve Wilbur Agilent Technologies, Inc. Everett, Washington **Michiko Yamanaka,** Agilent Technologies Tokyo Analytical Division, Tokyo, Japan

INTRODUCTION

The task of efficiently monitoring chemical and biological contaminants in imported and exported food can be overwhelming. Traditionally, analysis of metals in foods has required multiple techniques in order to cover the range of elements, concentrations, and food types. This approach is slow and expensive, so a more rapid, sensitive, and cost-effective screening test is necessary. The Agilent 7700x ICP-MS is capable of accurately analyzing a variety of foods for metals at trace and major levels using a single collision cell method. This method is simple to set up and operate routinely, and permits large numbers of samples to be quickly screened for total toxic metals. Samples that are found to contain metals where the potential toxicity is dependent on the chemical form can then be further analyzed for species composition as needed, using Agilent-supported hyphenated ICP-MS techniques such as LC-ICP-MS or GC-ICP-MS.

EXPERIMENTAL

To test the ability of the Agilent 7700x to analyze a variety of foods for a wide range of metals at highly variable concentrations, several certified reference food samples were analyzed. The 7700x was tuned using One-Click Plasma setting for robust plasma conditions and autotuned for optimum sensitivity, mass response, and minimal interferences. Operating conditions are shown in Table 1. To keep the method as quick and simple as possible, the Octopole Reaction System (ORS^3) was operated in a single mode, using helium (He) cell gas, which provides a reliable and effective cell method to remove all polyatomic interferences, regardless of the analyte or matrix composition. The following acquisition masses and integration times (Table 2) provided more than sufficient sensitivity to meet all certified values. Total run time per sample was less than 3 minutes.

Table 1. 7700 Autotuning Conditions

	Parameter	Value
Set by One-Click Plasma Setting	RF power (W)	1550
	Carrier gas flow (L/min)	0.99
	Spray chamber temp (°C)	2
	Sample depth (mm)	8
	Extract 1 lens (V)	0
Set by Autotune	CeO^+/Ce^+ (%)	1.114
	Ce^{++}/Ce^+ (%)	1.867
	Sensitivity cps/ppb	Li (62700), Y (92920), Tl (87080)

Traditionally, covering this range of concentrations for these elements would have required ICP-OES for the major elements (Na and Ca), graphite furnace AA for Pb and Cd, either a dedicated Hg analyzer or cold vapor AA for Hg and possibly hydride AA for As and Se. The Agilent 7700x ICP-MS running in He mode was able to measure all elements in a single run easily. Even elements such as Be and Hg, which would typically be acquired under no-gas conditions when using ICP-MS, demonstrated excellent sensitivity in He mode (Be DL = 28 ppt, Hg DL = 1.6 ppt).

Table 2. List of analytes and acquisition times (All elements were acquired in He mode)

Mass	Element	Integration time per mass (sec)	Replicates	
6	Li	0.3	3	Internal standard
9	Be	0.99	3	
23	Na	0.3	3	
40	Ca	0.3	3	
43	Ca	0.3	3	
45	Sc	0.3	3	Internal standard
51	V	0.3	3	
52- 53	Cr	0.3	3	
55	Mn	0.3	3	
56	Fe	0.3	3	
60	Ni	0.99	3	
63	Cu	0.3	3	
66	Zn	0.3	3	
72-74	Ge	0.3	3	Internal standard
75	As	0.99	3	
77-78, 82	Se	0.99	3	
95	Mo	0.99	3	
111	Cd	0.3	3	
115	In	0.3	3	Internal standard
121	Sb	0.99	3	
137	Ba	0.3	3	
159	Tb	0.3	3	Internal standard
202	Hg	0.99	3	
205	Tl	0.99	3	
208	Pb	0.3	3	
209	Bi	0.3	3	Internal standard
238	U	0.99	3	

Example calibration curves for several critical and difficult elements are shown in Figure 1.

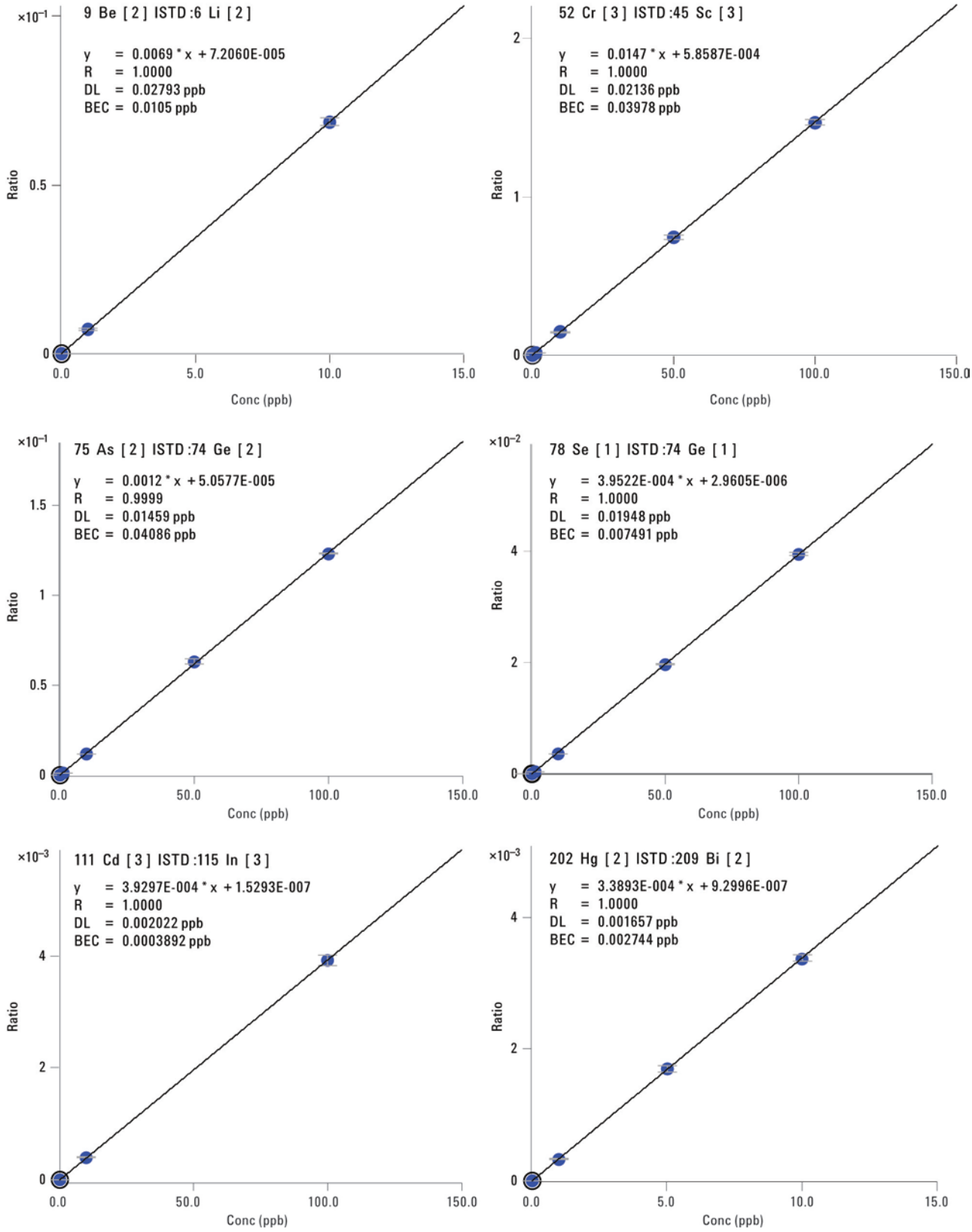

Figure 1. Calibrations for Be, Cr, As, Se, Cd, and Hg.

