Multiresidue Analysis of Pesticides in Olive Samples Using GC/MS/MS

Application Note

Food Testing and Agriculture

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Abstract

This application note describes an analytical method for the determination of 27 pesticide residues in olives. Olives have a high lipid content of about 80 to 85%, which can adversely affect pesticide recoveries and chromatographic systems. Therefore, a modified QuEChERS method for the extraction and analysis by gas chromatography/triple quadrupole (GC/MS/MS) multiple reaction monitoring method was employed. The method was validated for olives in terms of recovery, repeatability, and reproducibility. The results demonstrated that the method achieved acceptable quantitative recoveries of 70 to 120%, as recommended in SANCO/12571 [1], with RSDs < 20%. Limits of quantification at or below the regulatory maximum residue limits for the pesticides were achieved.

Introduction

Pesticide residue analysis is essential for the health of humans and animals, the import/export trade, and regulated control purposes. Many pesticide classes are used in agriculture, and most pesticides have regulatory guidelines, for example maximum residue levels (MRLs) in food, with analytical determination based on GC/MS or LC/MS/MS [2].

Methods using gas chromatography coupled to mass spectrometry (GC/MS) are based on selected ion monitoring (SIM), which is fairly sensitive, but identification potential and non-target/retrospective analysis capabilities are sacrificed. Determination of GC-amenable pesticides in food samples by using GC/MS/MS has emerged in the last decade as a valuable approach, which allows higher selectivity and sensitivity, while minimizing or even eliminating most chromatographic interferences [3]. Despite the increased sensitivity and selectivity of GC/MS/MS co-extract matrix components can cause matrix effects that can negatively impact results. An optimized sample preparation method can minimize these effects.

Materials and Methods

A modified EN extraction method was used for the extraction of 27 pesticides from olives. The solvent-modified method, which incorporated a mixture of nonpolar and polar aprotic solvents (ethyl acetate:cyclohexane:acetone) versus the polar aprotic solvent (acetonitrile) described in EN 15662 [4] was used to extract the pesticides from the high-lipid matrix associated with olives. A mixture of nonpolar and polar aprotic solvents can offer a wider polarity range for extractable compounds relative to the use of only a polar aprotic solvent, ACN. The pesticides included organochlorine, organophosphate, and pyrethroid classes.

Consumables and instruments

- Agilent Bond Elut QuEChERS EN Extraction Kit (p/n 5982-5650)
- Agilent Bond Elut QuEChERS Dispersive Kit, EN Method, 15 mL (p/n 5982-5156)
- Agilent SPE Bulk Sorbent, C18 Endcapped (p/n 5982-8082)
- Agilent J&W HP-5ms Ultra Inert GC column, 30 m × 0.25 mm (p/n 19091S-433UI)
- Agilent 7890A GC
- Agilent 7000A Triple Quadrupole GC/MS System
- Agilent 7693 ALS Injector

Solvents and pesticide standards

- Ethyl acetate, residue analysis purity, Baker
- Cyclohexane, residue analysis purity, Sharlau
- Acetone, residue analysis purity, Baker
- Solvent mixture: ethyl acetate:cyclohexane:acetone (1:1:4)
- Pesticide standards, Dr. Ehrenstorfer

GC conditions

Carrier:	Constant pressure, 22.0 psi
Oven temperature:	Initial 70 °C (2 min), 25 °C/min to 150 °C (0 min), 3 °C/min to 200 °C (0 min), 8 °C/min to 280 °C (10 min)
Injector temperature:	250 °C
Septum purge:	On, 3 mL/min
Purge flow split vent:	100 mL/min at 0.75 minutes
Injection:	Splitless, 1.0 µL
RTL compound:	Chlorpyrifos methyl

MS conditions

Ion source:	EPC
Source temperature:	280 °C
Collision gas:	He quench gas on, 2.35 mL/min N_2 collision gas on, 1.5 mL/min
Transfer line temperature:	280 °C
MS quad 1,2 temperature:	150 °C both
MS1/MS2 resolution:	Wide/wide
MRM settings:	See Table 1

Table 1. Retention time and MRM parameters for 27 pesticides.

