Application Note

Food & Beverage Testing

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Determination of Mycotoxins and Pesticides in Olives

Sample preparation using Agilent Captiva EMR–Lipid and the Agilent Ultivo triple quadrupole LC/MS

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Abstract

This application note describes an analytical method for the simultaneous determination of mycotoxins and pesticides in olives. The sample preparation method is based on a QuEChERS extraction followed by Agilent Captiva EMR–Lipid cleanup and analysis with an Agilent 1260 Infinity II Prime LC and Ultivo triple quadrupole MS (LC/MS/MS) coupled with an Agilent Jet Stream source (AJS). Compound transitions and optimized parameters were developed using Agilent MassHunter Optimizer software. The Captiva EMR–Lipid offers highly efficient removal of matrix interferences in olives, enabling the detection of analytes at low concentration levels. A total of 71 pesticides and six mycotoxins were simultaneously determined in a 14-minute run. This method is suitable for the extraction and determination of pesticides and mycotoxins in olives and can be recommended for implementation in routine analysis intended for screenings or monitoring tests for olives.

Introduction

Consumption of olives and olive oil is related to beneficial health effects, given their antitumor and anti-inflammatory properties.[1] Olive oil is rich in monounsaturated fatty acids and has a unique phenolic profile with interesting physiological properties.

As with any other crops, olives trees are subject to pests, diseases, and weeds, which can reduce production. Mycotoxigenic fungi can also attack olives and contaminate the crops with mycotoxins. These toxins may pose a health hazard to humans and livestock, causing acute poisoning and long-term effects such as immune deficiency and cancer.[2] Almost 25% of the world's harvested crops are spoiled by mycotoxins, which creates a significant annual loss of billions of dollars in the agricultural and industrial sectors.[3] Because of the chemical and thermal stability of mycotoxins, these toxins can survive food processing such as cooking and frying. Since most of the mycotoxins are fat soluble, they tend to accumulate in body fats, and so are difficult to excrete from the body.[4] Intended to protect fruits, pesticides are commonly applied to olive trees and olives during production, storage, and transportation. For this reason, many countries have set maximum residue levels (MRL) for olives, such as in the European Union, which established the MRL of pesticides in olives as a commodity.[5] As a result, reliable analytical methods for pesticides and mycotoxins determination in olives have become essential.

Experimental

Chemicals and reagents

Acetonitrile (ACN, pesticide grade), formic acid (99.8%), and HPLC-grade acetic acid were purchased from J.T. Baker (Phillipsburg, USA). Ultrapure water was obtained from a Milli-Q Gradient Water System (Millipore, Milford, USA). Reagent-grade sodium acetate was purchased from J.T. Baker (Xalostoc, Mexico).

Reference standards of pesticides (purity >97%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Mycotoxins standards were purchased from Fermentek Biotechnology (Jerusalem, Israel) and Sigma-Aldrich (Steinheim, Germany).

Solutions and standards

Individual standard stock solutions of pesticides and a mixture solution of all pesticides were prepared in ACN at 1 mg/L. The pesticides are classified as group 1.

Mycotoxins were divided into two groups according to their sensitivity in the LC/MS/MS system. Group 2 standard, containing aflatoxins B1, B2, G1, and G2, was prepared in ACN at 1 µg/mL; group 3 standard, containing citrinin and zearalenone, was prepared at 50 µg/mL in ACN.

A procedure internal standard (PIS) and an instrument internal standard (IIS) were used. The PIS was spiked into the samples before extraction and the IIS was added to the final dilution solvent (ACN/water, 1:1). The PIS containing 1,000 µg/L propoxur was prepared in ACN, and IIS containing 12.5 µg/L quinalphos was prepared in ACN. Solutions were stored in a freezer at ≤−18 °C.

Equipment and consumables

- Analytical precision balances (Sartorius, Germany)
- Mechanical shaker 3016 (GFL, Germany)
- Vortex Genie 2 (Scientific Industries, USA)
- Centrifuge Hareaus Varifugue (Thermo Fisher Scientific, Germany)
- Ultrapure water (18 MΩ cm), Milli-Q system from Millipore (Milford, USA)
- Agilent collection rack and funnel set for 16 × 100 mm test tubes, for Vac Elut 24 Manifold (part number 12234028)
- Automatic pipettes with variable volume (Eppendorf, USA)
- Agilent QuEChERS extraction kit, AOAC (part number 5982-5755CH)
- Agilent QuEChERS extraction kit, original (part number 5982-5550CH)
- Agilent QuEChERS extraction kit, EN (part number 5982-5650CH)
- Agilent ceramic homogenizers, 50 mL tubes (part number 5982-9313)
- Agilent Captiva EMR–Lipid cartridges, 3 mL, 300 mg (part number 5190-1003)
- Agilent vial, screw top, clear, certified, 2 mL (part number 5182-0714)
- Agilent InfinityLab Poroshell 120 SB-C18, 100 × 3 mm, 2.7 µm (part number 685975-302)
- Agilent InfinityLab Poroshell HPH-C18, 2.1 mm, 2.7 µm UHPLC guard (part number 821725-928)

Instrument conditions

Chromatographic separation was performed using an InfinityLab Poroshell 120 SB-C18 column connected to an UHPLC guard and installed on an Agilent 1290 Infinity II LC system.