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Agilent Technologies

The food certified reference materials were analyzed directly after microwave digestion. Between 0.5 g and 1 g of each sample was weighed (after determination of percent moisture) and digested using 6 mL of HNO_3 + 2 mL of H_2O_2 using microwave assisted digestion. All samples were brought to final volume of 100 mL using ultrapure water. Results are shown in Table 3. The trace elements, Ni, Mn, Cu, As, Se, Cd, Hg, and Pb exhibited excellent agreement with the certified values for all three samples. Slight deviations from certified values for Fe, Ca, and Zn were attributed to the digestion procedure rather than the analytical measurement.

CONCLUSIONS

Using a simple procedure based on microwave digestion and single He mode ICP-MS analysis, typical food samples can be quickly and accurately analyzed for trace and major element concentrations without the need for multiple sample preparations and analytical techniques. The Agilent 7700x using He mode alone can provide sensitive, accurate, interference-free analysis of a variety of metals in common foods. Because He mode is both sensitive and universal, it is applicable to trace analysis of all metals in any food sample digest. No prior information about the sample matrix or analyte elements present is required, as He mode removes all polyatomic interferences, regardless of the sample matrix.

Table 3. Measured and certified values for three certified reference food materials. Recoveries are dependent on digestion efficiency as well as analytical accuracy. All measured values are based on dry sample weight corrected for percent moisture. All certified elements are reported for each sample; not all samples are certified for all elements

	NRC-CNRC DORM3 Fish protein		NIST SRM 2976 Mussel tissue		NIST RM 8415 Whole egg powder	
Mass/element	Certified value (mg/kg)	Measured (mg/kg)	Certified value (mg/kg)	Measured (mg/kg)	Certified value (mg/kg)	Measured (mg/kg)
23 Na	–	–	–	–	3770 ± 340	3807
43 Ca	–	–	–	–	2480 ± 190	2703
52 Cr	–	–	–	–	0.37 ± 0.18	0.344
55 Mn	–	–	–	–	1.78 ± 0.38	1.64
56 Fe	347 ± 20	324.0	171 ± 4.9	158.5	–	–
60 Ni	1.28 ± 0.24	1.29	–	–	–	–
63 Cu	15.5 ± 0.63	14.4	4.02 ± 0.33	3.32	2.7 ± 0.35	2.61
66 Zn	51.3 ± 3.1	45.86	137 ± 13	121.2	–	–
75 As	6.88 ± 0.3	6.15	13.3 ± 1.8	12.57	–	–
78 Se	–	–	1.8 ± 0.15	1.87	1.39 ± 0.17	1.25
95 Mo	–	–	–	–	0.247 ± 0.023	0.215
111 Cd	0.29 ± 0.02	0.28	0.82 ± 0.16	0.794	–	–
202 Hg	0.355 ± 0.056	0.359	0.061 ± 0.0036	0.068	–	–
208 Pb	0.395 ± 0.050	0.398	1.19 ± 0.18	1.163	0.061 ± 0.012	0.055

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A Reliable and Routine GC/MS/MS Method for the Determination of Dioxins in Foodstuffs and Animal Feed

Agilent Technologies, Inc.

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are fat-soluble, highly toxic, ubiquitous environmental contaminants found at trace levels in all foodstuffs and animal feed. Current legislation in the European Union (EU) and the United States requires the confirmation of PCDD and PCDF congeners by GC-high resolution mass spectrometry (GC-HRMS). In the event of a food-related dioxin contamination incident, many samples must be analyzed in as short a time as possible in order to determine the extent of the contamination and the subsequent potential risk to human health.

Agilent Technologies has partnered with a leading European dioxin laboratory to develop a method based on GC/MS/MS for the trace analysis of PCDD and PCDF congeners in foodstuffs and animal feed. The method provides sensitive and reproducible results that are comparable to those obtained by GC-HRMS. The GC/MS/MS method meets the requirements of current EU legislation for the screening of PCDD and PCDF congeners in foodstuffs and animal feed, and has potential as an alternative confirmatory methodology for the determination of PCDD and PCDF congeners in official food and feed control, pending analytical quality criteria to be set by legislative bodies.

COMPOUNDS

- As specified in US and EU legislation
- 7 PCDD congeners
- 10 PCDF congeners

KEY BENEFITS

- Retention-time locked method for ease of chromatographic set-up.
- Capillary flow technology provides concurrent backflush for improved method robustness.
- Excellent linearity and response reproducibility for dioxins in foodstuffs and animal feed over the range of interest.
- Reproducible response even at low fg levels on column.
- Detection down to low pg WHO-TEQ/g.
- Chromatographic results that meet legislated screening requirements for EU methods.
- Mass Hunter software that is very powerful yet easy to master, providing excellent data review capabilities and easy, flexible reporting of data.

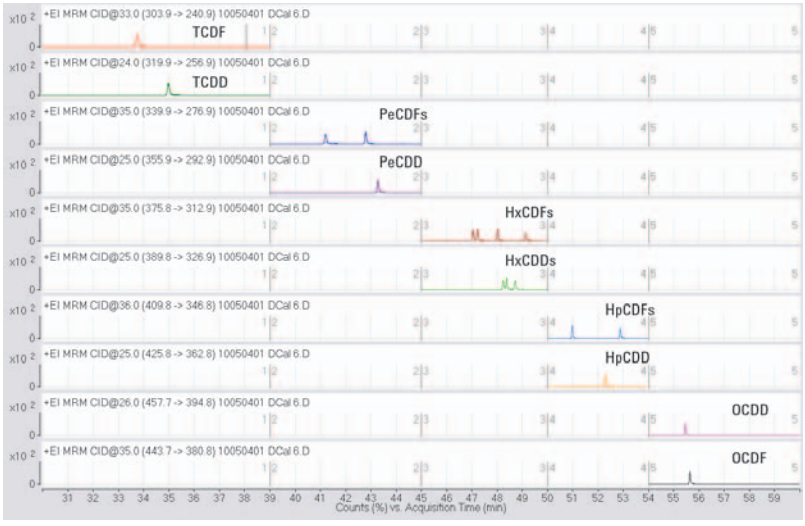
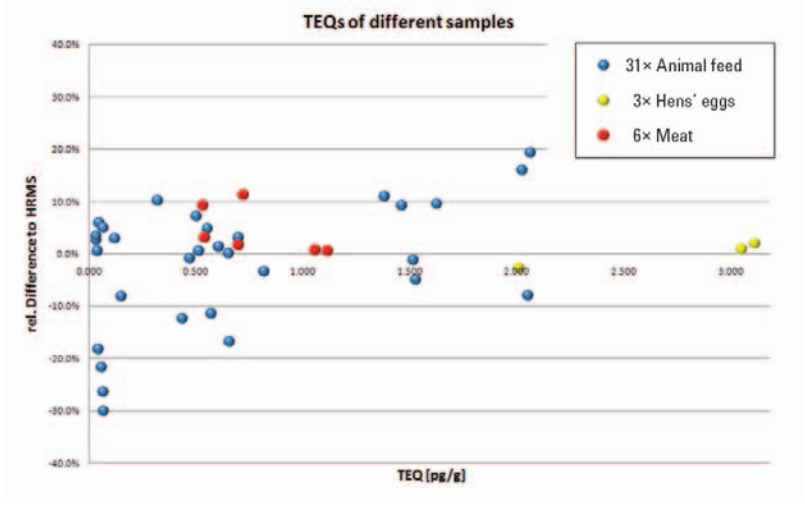


Figure 1. Chromatographic separation of native PCDD and PCDF congeners*

* Full analytical details are available in Agilent Technologies publication 5990-6594EN.

Figure 2. Relative difference in the sum of PCDD/PCDF congener quantitative results (TEQ WHO98



upperbound values) for 40 foodstuff and animal feed samples analyzed by GC-HRMS and GC/MS/MS

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Published in USA

The Measure of Confidence

A Prediction Model for Determining Wine Variety Using the Agilent LC/MS Q-TOF and Agilent

This work was reported in L. Vaclavik, O. Lacina, J. Hajslova, J. Zweigenbaum, Analytica Chimica Acta 2011, 685, 45 (http://www.sciencedirect.com/science/article/B6TF4-1GHWXP-1/2/e70f1f1928475f12c9341d8b67e05310).