Pesticide	RT (locked to chlorpyrifos methyl)
Chlorpropham	11.05
Heptenophos	9.737
Pirimiphos methyl	18.307
Hexachlorobenzene	12.377
Fonofos	13.889
Simazine	12.909
Terbufos	13.796
Terbuthylazine	13.810
Diazinon	14.466
Pirimicarb	15.677
Chlorpyrifos methyl	16.59
Chloropropylate	25.419
Parathion-methyl	16.594
Bifenthrin	28.839
Fenitrothion	18.072
Parathion	19.275
Chlorthal dimethyl	19.433
Chlorfenvinphos	21.557
Pendimethalin	20.991
Alpha-endosulfan	22.637
Procymidone	21.962
Beta-endosulfan	25.3158
Endosulfan sulfate	26.76
Acrinathrin	30.724
Aldrin	18.528
Chlorpyrifos	19.234
4,4′-Dichlorobenzophenone (dicofol)	19.201

Table 2 shows the time segments. MS1 and MS2 resolutions were wide/wide throughout. Dwell time was 10 ms.

Table 2. Time segments.

Compound	Precursor ion	Product ion	Collision energy
Time segment 2			
Chlorpropham	213	171	5
Chlorpropham	213	127	5
Heptenophos	124	89	20
Heptenophos	124	63	35
Time segment 3			
Hexachlorobenzene	284	249	20
Hexachlorobenzene	284	214	35
Time segment 4			
Fonofos	246	109	15
Fonofos	246	81	30
Simazine	201	186	5
Simazine	201	173	5
Terbufos	231	175	10
Terbufos	231	129	25
Terbuthylazine	214	132	10
Terbuthylazine	214	104	20
Time segment 5			
Diazinon	179	137	20
Diazinon	179	121	40
Fonofos	246	109	15
Fonofos	246	81	30
Terbufos	231	175	10
Terbufos	231	129	25
Terbuthylazine	214	132	10
Terbuthylazine	214	104	20
Time segment 6			
Pirimicarb	238	166	10
Pirimicarb	166	96	15
Terbacil	161	144	10
Terbacil	161	88	20
Time segment 7			
Chlorpyrifos methyl	286	271	20
Chlorpyrifos methyl	286	93	25
Methyl parathion	263	109	15
Methyl parathion	263	79	30
Time segment 8			
4,4′-Dichlorobenzophenone	139	111	15
4,4′-Dichlorobenzophenone	139	75	35
Aldrin	263	193	30
Aldrin	263	191	30
Chlorpyrifos	197	169	15
Chlorpyrifos	197	107	40
Fenitrothion	277	125	15
Fenitrothion	277	109	20
Parathion	291	109	10
Parathion	291	81	25
Pirimiphos methyl	305	290	10
Pirimiphos methyl	305	180	5
Time segment 9			
Chlorthal dimethyl	301	223	25
Chlorthal dimethyl	299	221	25
Time segment 10			
Chlorfenvinphos	267	159	15
Chlorfenvinphos	267	81	30
Pendimethalin	252	162	10
Pendimethalin	252	161	20
Time segment 11			
Endosulfan (alpha isomer)	241	206	10
Endosulfan (alpha isomer)	241	170	20
Procymidone	283	255	10
Procymidone	283	96	10
Time segment 13			
Endosulfan (beta isomer)	241	206	15
Endosulfan (beta isomer)	195	159	5
Time segment 14			
Endosulfan (beta isomer)	241	206	15
Endosulfan (beta isomer)	195	159	5
Chloropropylate	251	139	15
Chloropropylate	251	111	35
Time segment 15			
Endosulfan sulfate	272	237	20
Endosulfan sulfate	272	117	40
Time segment 16			
Bifenthrin	181	166	20
Bifenthrin	181	165	25
Time segment 17			
Acrinathrin	289	93	5
Acrinathrin	208	181	5

Sample preparation

Olives were picked from local trees. The olives were cut into thin slices and a portion of the sample (approximately 1 g) was used for the analysis. The rest was stored frozen. If the olives contained a stone, it was removed and an estimate of the percentage contribution of stone present in the final sample was calculated. This is a legislative requirement for LMR in calculations. This was done by taking a representative portion of the sample (two or three whole pieces) to evaluate the percentage of the stone, as follows. First, weigh the representative samples containing the stone. Then, remove the stones from the samples and weigh them. Finally, calculate the percentage associated with the stones using Equation 1.

$$\% \text{ H} = \frac{\text{Weight of stone}}{\text{Weight of sample}} \times 100 \qquad \text{Eqn.1}$$

Where:
% H = percentage of the stone

Procedure

1. Weigh 10.0 g ± 0.1 g of chopped sample in a 50-mL centrifuge tube.
2. Add 10 mL of the solvent mixture ethyl acetate:cyclohexane:acetone (1:1:4) then cap the tube and shake for at least 1 minute.
3. Add the Bond Elut QuEChERS extraction salt packet (p/n 5982-5650) to the sample tube; shake for 1 minute.
4. Centrifuge the tube at 4,000 rpm for 5 minutes.
5. Transfer 6 mL of the upper organic layer into a 15-mL Bond Elut dispersive tube (p/n 5982-5156) to which an additional 300 mg of C18EC (p/n 5982-8082) has been added.
6. Shake for at least 1 minute, then centrifuge at 4,000 rpm for 5 minutes.
7. Transfer 1 to 2 mL of the upper organic layer extract to a chromatography vial and seal tightly.