An Agilent Ultivo mass spectrometer with an AJS electrospray ion source was operated in dynamic MRM (dMRM) mode. All data acquisition and processing were performed using Agilent MassHunter software, version 12. The LC/MS/MS system conditions and parameters are shown in Table 1. Retention time, MRM transitions, and collision energy for target analytes are presented in Table 2.

Table 1. LC/MS/MS conditions.

	Parameter	Value
LC	Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm UHPLC guard: Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 5 mm, 2.7 µm
	Column Temperature	45 °C
	Injection Volume	2 µL
	Mobile Phase A	0.1% formic acid in water
	Mobile Phase B	0.1% formic acid in acetonitrile
	Gradient Program	Time (min) %A %B 0 80 20 5 80 20 9 10 90 9.25 10 90 11 5 95 13 5 95 14 80 20
	Postrun	1 min
	Flow	300 µL/min
	Total Run Time	15 min
MS	Ionization Mode	Positive
	Scan Type	Dynamic MRM
	MS1/MS2 Resolution	Unit/Unit
	Gas Temperature	11 L/min
	Gas Flow	250 °C
	Sheath Gas Flow	12 L/min
	Sheath Gas Temperature	350 °C
	Capillary Voltage	3,845 V

Table 2. Target analytes, retention time, MRM transitions, and collision energy.

Analyte	RT (min)	Transitions			
		Quantitative MRM Transition (*m/z*)	CE (V)	Qualitative MRM Transition (*m/z*)	CE (V)
Acephate	1.64	184 → 49.1	20	184 → 143	5
Acetochlor	8.05	270.1 → 148.1	17	270.1 → 224.2	9
Aflatoxin B1	5.84	313.1 → 128.2	89	313.1 → 241.2	41
Aflatoxin B2	5.55	315.1 → 77.2	117	315.1 → 287	29
Aflatoxin G1	5.83	329.1 → 115	89	329.1 → 213.8	41
Aflatoxin G2	5.03	331.1 → 201	41	331.1 → 245.1	33
Aldicarb sulfone	1.86	240.1 → 58.1	37	240.1 → 63.1	37
Azinphos-methyl	7.32	318 → 125	24	318 → 260.9	4
Azoxystrobin	7.49	404.1 → 329.1	32	404.1 → 372.1	8
Bitertanol	7.92	338.2 → 99.1	10	338.2 → 269.3	4
Boscalid	7.76	316.1 → 165	29	316.1 → 247.1	17
Bromuconazole I-II	7.55	376 → 122.8	81	376 → 158.8	41
Bupirimate	7.14	317.2 → 166.1	33	317.2 → 210.2	20
Buprofezin	8.54	306.2 → 116.1	10	306.2 → 201.1	5
Cadusafos	8.61	271.1 → 130.7	21	271.1 → 158.9	12
Carbaryl	6.44	202.1 → 127.1	28	202.1 → 145.1	4
Chlorfenvinphos	8.16	358.9 → 99.2	28	358.9 → 155	8
Citrinin	6.89	251.1 → 90.9	61	251.1 → 115	69
Cyazofamid	8.13	325 → 108	8	325 → 261	4