INTRODUCTION

Wine is a beverage produced and consumed throughout the world and is a highly valued commodity. Its classification and authenticity can be very important. The constituents of wine are complex and include compounds that impart taste, color, and other characteristics that determine the quality of the beverage. One component is the type of grape used and the question this study examines is whether there are specific compounds in wine that distinguish one grape from another. Using wines obtained from around the world, the power of accurate mass and high resolution is put to use by analyzing three varieties of wine: Pinot Noir, Merlot, and Cabernet Sauvignon. Using Agilent's Mass Profiler Professional software, the resulting single MS data containing over 26000 entities are statistically evaluated. Once filtered on differences, principle component analysis shows that the wine variety can be grouped by specific compounds found in the wine samples. With this knowledge, a model based on partial least squares differentiation is made and unknown wines can be classified. All this is done without knowing the identity of the marker compounds that can distinguish one grape from another.

Using the Agilent 6530 Accutate-Mass Q-TOF LC/MS, MS/MS can be performed on the ions shown to correlate specific grapes, and identification can be pursued with the excellent accurate mass measurements. It is noted that the identification of true unknowns, compounds not found in any database, is a difficult task. However, identification is not necessary for this type of determination and even the unidentified compounds can be used with their MS/MS signatures for routine classification. This study demonstrates the power of the Agilent LC/MS Q-TOF in combination with Mass Profiler Professional's multivariate statistical capabilities designed specifically for MS data processing.

KEY BENEFITS

- Agilent 6500 Series Q-TOF LC/MS provides the sensitivity, mass accuracy and resolution needed to separate unique compounds recognizing a wine's variety.
- Mass Profiler Professional provides the needed multivariate statistics directly on accurate mass spectral data.
- Mass Profiler Professional provides powerful models for prediction analysis.
- Comprehensive workflow wizards guide the experimenter through statistical analysis of their data to obtain powerful correlations and visualization of the results.
- With Q-TOF MS/MS, the identity of marker compounds may be obtained.

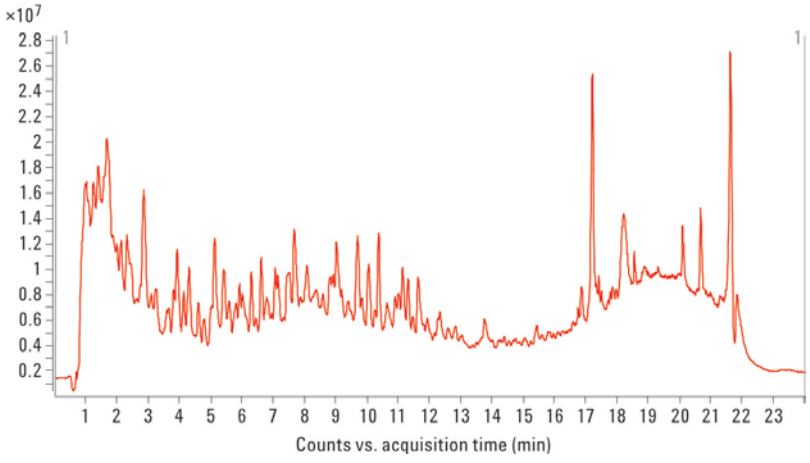

Figure 1. Total Ion Chromatogram on wine sample injected directly shows the complexity of the data

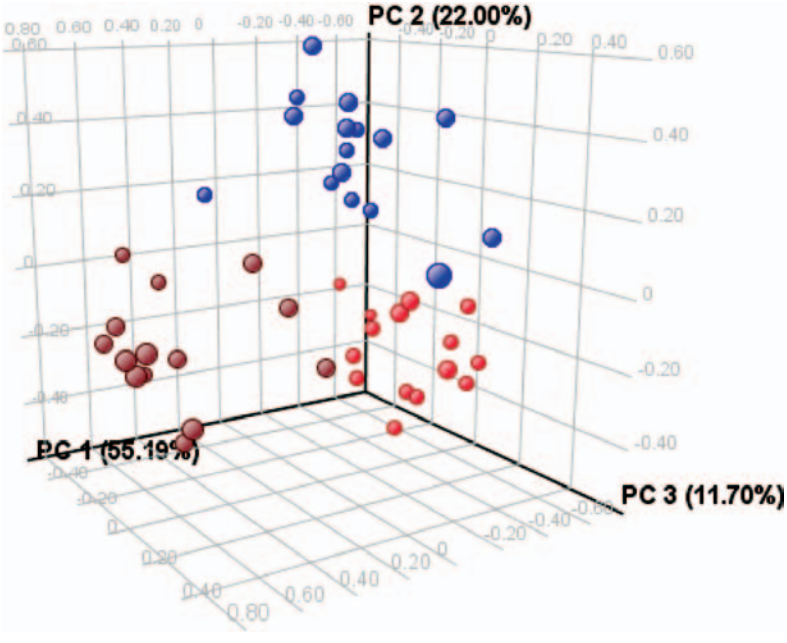

Figure 2. Principal component analysis of filtered results shows that Merlot, Cabernet Savagnoun, and Pinot Noir wines can be distinguished by these marker compounds.

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SIMULTANEOUS ANALYSIS OF HUNDREDS OF PESTICIDES IN FOOD SAMPLES USING THE AGILENT 6400 SERIES TRIPLE QUADRUPOLE LC/MS

The Challenge

More than 1000 pesticides are currently used worldwide in the treatment of soil and crops. Many countries have established allowable levels of those pesticides in food to protect consumers. While these maximum residue levels (MRLs) vary, the default tolerance is 10 parts per billion (ppb). However, the MRL for pesticides in baby food can be as low as 4 ppb.

These pesticides have to be monitored as part of the quality control of food, especially fruits and vegetables, challenging food producers to detect and quantify hundreds of compounds present at minute levels. Since analyzing all of these compounds separately is not feasible, multi-compound methods are required. However, the ability to monitor hundreds of pesticides at once is a challenging problem for chromatography and mass spectrometry.

Meeting the Challenge

A method that can simultaneously detect and quantify hundreds of pesticides requires a liquid chromatography (LC) system that can generate very sharp peaks and rapid separations. It also requires a triple quadrupole mass spectrometer (MS) that can provide excellent selectivity and sensitivity in very complex matrices, along with short dwell times that allow the analysis of peaks only seconds in width.

Rapid and effective development of multi-pesticide methods also requires a reliable and fast sample extraction method, as well as access to a database that contains method parameters for hundreds of compounds.

The Agilent Solution

Development of methods that can screen and quantify hundreds of pesticides can be a daunting task. Agilent provides an effective solution that addresses all of the key requirements for successful method development. The Pesticides Dynamic MRM Database Application Kit includes a database with conditions, transitions and retention times for more than 750 compounds that can be used to generate custom methods. In addition, a pre-configured method for 300 pesticides is included with the kit.

We recommend Bond Elut QuEChERS extraction and dispersive kits for optimal sample prep. A ZORBAX Eclipse Plus UHPLC column provides excellent resolution on short columns for high sensitivity and rapid separations. The 1200 series LC systems utilize the UHPLC columns to provide very fast separations (<20 minutes) and very sharp peaks for the 300 pesticide method that maximize the number of components that can be identified and quantified (Figure 1).