Results and Discussion

All target pesticides were separated and well detected by the HP-5ms Ultra Inert GC column. With the powerful selectivity provided by GC/MS/MS, the MRM chromatograms of the matrix blank did not show any interference peaks with the target analytes. Figure 1 shows the GC/MS/MS chromatogram of a 20 µg/kg-fortified olive extract processed by the modified EN QuEChERS extraction method. Figure 2 shows the exceptional quantitative analysis of pesticides considered to be more problematic in olives and olive oil analysis, such as terbuthylazine and chlorpyrifos.

Calculations

Once the pesticide concentration in the vial was obtained from the calibration curve, we worked out sample concentrations in mg/kg using Equation 2.

$$C_{Pesticide}(mg/kg) = C_{Pesticide}(\mu g/L) \times \frac{10}{W} \times 1e - 3 \times [1 - (\frac{\% H}{100})] \qquad \text{Eqn.2}$$

Where:
$C_{Pesticide}$ = concentration (µg/L) obtained from the calibration curve

W = initial weight of the sample taken for the test in g (10.0 g)

$\% H$ = percentage contribution of stones (if necessary)

The final result is expressed in I.S. units, and results given in µg/kg.

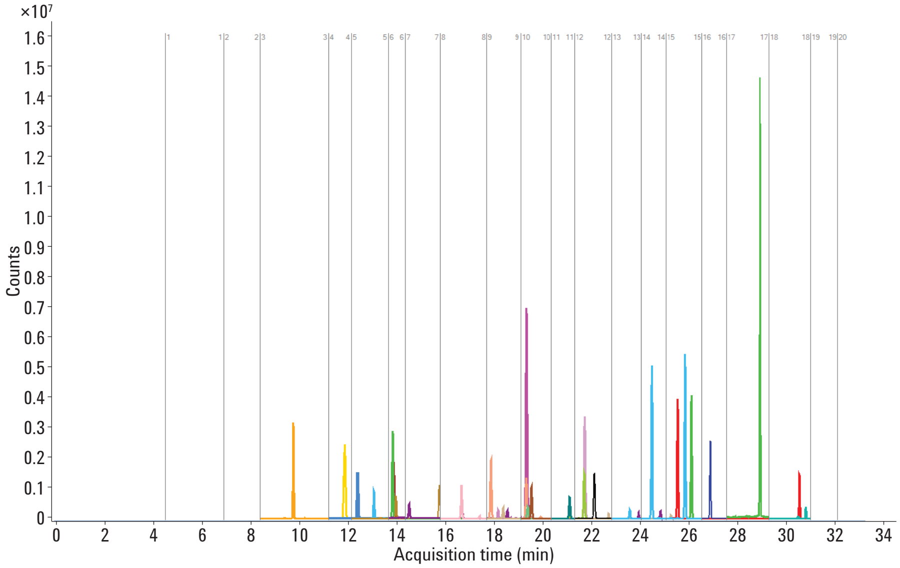
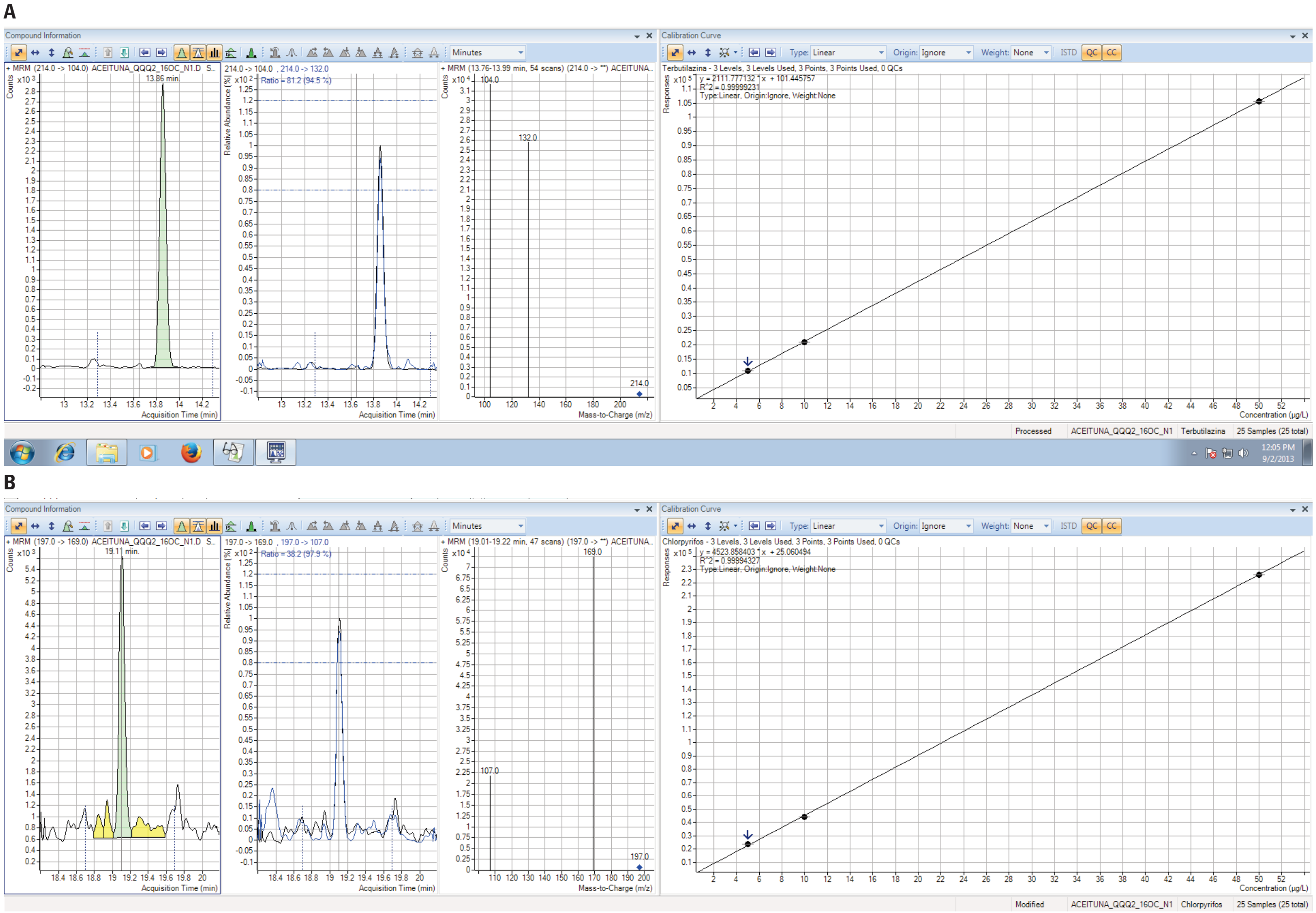