Analyte	RT (min)	Transitions			
		Quantitative MRM Transition (*m/z*)	CE (V)	Qualitative MRM Transition (*m/z*)	CE (V)
Diazinon (Dimpylate)	8.58	305.1 → 97	40	305.1 → 169.1	32
Diethofencarb	7.39	268.2 → 124	30	268.2 → 226.1	0
Dimethoate	3.58	230 → 125	16	230 → 198.8	0
Dimethomorph(E)	7.17	388.1 → 273.1	32	388.1 → 301	24
Diniconazole	8.13	326.1 → 70	25	326.1 → 159	28
Diuron	6.65	233 → 72	20	233 → 160.1	29
Ethion	9.33	385 → 142.8	24	385 → 199	12
Ethirimol	1.66	210.2 → 98	32	210.2 → 140.1	20
Ethoprophos	7.77	243.1 → 97	30	243.1 → 130.9	15
Etoxazole	9.62	360.2 → 113	60	360.2 → 141	32
Fenamidone	7.53	312 → 92.2	28	312 → 236.2	8
Fenazaquin	10.4	307.2 → 147.1	16	307.2 → 161.1	10
Fenbuconazole	7.77	337.1 → 70	33	337.1 → 125.1	40
Fenhexamid	7.64	302.1 → 55.1	40	302.1 → 97.1	20
Fenpropimorph	6.16	304.3 → 132	40	304.3 → 147	28
Fenpyroximate	9.71	422.2 → 107	64	422.2 → 366.2	12
Flufenoxuron	9.08	489 → 140.9	56	489 → 158	20
Fluquiconazole	7.70	376 → 272.2	40	376 → 348.9	21
Flusilazole	7.76	316.1 → 165	24	316.1 → 247.1	12
Flutolanil	7.85	324.1 → 92.9	37	324.1 → 144.9	65
Flutriafol	6.37	302.1 → 108.9	40	302.1 → 122.9	33
Haloxyfop-2-ethoxyethyl	8.84	434.1 → 90.8	45	434.1 → 316.1	17
Hexaconazole	7.97	314.1 → 124.8	40	314.1 → 159	30
Hexythiazox	9.47	353.1 → 168.1	24	353.1 → 227.9	8
Imidacloprid	3.50	256 → 175	12	256 → 208.9	12
Iprovalicarb	7.44	321.2 → 115.9	17	321.2 → 203.1	5
Linuron	7.39	249 → 160.1	20	249 → 182	17
Mecarbam	8.10	330 → 97.1	45	330 → 227	15
Mepanipyrim	7.8	224.1 → 192.1	29	224.1 → 207.9	17
Methamidophos	1.62	142 → 94	10	142 → 125	10
Methomyl	2.05	163.1 → 88	0	163.1 → 106	4
Monocrotophos	1.70	224.1 → 127	10	224.1 → 193	0
Myclobutanil	7.52	289.1 → 70.1	16	289.1 → 125.1	32
Omethoate	1.63	214 → 109	24	214 → 125	16
Oxadixyl	5.62	279.1 → 132.3	32	279.1 → 219.2	5
Oxamyl	1.82	237.1 → 72	12	237.1 → 220.1	0
Paclobutrazol	7.16	294.1 → 70.1	16	294.1 → 125.2	36
Penconazole	8.07	284.1 → 70.1	15	284.1 → 158.9	37
Phosalone	8.59	368 → 110.9	44	368 → 182	8
Picoxystrobin	8.22	368.1 → 145	20	368.1 → 205.2	4
Pyrazophos	8.46	374.1 → 194.1	37	374.1 → 222.2	21
Pirimiphos-methyl	8.59	306.2 → 108.1	30	306.2 → 164.1	20
Profenofos	8.91	373 → 302.9	17	373 → 344.8	9
Propargite	9.58	368.1 → 175.2	8	368.1 → 231.2	0
Propham	6.99	180.1 → 120	12	180.1 → 138.1	4
Propiconazole	8.18	342.1 → 123	60	342.1 → 159	32

Optimization of the extraction procedure

Five preliminary studies (Figure 1) were conducted to evaluate method accuracy, precision, and matrix cleanliness. For all tests, the olive paste samples were spiked (n = 3) with two concentrations for pesticides and mycotoxins simultaneously.

Analyte	RT (min)	Transitions			
		Quantitative MRM Transition (*m/z*)	CE (V)	Qualitative MRM Transition (*m/z*)	CE (V)
Propyzamid	7.68	256 → 172.9	21	256 → 190	15
Pyridaben	10.20	365.1 → 147.2	20	365.1 → 309.1	4
Simazine	5.76	202.1 → 104	30	202.1 → 132	17
Spiroxamine	6.20	298.3 → 100.1	32	298.3 → 144.1	16
Tebufenozide	9.06	334.2 → 145	37	334.2 → 147.1	24
Tebufenpyrad	8.09	353.2 → 133	16	353.2 → 297.1	0
Thiabendazole	1.64	202 → 65	52	202 → 175	24
Thiodicarb	6.04	355.1 → 88.1	8	355.1 → 108.1	8
Triadimefon	7.64	294.1 → 197.2	8	294.1 → 225.1	20
Triazophos	7.88	314.1 → 118.9	37	314.1 → 162	17
Triticonazol	7.32	318.1 → 69.9	17	318.1 → 124.9	40
Zearalenone	7.33	319.2 → 127.8	65	319.2 → 301.2	5