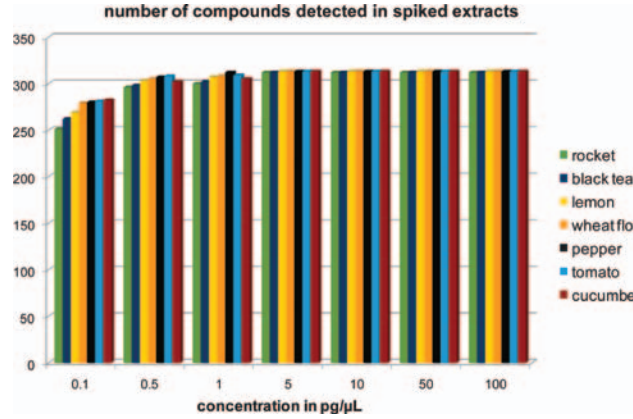

Figure 2. The number of compounds detected in extracts of a variety of fruits and vegetables spiked with the 300 pesticide mix at concentrations from 0.1 to 100 ppb, using the 1290 Infinity UHPLC and the 6490 Triple Quadrupole LC/MS.

The Agilent 6400 series Triple Quadrupole LC/MS systems are also used with the kit to provide fast, multi-analyte quantification. They utilize Dynamic MRM, which monitors analytes only when they are eluting from the LC, to shorten cycle times and maximize the number of compounds detected in a chromatogram without dividing it into segments. The Jet Stream and Ion Funnel technologies also available with the 6400 series provide the sensitivity to detect as low as sub-ppb levels of pesticides in a wide range of fruit and vegetable matrices. (Figure 2).

For more details on the Pesticides Dynamic MRM Database Application Kit, 1200 Infinity Series LC systems, and 6400 Series Triple Quadrupole LC/MS systems for pesticide applications, visit the Agilent Technologies web site at: www.agilent.com.

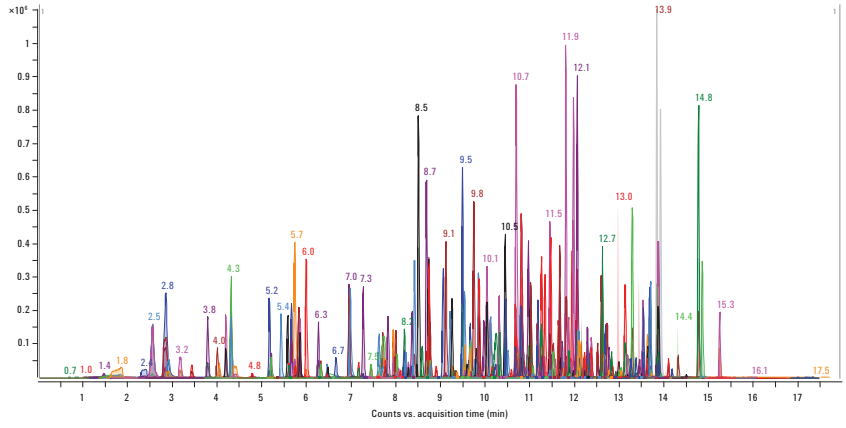

Figure 1. Extracted Ion Chromatogram (EIC) of a 300-compound pesticide mixture using the Agilent 1290 Infinity UHPLC. The retention time is shown above each peak.

Triple Quadrupole LC/MS Analysis of Aflatoxins in Various Food Samples

Agilent Technologies, Inc.

INTRODUCTION

Triple quadrupole LC/MS analysis of aflatoxins in food samples eliminates the need for expensive immuno-affinity cleanup columns and reduces the probability of false positives to almost zero.

Aflatoxins are highly carcinogenic secondary metabolites of the molds *aspergillus flavus* and *aspergillus parasiticus* and are found in grains, corn, peanuts, and other foodstuffs. The action level for these toxic naturally occurring compounds is typically 20 ppb around the world for food and animal feed. Japan has a more aggressive limit of 10 ppb for Aflatoxin B1 and is now changing that to 10 ppb total aflatoxin. This method uses the highly selective and sensitive triple quadrupole LC/MS/MS in Multiple Reaction Monitoring (MRM) mode and can achieve sensitivities in food matrices that are well below these action levels. In addition, the expensive immuno-affinity solid phase extraction (SPE) is compared, in this limited study, to the very inexpensive dispersive solid phase extraction (DSPE) and found to be equivalent.

The method was developed with the Agilent 6460 Triple Quadrupole LC/MS with focusing Agilent Jet Stream Technology and the Agilent 1200 Series LC including the Agilent 1260 Infinity Binary Pump and the 1200 Series High Performance Well Plate Sampler SL Plus. This high-performance method achieves near baseline separation of each of the four compounds in less than 5 minutes. In addition, the method uses three transition ions: one for quantitation and two for qualifiers providing sensitive and accurate quantitation and confirmation by comparing ion ratios and retention times to standards. This eliminates false positives and the need for further analysis to obtain confirmation. The use of stable isotope internal standards added to the samples prior to extraction provides correction for any matrix affects in both extraction and analysis, and assures near zero probability of false negatives.

The Agilent LC/MS/MS provides the necessary limits of reporting for aflatoxins and reduces false positives and negatives to the lowest levels of probability. This provides the assurance needed for a safe food supply. With DSPE for sample preparation, the day-to-day method is cost-effective and efficient.

KEY BENEFITS

- Agilent 6460 Triple Quadrupole LC/MS provides high sensitivity for carcinogenic aflatoxins with limits of detection (LODs) well below action levels in food and feed.
- The highly selective LC/MS/MS requires less expensive sample preparation.
- Quantitation and confirmation are performed in one analysis.
- More samples can be analyzed and reported faster.
- Agilent provides everything needed from dispersive SPE material to columns and supplies.

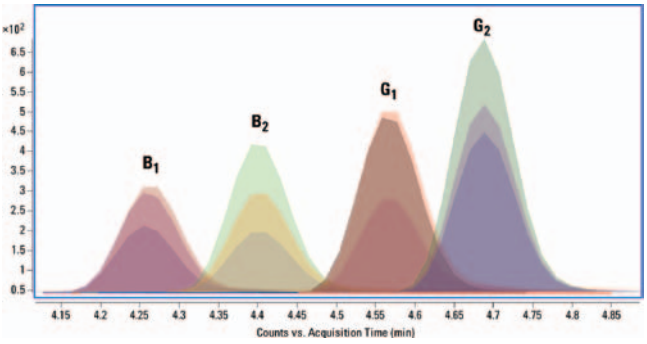

Figure 1. LC/MS/MS chromatogram of aflatoxin B_1, B_2, G_1, and G_2 standards at 1 ppb

Food matrix	B_1 LOD (ng/g)	B_2 LOD (ng/g)	G_1 LOD (ng/g)	G_2 LOD (ng/g)
Corn	0.060	0.085	0.100	0.033
Wheat	0.012	0.037	0.150	0.110
Peanut	0.056	0.069	0.050	0.140
Walnut	0.093	0.098	0.120	0.040
Average	0.055	0.072	0.105	0.080
Mass On-Column	275 fg	360 fg	525 fg	400 fg

Figure 2. LOD results observed for Aflatoxins B_1, B_2, G_1, and G_2 with dispersive SPE sample preparation

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Identification and Quantitation of Pesticides in Chamomile and Ginger Extracts Using an Agilent 6460 Triple Quadrupole LC/MS System with Triggered MRM

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ABSTRACT

This application note describes the use of triggered Multiple Reaction Monitoring (tMRM) for the analysis of pesticide residues applied to chamomile and ginger extracts. The analysis is performed using the Agilent 1290 LC system coupled to a 6460 Triple Quadrupole LC/MS with tMRM acquisition. Two examples of false positive identifications were explored: tebuthiuron in chamomile and tebufenpyrad in ginger. Both compounds were quantitated and confirmed with library matching in a single analytical run. False positive identification was avoided by using library matching and tMRM acquisition.

INTRODUCTION

Modern multiresidue methods for pesticide analysis typically cover hundreds of compounds of different chemical classes. This same method is also typically applied to different matrices. Commonly, this type of analysis is performed using a fast-scanning instrument—usually a triple quadrupole—which is set up to acquire two MRM transitions (one quantitative and one confirmatory transition) for each of the chosen analytes.