Figure 1. Overlay of MRM transitions of 27 pesticides spiked in olives at 20 µg/kg obtained using GC/MS/MS.



Type: Linear Origin: Ignore Weight: None

Terbutilazina - 3 Levels, 3 Levels Used, 3 Points, 3 Points Used, 0 QCs

y = 2111.777132 * x + 101.445757
R^2 = 0.99999231
Type:Linear, Origin:Ignore, Weight:None

Processed ACEITUNA_QQQ2_16OC_N1 Terbutilazina 25 Samples (25 total)

Type: Linear Origin: Ignore Weight: None

Chlorpyrifos - 3 Levels, 3 Levels Used, 3 Points, 3 Points Used, 0 QCs

y = 4523.858403 * x + 25.060494
R^2 = 0.99994327
Type:Linear, Origin:Ignore, Weight:None

Modified ACEITUNA_QQQ2_16OC_N1 Chlorpyrifos 25 Samples (25 total)

Figure 2. A) Terbuthylazine at 5 µg/kg g/kg, R^2 = 0.9999. B) Chlorpyrifos at 5 µg/kg, R^2 = 0.9999.

Linearity and limit of quantification (LOQ)

The linearity calibration range for all the pesticides tested was 5 to 60 µg/kg. Calibration curves using spiked matrix blanks were made at 5, 10, 20, and 60 µg/kg, where 60 µg/kg was at least 120% of the upper limit. The calibration curves were generated by plotting the relative responses of the analytes. The 10 µg/kg quantification limits established for all pesticides were at or below the MRLs of these pesticides in fruits and vegetables. The correlation coefficients (R^2) for all compounds were > 0.9999.