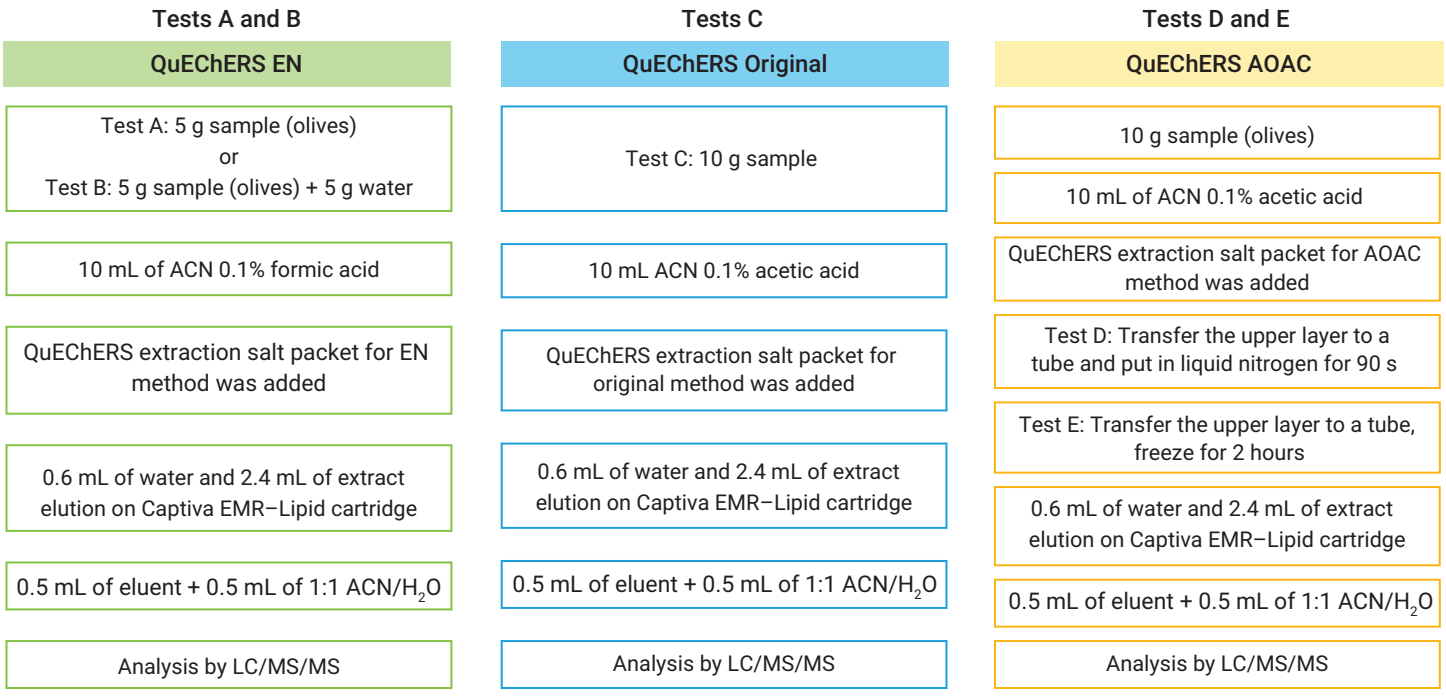

Figure 1. Preliminary extraction protocols scheme.

Final extraction procedure (Figure 2)

1. Weigh 10 ±0.05 g of olive sample into a 50 mL centrifuge tube.
2. Spike with a PIS at 10 ng/kg followed by four spiking levels, cap tightly, vortex, and equilibrate for approximately 15 minutes.
3. Add 10 mL of acetic acid 1% in acetonitrile and shake for 4 minutes.
4. Add a Bond Elut QuEChERS AOAC extraction kit, and shake for 1 minute.
5. Centrifuge at 4,000 rpm for 5 minutes.
6. Transfer the upper layer to a 15 mL tube and place the tubes for 90 seconds in liquid N_2.
7. Transfer 2.4 mL of supernatant into a new tube. Add 0.6 mL of water to mix gently, then load the sample mixture onto a Captiva EMR–Lipid cartridge, 3 mL, 300 mg, and allow gravity flow.
8. Transfer 0.5 mL of eluent to a vial, then dilute with 0.5 mL of ACN:H_2O (1:1, v:v).