In Europe, the analysis of pesticides in food products is based on commission regulation (EC) No. 396/2005[1] and its annexes, which specify the maximum residue limits for pesticides in different products. As of March 11, 2008, there are maximum residue limits (MRLs) defined for more than 170,000 matrix-pesticide combinations by the European Union. Guideline SANCO/10684/2009[2] sets criteria for method validation and quality control procedures for pesticide residue analysis in food and feed. For LC/MS triple quadrupole analysis, the identification criteria include retention time, *m/z* value, and abundance data. In addition, the retention time of the analytes must not vary beyond 2.5%. Multiple reaction monitoring with two or more product ions and a constant ion ratio have specified tolerances of ±20% to 50% depending on their relative intensity to the base peak.

For this work, 51 pesticides were analyzed using tMRM acquisition with an Agilent 6460 triple quadrupole LC/MS. We examined two pesticides in particular for which there have been reports of false positives in the past: tebuthiuron (a broad-spectrum herbicide recently reported for a chamomile sample) and tebufenpyrad (a pyrazole acaricide and insecticide reported falsely for ginger). These two pesticides exemplify two common analytical scenarios under the applied method conditions. Tebuthiuron is well-resolved from the neighboring endogenous matrix interference showing both primary MRM transitions in a similar ratio. Tebufenpyrad co-elutes with an endogenous ginger compound that shares the same primary MRM transitions. For both analytes in a conventional MRM analysis, the endogenous compounds in the matrix may result in a false positive result. However, tMRM analysis was able to differentiate the endogenous compound in ginger from tebufenpyrad contamination using eight additional product ions for the observed contaminant to perform library confirmation. In addition to the retention time for tebuthiuron, which can be influenced by the sample matrix, tMRM analysis allowed the unambiguous identification of tebuthiuron offering an enhanced level of confirmation. The power of tMRM acquistion comes from its ability to provide quantitative and qualitative data in a single analytical injection.

The tMRM analysis starts with a MRM scan of the designated primary MRM transitions for each compound, covering a range of possible target analytes. When the signal for a given primary transition reaches a user-defined threshold, the secondary transitions are triggered automatically. Each compound is allowed a total of 10 MRM transitions in tMRM mode. These 10 transitions include the primary and secondary MRM transitions in any combination (one primary and nine secondary MRMs, two primary and eight secondary, etc). This type of acquisition maximizes the dwell time for all possible target analytes in the primary MRM screening phase, and then acquires sufficient MRM data for the detected analytes to compose a product ion spectrum. The generated product ion spectra can be used for library searching, so that at the end of the tMRM analysis rigorous quantitative data and a product ion spectrum with the accompanying confirmatory library match are acquired. By applying the optimized collision energy and dwell time for each product ion, tMRM is significantly more sensitive than conventional product ion scanning.

EXPERIMENTAL

Sample Preparation

Samples have been prepared according to §64 LFGB QuEChERS[3] without modification. Ten grams of homogenized ginger sample were extracted with 10 mL of acetonitrile. For the chamomile extract, the sample amount was reduced to 2 g and samples were diluted with 10 mL water before extraction. $MgSO_4$, NaCl, and sodium citrate were added and then centrifuged for 5 minutes at 3000 rpm. Clean-up was performed by dispersive SPE. Six mL of the supernatant was transferred to a d-SPE tube with 900 mg $MgSO_4$ and 150 mg PSA. For the chamomile sample, 45 mg of graphitized carbon black was also added. After centrifugation, 5 ml of the supernatant were stabilized with 50 µL of 5% formic acid in acetonitrile.

Table 1. LC Conditions

LC column	ZORBAX Eclipse Plus C-18 RRHD column 100 x 2.1 mm, 1.8 µm @ 30 °C
Mobile phase	A = 5 mM ammonium formate in water B = 5 mM ammonium formate in methanol
Gradient program	5% B for 0.2 minutes; ramp up to 30% B over 2 minutes; ramp up to 100% B over 8.3 minutes; hold for 2.5 minutes; bring to 5% B; hold for 2 minutes
Flowrate	0.500 mL/min
Injection volume	2 µL

Table 2. MS Conditions

Ionization mode	Agilent Jet Stream positive and negative mode
API drying gas	7 L/min @ 200 °C
API nebulizing gas	35 psi
Sheath gas	12 L/min @ 375 °C
Nozzle voltage	+300/-500 V
Capillary voltage	+3500/-3000 V
Cycle time	500 ms
Interscan delay	3.5 ms
Total number of MRMs	390
Maximum number of concurrent MRMs	84
Minimum dwell time	3.64 ms
Maximum dwell time	146.5 ms

LC/MS ANALYSIS

Instrumentation

The Agilent 1290 Infinity LC system is coupled to an Agilent 6460 triple quadrupole LC/MS.

LC CONDITIONS

Table 1 shows the LC parameters used for analysis of pesticides in ginger and chamomile extracts using tMRM acquisition.

MS CONDITIONS

Table 2 shows the MS parameters used for the analysis.

RESULTS AND DISCUSSION

This LC/MS method separated and detected 51 pesticides. Although the tMRM experiment allowed a total of 10 MRM transitions per compound, the compounds in this analysis utilized two primary transitions and up to seven secondary transitions per compound. Figure 1 shows the overall Total Ion Chromatogram (TIC) and Extracted Ion Chromatogram (EIC) for a quality control standard of all of the pesticides included in this method at the minimum reporting level (MRL).

In order to acquire qualitative and quantitative information in a single analytical run, tMRM acquisition was used.

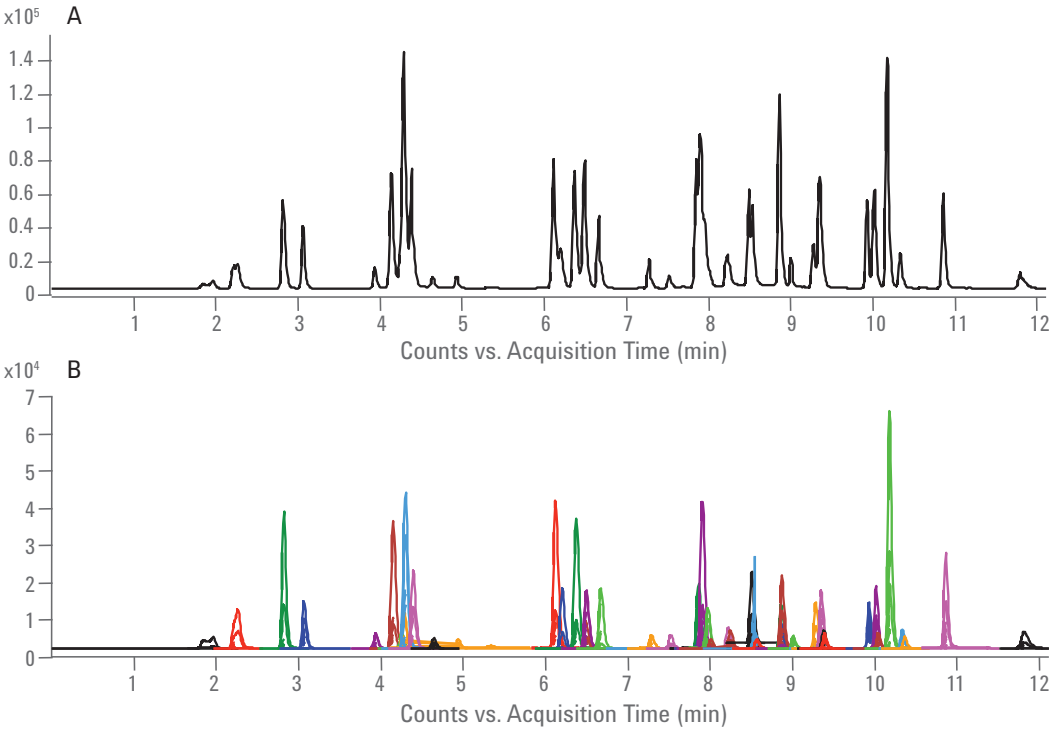

Figure 1. Total Ion Chromatogram (A) and Extracted Ion Chromatogram (B) for 51 pesticides at the minimum reporting level (10 ng/mL)

The first analyte of interest was tebuthiuron in chamomile extract. Chamomile contains an endogenous compound that shares the same mass and a similar retention time to tebuthiuron. Figure 2 shows two chromatograms: the one on the left represents a tebuthiuron standard injection at 50 ppb and the one on the right represents an injection of a blank chamomile extract (one that was known not to contain tebuthiuron). The data showed excellent peak shape and signal for tebuthiuron, and that there are no co-eluting target analytes in our mix of 51 pesticide compounds of interest. Fortunately, the native compound (although very similar in mass to charge ratio and retention time to tebuthiuron) fell beyond the acceptable SANCO retention time variance guidelines for this type of pesticide analysis, but still could be mistakenly identified as tebuthiuron.