Recovery and reproducibility

Recovery and reproducibility were evaluated by spiking pesticide standards in comminuted olive samples at levels of 5, 10, and 20 µg/kg, which were then prepared by the EN QuEChERS extraction method and the solvent-modified cleanup method. These QC samples were quantitated against the matrix spike calibration curve. The analysis was performed in replicates of five at each level. The recovery and reproducibility (RSD) data are shown in Table 3. It can be seen that the 27 pesticides had excellent recoveries and precision.

Table 3. Recovery %, (RSD%), and reproducibility of pesticides in olives with Agilent Bond Elut QuEChERS EN extraction method and solvent modified dispersive SPE kit for fruits and vegetables with fats and waxes.

Compound	**L1**	**L2**	**L3**
4,4′-Dichlorobenzophenone	93.94 (7.32)	92.95 (7.62)	106.43 (6.28)
Acrinathrin	101.92 (6.66)	95.13 (14.01)	105.25 (2.00)
Aldrin	90.46 (12.86)	90.36 (9.60)	100.00 (10.52)
Bifenthrin	96.50 (9.12)	95.88 (7.87)	104.29 (8.01)
Chlorpyrifos	93.15 (4.43)	86.07 (14.21)	108.10 (5.53)
Chlorthal dimethyl	98.78 (5.78)	96.05 (7.47)	99.96 (16.41)
Chlorfenvinphos	93.10 (6.04)	89.01 (11.80)	106.86 (6.38)
Chloropropylate	91.24 (7.46)	86.68 (7.88)	108.70 (5.41)
Chlorpyrifos methyl	100.40 (11.72)	92.70 (8.63)	110.22 (3.23)
Chlorpropham	98.38 (7.30)	91.00 (15.89)	105.07 (6.86)
Diazinon	94.11 (10.24)	94.68 (14.24)	108.81 (5.98)
Endosulfan (*alpha* isomer)	89.13 (16.31)	93.97 (11.88)	105.55 (5.64)
Endosulfan *beta*	83.90 (21.05)	93.06 (10.03)	98.25 (4.41)
Endosulfan sulfate	94.67 (4.76)	93.48 (7.74)	109.58 (4.75)
Fenitrothion	91.48 (8.74)	86.33 (17.91)	109.38 (10.91)
Fonofos	89.55 (5.21)	91.06 (9.75)	104.63 (7.52)
Heptenophos	97.24 (4.02)	93.55 (17.02)	101.78 (12.40)
Hexachlorobenzene	95.25 (3.52)	87.10 (8.51)	105.62 (7.34)
Parathion-methyl	94.00 (15.54)	85.27 (10.50)	104.57 (7.81)
Parathion	86.26 (11.20)	81.57 (10.98)	94.98 (10.91)
Pendimethalin	90.25 (12.40)	81.78 (7.91)	96.00 (4.64)
Pirimicarb	84.52 (5.09)	76.15 (5.43)	89.66 (10.16)
Pirimiphos methyl	93.02 (7.29)	85.34 (13.69)	106.92 (5.54)
Procymidone	90.75 (7.97)	85.04 (3.41)	98.88 (7.29)
Simazine	94.12 (4.59)	90.17 (11.80)	105.81 (8.75)
Terbufos	105.06 (8.45)	98.92 (9.06)	112.62 (6.92)
Terbuthylazine	97.31 (9.04)	91.66 (7.59)	105.34 (9.99)

Level	**Calibration points (µg/L)**	**Limit of quantification (µg/kg)**
L1	5.0 (at least 70% LQ)	10.0
L2	10	
L3	20	
L4	60 (at least 120% of upper limit)	

Conclusions

The Agilent Bond Elut QuEChERS EN buffered extraction kits and dispersive-SPE kits for fruits and vegetables containing fats and waxes, with a modified-solvent mixture, provided a simple, fast, and effective method for the purification of representative pesticides in olives. The recovery and reproducibility, based on matrix-spiked standards, were acceptable for multiclass pesticide determination in olives. The matrix effects from the olives did not interfere with the quantitation of target compounds. The LOQs of the pesticides were at or below regulated MRLs in foods. As the selected pesticides represented a broad variety of different classes and properties, Bond Elut QuEChERS EN extraction and dispersive-SPE with modified solvent mixtures are excellent choices for other pesticides in similar food matrixes.

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