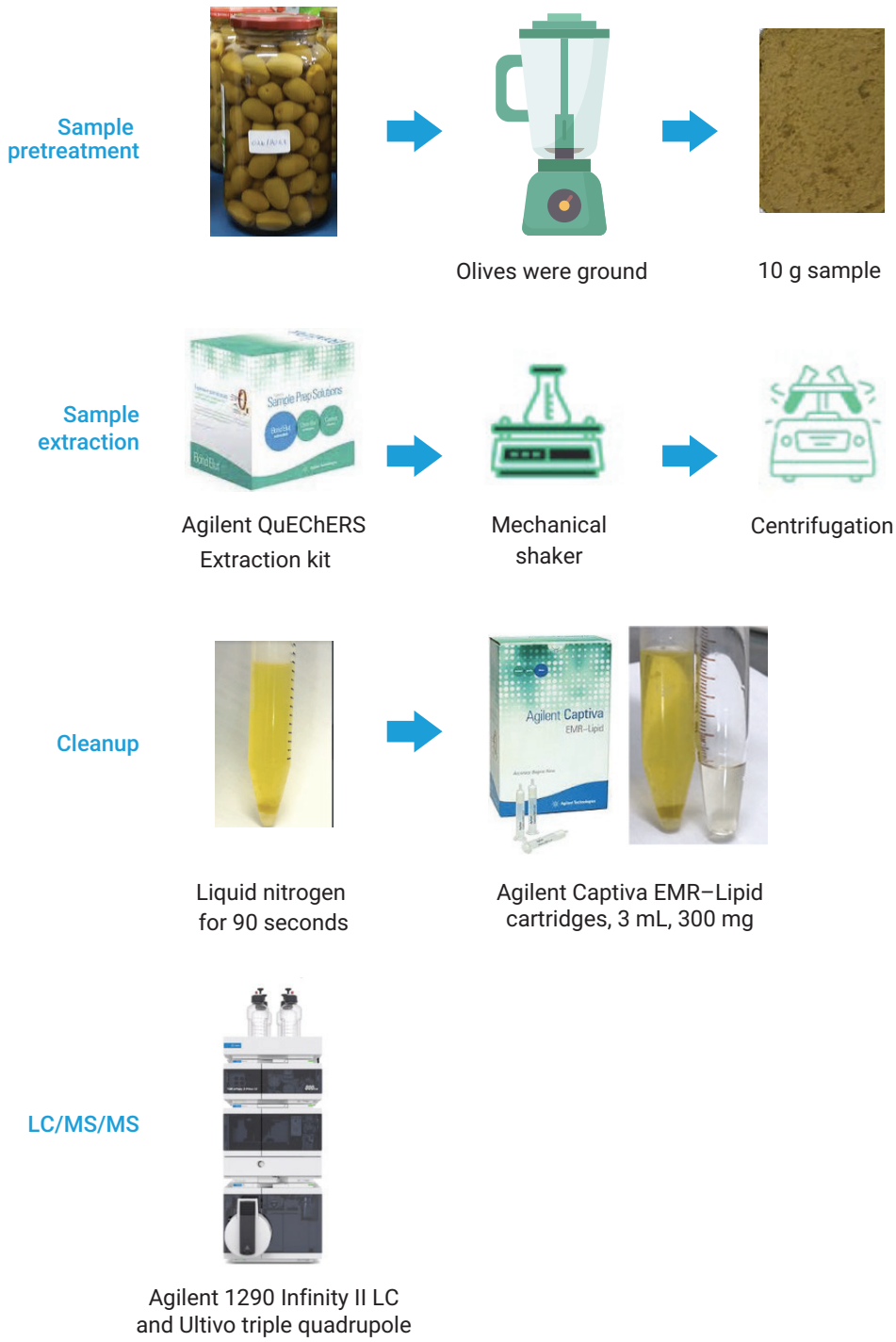

Figure 2. Sample preparation procedure for pesticide and mycotoxin extraction in olives.

Results and discussion

Development of LC/MS/MS method

For each analyte, MRM transitions, as well as fragmentor voltages, collision energies, and most abundant ions were optimized using MassHunter Optimizer software with flow injection. Analyte peaks from both product ions in the extracted ion chromatograms fully overlapped, and the ion ratio from sample extracts were within the range ±30% (relative) of the average of calibration standards from the same sequence.

Sample preparation optimization

The chromatographic profiles obtained in the five tests were also evaluated for selection of the best QuEChERS approach. To investigate the matrix effect of samples prepared by different methods, the final matrix blank extract was postspiked with 50 ng/mL of standard and the overall TIC peak intensities were compared. As demonstrated in Figure 3, overall, TIC chromatograms of sample prepared with methods D and E presented higher intensity for both polar and nonpolar targets than methods A, B and C; this indicates that higher recovery and the lower matrix effect were provided by these two methods. Lesser matrix effects, along with higher target intensities result in more analytes being quantified with more reliable and consistent results at the LOQ level. This confirmed the necessity of adding a step of freezing out before Captiva EMR–Lipid cleanup.

Further comparison of tests D and E was conducted for the liquid N_2 and freezer freezing out procedure by evaluating the targets recoveries. Although the sample freezing out in a freezer is more typical and practical in food testing labs, it was found that the freezer freezing out method resulted in mycotoxin loss. Especially for aflatoxins, it caused approximately 50% loss; for citrinin and zearalenone, it resulted in almost complete loss.

Table 3 shows a comparison study for the targets recovery in samples extracted using procedures E and D. Procedure D was therefore chosen as the final optimized sample preparation method.

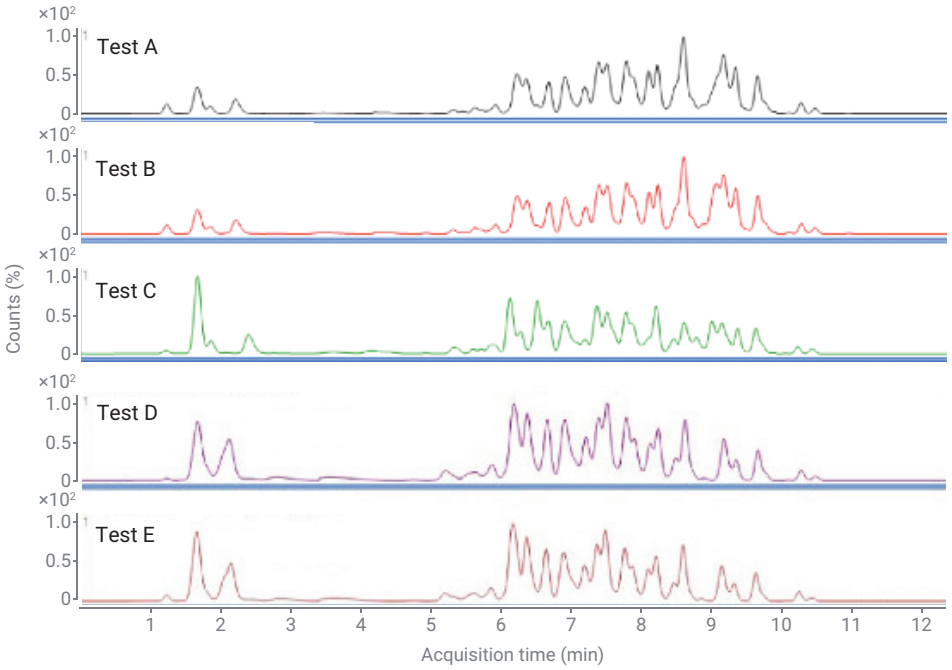

Figure 3. Total ion chromatograms of olive matrix blanks postspiked with standard at 50 ng/mL.