The retention time for the native compound had a 3.18% difference from the tebuthiuron standard (2.5% is the maximum deviation allowed), and the qualifier-to-quantifier ion ratio was 189.9% of the expected ion ratio for tebuthiuron. (The SANCO cutoff is 120% of the expected value.) Although the native chamomile extract compound in this case was similar to tebuthiuron, it would likely be rejected as a match by applying the SANCO guidelines. In this case, tMRM analysis gave definitive proof that the endogenous compound is not tebuthiuron, beyond the fact that the retention time for these two compounds differed enough to force rejection by SANCO guidelines.

tMRM analysis was able to definitively identify the endogenous chamomile compound by library matching. In addition, tMRM analysis was able to qualitatively confirm that the endogenous chamomile compound was not tebuthiuron. However, quantitative analysis was performed on tebuthiuron spiked into a blank chamomile extract in order to demonstrate that in addition to qualitative confirmation, tMRM acquisition is able to acquire reliable quantitative data that one would expect from a high-performance triple quadrupole mass spectrometer. Even though these compounds elute relatively close to one another and triggered the acquisition of secondary transitions in both cases, a blank chamomile extract spiked with tebuthiuron generated a linear calibration curve with an R^2 value equal to 0.9997 (Figure 3).

Five replicate injections of tebuthiuron at 10 ppb spiked into chamomile extract had a %RSD value of 0.94. Five replicate injections of the entire 51 pesticide mix spiked into chamomile extract at 10 ppb had a %RSD value of 1.10. This method was found to produce linear and reproducible quantitative results.

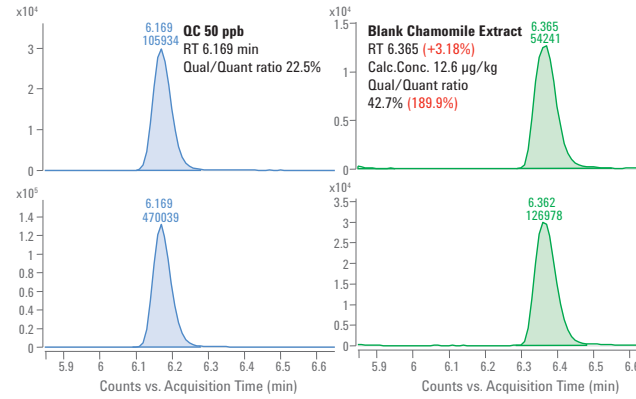

Figure 2. Primary MRM traces for tebuthiuron in 50 ppb quality control standard (left) and in a blank chamomile extract (right). The top chromatograms represent the first qualifier traces for tebuthiuron (m/z 229.1 > 116.0) and the bottom chromatograms represent the quantifier traces (m/z 229.1 > 172.1)

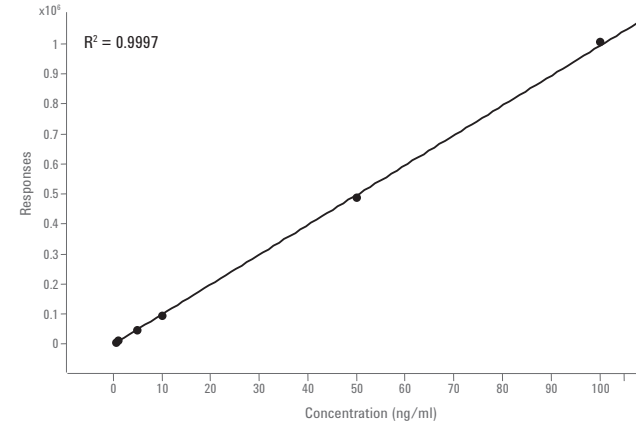

Figure 3. Calibration curve for tebuthiuron in a blank chamomile extract from 1 ppb to 100 ppb. The R^2 value for the calibration curve is 0.9997

In the case of tebufenpyrad in ginger extract, tMRM analysis was critical in avoiding a false positive result for tebufenpyrad, even if SANCO guidelines were applied. Figure 4 shows the chromatograms for the primary MRM transitions for tebufenpyrad in a 50 ppb quality control standard (on the left) versus an injection of a blank ginger extract that did not contain tebufenpyrad (on the right).

In this case, the endogenous ginger compound (shown on the right in Figure 4) varied only in retention time from the tebufenpyrad standard by 0.47% (well within regulatory guidelines). The qualifier-to-quantifier ion ratio of the endogenous ginger compound was 123.1% of the expected ion ratio for tebufenpyrad, and 120% is the regulatory cutoff set by SANCO guidelines. This endogenous compound was very similar to tebufenpyrad and would most likely give a false positive result using standard acquisition techniques. However, tMRM acquisition gave valuable qualitative data that could be used in library matching for definitive confirmation.

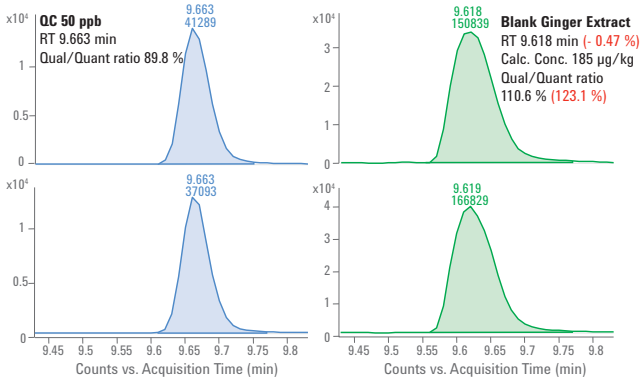

Figure 4. Primary MRM traces for tebufenpyrad in 50 ppb quality control standard (left) and in a ginger extract (right). The top chromatograms represent the quantifier traces for tebufenpyrad (m/z 334.1 > 117.1) and the bottom chromatograms represent the first qualifier traces (m/z 334.1 > 145.1)

Figure 5 shows the library search results for the native ginger compound with similar retention time, quantifier, and qualifier ions to tebufenpyrad. The bottom window shows the stored library spectrum and the upper window shows the spectrum that has been acquired for the native ginger compound. The mirrored spectra in the central window allowed for a simple comparison of the acquired versus the library spectrum. Although this co-eluting compound would commonly give a false positive result in typical quantitative MRM analyses, we see here that there are many peaks present in the tebufenpyrad tMRM library spectrum that were missing from the native ginger compound spectrum. As a result, the library match score was only 70.34 out of 100, and we were able to confidently reject the native compound as tebufenpyrad.

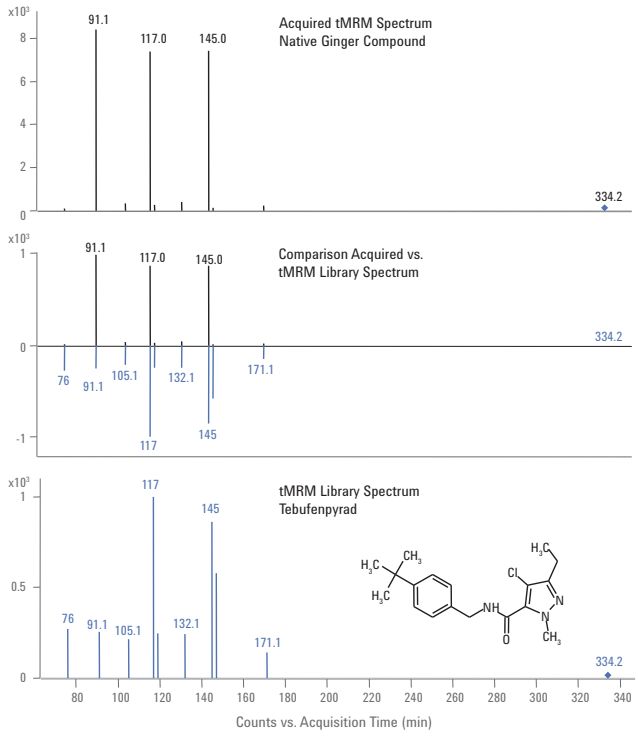

Figure 5. Library match result for the native ginger compound searched against the tMRM library spectrum for tebufenpyrad yielded a library match score of 70.34, allowing rejection of the native compound as tebufenpyrad and avoiding a positive result.

CONCLUSIONS

The analyses of pesticides in chamomile and ginger extracts with tMRM acquisition achieved accurate quantitative analysis with the confidence of library matching in a single analytical run. Tebuthiuron and tebufenpyrad were successfully distinguished from nearby or co-eluting endogenous compounds, and false positives were successfully averted with the inclusion of qualitative analysis with library matching. tMRM acquisition is a data-dependent scan mode capable of providing quantitative and qualitative data on a single instrument, in a single injection.

REFERENCES

(1) Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC (including amendments as of 18 March 2008).

(2) European Guideline SANCO/10684/2009: Method validation and quality control procedures for pesticide residues analysis in food and feed.

(3) Official collection of test procedures according to §64 law on food and animal feed (LFGB), Beuth-Verlag.

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Analysis of Plant Stanyl Fatty Acid Esters in Enriched Margarine Using an Online Coupled Agilent 1220 Infinity LC-7890 GC System

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ABSTRACT

The work described here involved the investigation of intact plant stanyl fatty acid esters in an enriched commercial margarine using an online coupled Agilent 1220 Infinity LC-7890A GC system. The lipid extract was directly analyzed without prior purification steps. The LC fraction of plant stanyl esters was transferred online into the GC system using the solvent vent mode of the multimode inlet for solvent evaporation. The online LC-GC combination showed very good linearity and repeatability.

INTRODUCTION

Plant steryl and stanyl esters (Figure 1) are added to food products like skimmed milk-drinking yogurts or margarines because of their cholesterol-lowering properties. The capillary gas chromatographic investigation of plant stanyl fatty acid esters from skimmed milk products can be performed directly after the lipid extraction[1]. However, the presence of di- and triglycerides may hamper the direct GC quantification. Therefore, the analysis in lipid extracts from foods with high fat contents like margarine requires a fractionation prior to the GC separation by laborious offline techniques, such as Thin Layer Chromatography (TLC) or Solid Phase Extraction (SPE). The online coupling of LC and GC offers an efficient and elegant alternative. The plant stanyl esters can be fractionated by liquid chromatography and transferred online into the GC system. In this way, the pre-fractionation step and the capillary gas chromatographic analysis of the transferred LC fraction are performed in a closed system in one run. Hence, the risk of sample loss and contamination is reduced and the approach results in better repeatability[2, 3].

In a recently published paper[1], the analysis of plant stanyl esters in enriched margarines using an online LC-GC system equipped with a loop-type interface was reported. Using the loop-type interface, the solvent evaporation was performed in the GC capillary columns by means of a pre-column system in combination with an early solvent vapor exit. Due to the high solvent amounts which were loaded on the pre-column system with each transfer, a loss of resolution was observed after a few runs.

The online coupling of an Agilent 1220 Infinity LC system and an Agilent 7890A GC system, with a 2-position/6-port switching valve using the solvent vent mode of the multimode inlet of the GC, allowed for the evaporation of the solvent prior to the capillary column[4]. A pre-column system and/or a solvent vapor exit were not necessary. This combination was already suitable for the analysis of cholesteryl esters[4].

The use of an Agilent online coupled LC-GC combination for the quantification of plant stanyl esters in enriched margarine is presented here.

EXPERIMENTAL

Chemicals and materials

The plant stanyl ester mixture "plant stanol ester, STAEST-115" was provided by Raisio Group (Raisio, Finland). The internal standard cholesteryl palmitate (≥ 98%) was obtained from Sigma Aldrich (Taufkirchen, Germany).

Benecol (taste-type Kevyt kasvirasvalevite 32%, with added plant stanyl esters) margarine was purchased in a supermarket in Finland. The plant stanol content was labeled as 8 wt-%, total lipids as 32 wt-%.

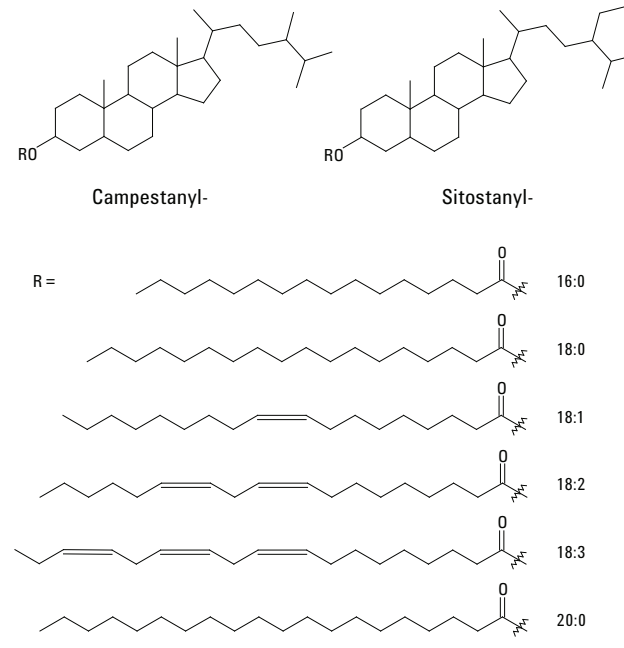

Figure 1. Structures of plant stanyl fatty acid esters

SAMPLE PREPARATION OF MARGARINE[1]

The margarine sample (20–40 mg, accuracy of ± 0.1 mg) was weighed into a vessel; internal standard (cholesteryl palmitate, 750 µg), 5 mL of n-hexane/MTBE (3:2) and sodium sulfate (anhydrous) were added and sonicated for 1 minute. The solution was filtered through a 0.45 µm membrane filter assembled with a 5 mL syringe. The vessel and the filter were washed twice with 5 mL *n*-hexane/MTBE (3:2). After dilution (1:5) of the combined extracts, the solution was used for online LC-GC analysis.

QUANTIFICATION

The five-point calibration functions of nine individual stanyl esters were generated in a range of 0.2 – 1.0 µg of total stanyl ester ("plant stanol ester, STAEST-115") per 2 µL i.v. Each calibration point was done in triplicate. Linear regression analysis was performed in coordinate ratios of areas (individual stanyl ester/IS) and amounts (individual stanyl ester/IS).

EQUIPMENT

The coupling of the Agilent 1220 Infinity LC system to the Agilent 7890A GC system was accomplished using an Agilent 2-position/6-port switching valve equipped with a 200 µL sample loop (Table 1). The evaporation of the eluent was performed using the temperature programmable MultiMode (MM) Inlet in the Programmable-Temprature Vaporizing (PTV) solvent vent mode[4].