Table 3. Comparison of accuracy and precision (%) of pesticides extracted by tests D and E.

Pesticides	Recovery ±RSD (%)	
	Test D	Test E
Aldicarb-sulfone	117 ±3	Not detected
Bitertanol	78 ±11	50 ±2
Dimethoate	88 ±2	51 ±5
Flufenoxuron	88 ±11	10 ±3
Linuron	88 ±2	43 ±1
Penconazole	83 ±2	Not detected
Thiodicarb	78 ±1	46 ±8
Triadimefon	94 ±9	27 ±17

Validation results

Linearity was determined using calibration curves spiked into the olive extract. The deviation of back-calculated concentration from true concentration was within ±20%. Table 3 shows the pesticides and mycotoxins linear range for the matrix-matched calibration curves. As an example, the calibration curve of acetamiprid in solvent calibration standards and matrix-matched standards is plotted in Figure 4. Most pesticides presented a linear range from 10 to 150 ng/g. Aflatoxins B1, G1, and G2 and ochratoxin A gave a linear range from 2 to 100 ng/g and aflatoxin B2 from 10 to 100 ng/g. For citrinin and zearalenone, the linear range was from 500 to 5,000 ng/g.

The matrix effect (ME) was evaluated from the same analytical sequence of standard solutions used to assess linearity, and the results are summarized in Table 3. Positive ME indicates matrix enhancement, while negative ME suggests matrix suppression. For the entire targets, the developed method provides negligible ME (0 ±20) for 28% of targets, acceptable ME (±50 to ±20, suppression or enhancement) for 42% of targets, and significant ME (±50 to ±100) for the remaining 28% of targets. Due to the high complexity of a high oil content and intermediate water content, more target analytes were impacted by matrix ion suppression.

The limits of quantitation (LOQ) obtained for all targets meet the European Union MRL requirement of 10 ng/g for pesticides in olives.

The acceptance criteria were in accordance with SANTE/11312/2021[6] recoveries (70% to 120%) and RSD values were less than 20%. As shown in Table 4, all pesticides presented LOQs equal to 10 ng/g. For mycotoxins aflatoxins B1, G1, and G2, the method LOQs were 2 ng/g; and for aflatoxin B2, the LOQ was 10 ng/g. Citrinin and zearalenone presented a LOQ of 500 ng/g.

To compare the LOQs obtained in the method with European regulations, the Food and Agriculture Organization of the United Nations (FAO) was consulted. With the goal of protecting public health, the Commission Regulation EC No. 1881/2006 sets forth maximum levels for certain contaminants in foodstuffs to keep them at levels that are toxicologically acceptable. Zearalenone MRLs can vary in food from 20,000 to 400,000 ng/g depending on the type of foodstuff, while the citrinin MRL is fixed at 2,000,000 ng/g. Aflatoxins MRLs are presented as B1 and the sum of B1, B2, G1, and G2, and their values can be between 100 to 15,000 ng/g, also depending on the type of foodstuff. No value has been established for olive matrix yet, but the LOQs provided in this method could be used to meet current FAO limits.[7]

Brazil has also set up MRLs by ANVISA (National Health Surveillance Agency) on RDC nº 7/2011. Depending on the sample type (milk, nuts, cheese, coffee, rice, baby food, and others are listed), zearalenone can vary from 20,000 to 1,000,000 ng/g, while aflatoxins B1, B2, G1, and G2 may vary from 1,000 to 20,000 ng/g. Citrinin was not established in this resolution, and olive matrix is not mentioned[8] by the FAO. In both legislations, the lowest MRLs are defined for food for babies, lactating women, and early childhood.

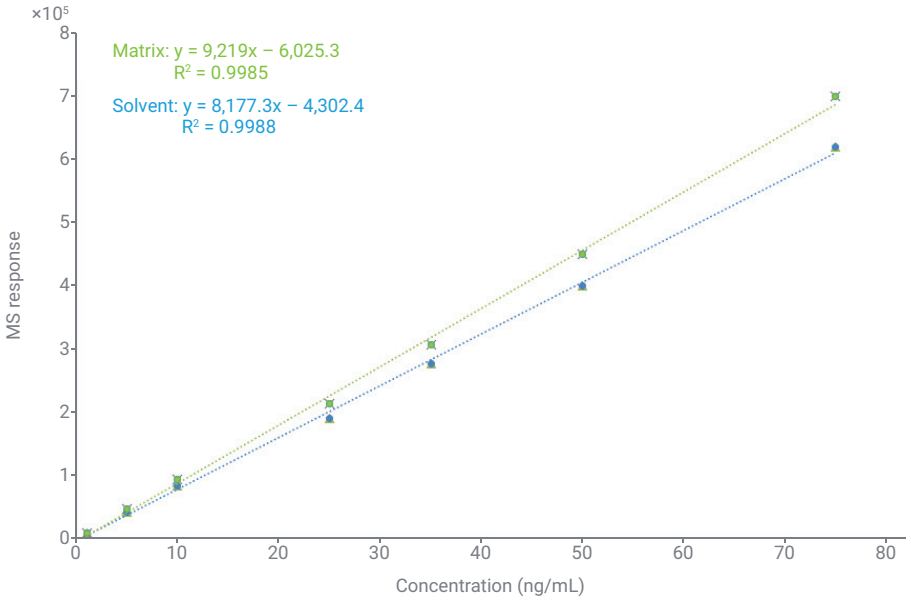

Figure 4. Acetamiprid calibration curves in neat solvent and matrix-matched standards.

Table 4. Limit of quantification (LOQ, ng/g), ME, calibration linear range and R^2, accuracy (n = 6), and RSD% for pesticides and mycotoxins.

Analyte	LOQ (ng/g)	Calibration Curve			Accuracy and RSD%			
		Matrix Effect (%)	R^2	Linear Range (ng/g)	Level 1[a]	Level 2[a]	Level 3[a]	Level 4[a]
Acephate	10	–92	0.9955	10–150	109, 5	101, 6	89, 6	89, 16
Acetochlor	10	–4	0.9990	10–150	99, 7	77, 7	80, 11	98, 7
Aflatoxin B1	2	–63	0.9994	2–100	119, 7	105, 7	95, 3	95, 9
Aflatoxin B2	10	–46	0.9989	10–100	N.A.	113, 6	98, 3	95, 4
Aflatoxin G1	2	1	0.9994	2–100	106, 16	117, 8	95, 5	91, 8
Aflatoxin G2	2	–32	0.9995	2–100	111, 12	104, 4	92, 5	93, 3
Aldicarb sulfone	10	–84	0.9975	10–150	117, 4	105, 5	97, 2	80, 8
Azinphos-methyl	10	–51	0.9847	10–150	87, 9	103, 13	107, 5	93, 12
Azoxystrobin	10	10	0.9980	10–100	119, 4	109, 2	111, 4	90, 9
Bitertanol	10	–43	0.9994	10–150	107, 12	92, 8	95, 10	79, 12
Boscalid	10	–33	0.9829	10–150	92, 11	94, 3	98, 6	78, 15
Bromuconazole I-II	10	–54	0.9995	2–150	102, 10	90, 3	92, 3	81, 13
Bupirimate	10	–31	0.9978	10–150	104, 4	97, 4	98, 3	81, 12
Buprofezin	10	–17	0.9995	10–150	97, 3	83, 3	81, 8	80, 3
Cadusafos	10	–17	0.9965	10–150	97, 3	87, 11	97, 18	94, 15
Carbaryl	10	–60	0.9997	10–150	106, 9	98, 8	97, 8	99, 8
Chlorfenvinphos	10	–12	0.9997	10–150	107, 14	96, 12	104, 12	104, 13
Citrinin	500	–22	0.9234	500–5,000	N.A.	76, 4	93, 1	93, 3
Cyazofamid	10	–32	0.9878	10–150	116, 11	98, 15	119, 6	118, 9
Diazinon	10	–14	0.9971	10–150	93 ,9	91, 4	93, 7	95, 7
Diethofencarb	10	–7	0.9841	10–150	101, 8	101, 6	104 ,6	101, 4
Dimethoate	10	–37	0.9980	10–150	114, 3	98, 2	97, 2	100, 2
Dimethomorph(E)	10	22	0.9958	10–150	108, 4	101, 4	102, 5	101, 4
Diniconazole	10	–42	0.9992	10–150	98, 14	86, 11	88, 13	89, 12
Diuron	10	–70	0.9989	10–150	102, 13	101, 6	101, 5	102, 2
Ethion	10	–43	0.9998	10–150	87, 13	80, 9	84, 8	81, 3
Ethirimol	10	–81	0.9929	10–150	81, 4	71, 5	57, 3	55, 5
Ethoprophos	10	–12	0.9860	10–150	90, 8	94, 7	96, 8	90, 7
Etoxazole	10	–11	0.9996	10–150	87, 3	81, 3	79, 3	78, 3
Fenamidone	10	–43	0.9992	10–150	116, 6	103, 4	103, 5	103, 5
Fenazaquin	10	–21	0.9997	2–150	68, 2	60, 3	57, 6	57, 4
Fenbuconazole	10	–8	0.9818	10–150	91, 18	97, 12	94, 9	93, 9
Fenhexamid	10	–60	0.9983	10–150	110, 16	91, 10	92, 10	92, 7
Fenpropimorph	10	–33	0.9989	10–150	70, 2	63, 3	63, 3	63, 2
Fenpyroximate	10	–21	0.9993	10–150	81, 4	72, 3	70, 3	71, 3
Flufenoxuron	10	–37	0.9991	10–150	93, 15	96, 17	93, 10	93, 8
Fluquiconazole	10	–50	0.9988	10–150	97, 14	95, 9	94, 9	90, 9
Flusilazole	10	–33	0.9828	10–150	92, 11	94, 3	98, 6	94, 7
Flutolanil	10	–16	0.9971	10–150	109, 13	103, 8	105, 9	100, 8
Flutriafol	10	–87	0.9971	10–150	118, 7	113, 11	108, 9	108, 8
Haloxyfop-2-ethoxyethyl	10	–35	0.9986	10–150	95, 11	95, 6	96, 9	97, 7
Hexaconazole	10	–51	0.9982	10–150	88, 7	84, 9	86, 10	85, 8
Hexythiazox	10	–42	0.9974	10–150	94, 12	76, 10	70, 3	72, 8