Table 1. Liquid and gas chromatographic conditions

CHROMATOGRAPHIC CONDITIONS

LC conditions	
Injection volume:	2 µL
Eluent:	*n*-hexane/tert-butylmethyl ether (96:4, v/v)
Column temperature:	27 °C
Column flow:	0.200 mL/min
Column type:	Eurospher-100Si (250 x 2 mm id, 5 µm)
Wavelength:	205 nm
LC controlled interface	
Transfer valve:	4.25 min: Position 1 → 2 7.50 min: Position 2 → 1
GC start:	4.20 min: Change contacts switch contact A to closed 4.25 min: Change contacts switch contact A to open
GC conditions	
Front MM inlet:	Mode: Solvent vent Carrier: H_2 Pressure: 7.8 psi Septum purge flow: 3 mL/min Vent pressure: 4 psi until 0.5 min Vent flow: 1000 mL/min Temperature program: Initial: 50 °C for 0.5 min Rate 1: 900 °C/min to 350 °C for 2 min Purge flow to split vent: 2.5 mL/min at 0.5 min Gas saver: 20 mL/min after 5 min
Column 1:	Column type: Restek Rtx-200MS: 30 m × 250 µm; 0.1 µm df; Constant flow: 1.5 mL/min
Column 2:	Transfer line, controlled by PCM C-1 Pressure program: Initial: 5 psi for 0.3 min Rate 1: 10 psi/min to 20 psi
Oven:	Temperature program: Initial: 40 °C for 2 min Rate 1: 100 °C/min to 100 °C for 0 min Rate 2: 15 °C/min to 310 °C for 2 min Rate 3: 1.5 °C/min to 340 °C for 3 min
Detector:	FID: 360 °C (H_2: 30 mL/min, Air: 400 mL/min; Makeup: 25 mL/min)

RESULTS AND DISCUSSION

The chromatograms obtained by online LC-GC analysis of a margarine enriched with plant stanyl esters are presented in Figure 2. The LC-fractionation (Figure 2a) was performed isocratically on a silica gel column with *n*-hexane/MTBE (96+4; v+v) as mobile phase. The plant stanyl esters eluted after approximately 4 minutes. The transfer was performed 4.25 minutes after injection. The transfer conditions for the analysis of cholesteryl esters[4] were also suitable for plant stanyl esters. The GC separation of the transferred fraction was similar to that reported for the online LC-GC analysis via a loop-type interface[1]. The intact plant stanyl fatty acid esters were distinguishable according to their carbon number and, in the case of unsaturated fatty acid moieties, to the number of double bonds; only the esters of saturated and monounsaturated fatty acids of the same chain length eluted at the same time.

Under the employed online LC-GC conditions, using the Agilent Multimode Inlet for the solvent evaporation[4], the solvent load on the GC capillaries was low in comparison to the loop-type coupled system. Even after 600 transfers, no loss of resolution was observed using the online LC-GC combination.

For the calibration, linear regression analysis was performed in the coordinate ratios of areas (individual stanyl ester/IS) and amounts (individual stanyl ester/IS). The correlation coefficients of the calculated calibration functions (R^2) were in the range of 0.995 – 0.999, showing very good linearity of the online LC-GC/FID detector response.

The repeatability was determined by 10-fold injections of the same sample solution. The coefficients of variation were low (< 9%) for all plant stanyl esters (Table 2). The quantitative results were comparable to those obtained by means of the loop-type interface coupled online LC-GC[1].

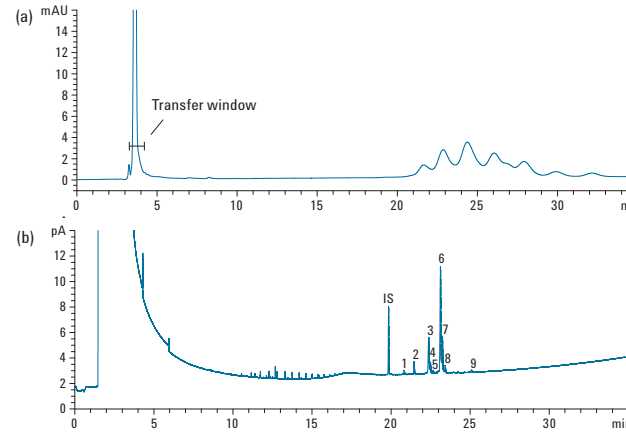

Figure 2. Analysis of plant stanyl esters in enriched margarine by online LC-GC/FID; (a) LC-chromatogram and (b) GC-chromatogram of the transferred LC-fraction; peak numbering according to Table 2; (IS) internal standard cholesteryl palmitate

Table 2 Coefficients of variation of plant stanyl esters

		CV [%][b]			
No.[a]	**Stanyl ester**	**Extract 1**	**Extract 2**	**Extract 3**	**Amount [g/100 g][c]**
1	Campestanyl-16:0/16:1	7.7	2.0	5.1	0.18 ± 0.01 (8.1)
2	Sitostanyl-16:0/16:1	5.7	0.7	2.6	0.47 ± 0.02 (5.3)
3	Campestanyl-18:0/18:1	1.1	0.8	0.6	2.10 ± 0.06 (2.8)
4	Campestanyl-18:2	3.7	1.6	3.2	0.73 ± 0.04 (5.2)
5	Campestanyl-18:3	8.8	2.8	6.9	0.30 ± 0.02 (7.6)
6	Sitostanyl-18:0/18:1	1.1	0.9	1.5	6.36 ± 0.22 (3.5)
7	Sitostanyl-18:2	1.9	1.5	2.0	2.17 ± 0.11 (5.1)
8	Sitostanyl-18:3	6.5	2.2	7.2	0.81 ± 0.06 (8.0)
9	Sitostanyl-20:0/20:1	4.8	2.5	5.5	0.19 ± 0.01 (7.0)
Total stanyl esters		1.6	0.7	1.6	13.3 ± 0.5 (3.7)
Esterified sterols		1.6	0.7	1.6	8.1 ± 0.3 (3.7)

[a] Peak number correspond to Figure 2b
[b] Coefficient of variation [CV] determined by 10-fold injections of the same sample solution
[c] Values represent average ± standard deviations of 30 analyses (coefficient of variation [%])

CONCLUSION

Online coupling of an Agilent 1220 Infinity LC system and an Agilent 7890A GC system was shown to be suitable for the quantitative analysis of plant stanyl fatty acid esters in enriched margarine. The online LC-GC system was characterized by easy handling and a very robust separation performance for both dimensions. Therefore, the Agilent online LC-GC combination can be a valuable tool for the routine analysis of plant steryl and stanyl esters in functional foods.

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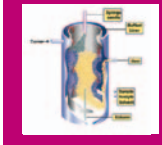

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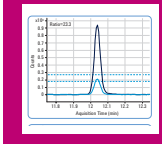

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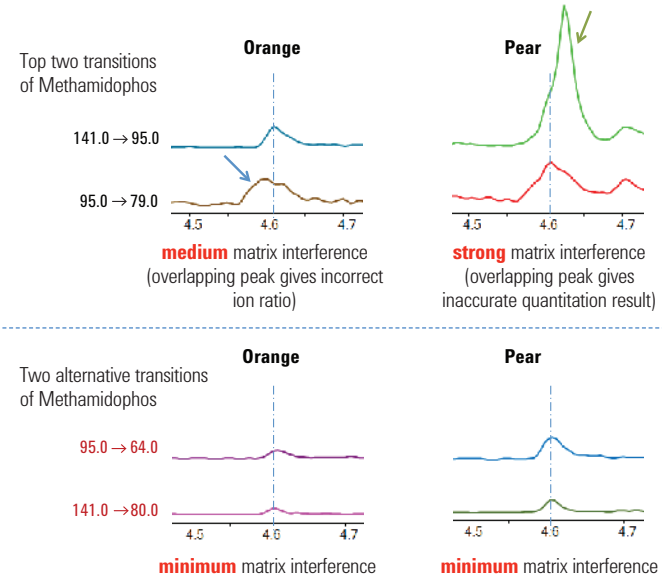

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