Analyte	LOQ (ng/g)	Calibration Curve			Accuracy and RSD%			
		Matrix Effect (%)	R^2	Linear Range (ng/g)	Level 1[a]	Level 2[a]	Level 3[a]	Level 4[a]
Imidacloprid	10	116	0.9986	10–150	120, 3	105, 1	102, 2	103, 3
Iprovalicarb	10	8	0.9962	10–150	108, 7	103, 2	107, 6	102, 4
Linuron	10	–25	0.9374	10–150	83, 16	90, 17	109, 12	101, 10
Mecarbam	10	–11	0.9844	10–150	107, 12	105, 14	108, 17	102, 14
Mepanipyrim	10	–39	0.9953	10–150	71, 11	76, 9	79, 9	102, 14
Methamidophos	10	–87	0.9991	2–150	88, 6	79, 3	79, 3	77, 3
Methomyl	10	–66	0.9991	10–150	108, 3	95, 3	95, 3	96, 3
Monocrotophos	10	–79	0.9931	10–150	105, 15	86, 8	81, 6	81, 5
Myclobutanil	10	–15	0.9895	10–150	108, 12	106, 14	108, 10	104, 8
Omethoate	10	–90	0.9978	10–150	100, 8	84, 6	76, 6	77, 6
Oxadixyl	10	–23	0.9982	10–150	107, 5	90,± 4	85, 3	85, 4
Oxamyl	10	–68	0.9978	10–150	120, 3	105, 4	101, 3	101, 3
Paclobutrazol	10	–52	0.9864	10–150	97, 7	102, 5	107, 5	104, 3
Penconazole	10	–35	0.9925	10–150	89, 15	88, 10	93, 9	93, 7
Phosalone	10	–34	0.9941	10–150	72, 11	114, 19	96, 19	106, 19
Picoxystrobin	10	–16	0.9971	10–150	112, 6	105, 6	106, 6	108, 5
Pyrazophos	10	–11	0.9910	10–150	94, 20	90, 7	101, 16	98, 17
Pirimiphos-methyl	10	–20	0.9972	10–150	85, 10	85, 6	88, 9	87, 7
Profenofos	10	–36	0.9972	10–150	98, 9	74, 10	75, 12	74, 10
Propargite	10	–27	0.9991	10–150	92, 9	83, 8	79, 6	81, 7
Propham	10	–29	0.9993	10–150	105, 13	99, 8	98, 6	100, 6
Propiconazole	10	–38	0.9985	10–150	89, 13	88, 11	91, 12	94, 8
Propyzamid	10	-–44	0.9994	10–150	107, 12	92, 7	85, 11	88, 6
Pyridaben	10	–20	0.9993	10–150	118, 4	91, 4	77, 8	74, 6
Simazine	10	–71	0.9976	10–150	89, 3	78, 3	73, 3	75, 2
Spiroxamine	10	–20	0.9986	10–150	73, 2	70, 3	73, 2	75, 1
Tebufenozide	10	–2	0.9902	10–150	117, 10	109, 11	118, 13	111, 9
Tebufenpyrad	10	–47	0.9992	10–150	73, 7	72, 15	71, 7	75, 9
Thiabendazole	10	–91	0.9929	10–150	92, 7	73, 9	72, 12	70, 12
Thiodicarb	10	63	0.9996	2–150	112, 4	99, 3	97, 3	97, 3
Triadimefon	10	–28	0.9991	10–150	110, 5	93, 8	91, 6	86, 7
Triazophos	10	–11	0.9997	10–150	116, 10	102, 8	103, 11	100, 11
Triticonazol	10	–53	0.9990	10–150	90, 14	89, 7	92, 3	94, 3
Zearalenone	250	–50	0.9659	100–5,000	N.A.	99, 13	97, 14	98, 10

[a] Analytes have a spiking level of: Group 1: 10, 20, 50, and 70 ng/g. Group 2: 2, 5, 10, and 20 ng/g. Group 3: 100, 250, 500, and 1,000 ng/g.
N.A. = not available

Conclusion

The Agilent Ultivo LC/MS/MS demonstrated great performance in pesticides and mycotoxins determination in olives, and was shown to be accurate, robust, and sensitive. In general, extraction with 10 g of the sample instead of 5 g proved to be advantageous in terms of method sensitivity, with an acceptable matrix effect.

The analytical method enabled good accuracy and precision. In validation, 77 compounds (71 pesticides and six mycotoxins) were successfully validated. The method can be applied in olive analysis to reach MRLs requested by FAO and Brazilian ANVISA for mycotoxins. This study demonstrated that the method is suitable for simultaneous extraction and determination of pesticides and mycotoxins in olives and can be recommended for implementation in routine analysis intended for screening or monitoring programs.

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