



QuEChERS Sample Preparation For The Analysis Of Pesticide Residues In Olives*

UCT Product Number:

ECMSSC50CT-MP (4 g MgSO₄, 1.0 g NaCl)

ECQUEU122CT (2 mL centrifuge tube, 150 mg MgSO₄, 50 mg PSA, 50 mg C18 and 50 mg GCB)

CUMPSC1875CB2CT – For better recovery of planar pesticides (2 mL centrifuge tube, 150 mg MgSO₄, 50 mg PSA, 50 mg C18, 7.5 mg GCB)

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Summary

This application is a summary of the original paper “Evaluation of the QuEChERS sample preparation approach for the analysis for pesticide residues in olives”*. It describes the use of QuEChERS for the extraction and clean-up of 16 pesticide residues contained in olives. LC-MS/MS with positive ESI was used for pesticides that are difficult to detect by GC-MS. Matrix matched calibration standards were used to compensate for matrix effects. The method achieves acceptable quantitative recoveries of 70–109% with RSDs <20% for DSI-GC-MS and 88–130% with RSDs <10% for LC-MS/MS, and LOQ at or below the regulatory maximum residue limits. Analyte protectants were used with DSI to improve analyte peak shapes and intensities.

Analytes Covered in this Method

Analyte	CASRN
Ometholate	1113-02-6
Dimethoate	60-51-5
Simazine	122-34-9
Diazinon	65863-03-8
p,p'-DDE	82413-20-5
Diuron	56449-18-4
Carbaryl	63-25-2
Malathion	121-75-5
Fenthion	55-38-9
Methidathion	950-37-8
Napropamide	15299-99-7
Oxyfluorfen	42874-03-3
Carfentrazone-ethyl	128639-02-1
Phosmet	732-11-6

Pyriproxyfen	95737-68-1
Deltamethrin	64121-95-5

1. Sample Extraction

- a) Weigh 10 g of homogenized sample into a 50 mL centrifuge tube
- b) Add 10 mL of acetonitrile (MeCN)
- c) Add contents of **ECMSSC50CT-MP**
- d) Shake vigorously by hand for 1 minute
- e) Centrifuge @ 3450 rcf for 1 minute

2. Dispersive Clean-Up

- a) Transfer 1 mL the supernatant to a micro-centrifuge tube **ECQUEU122CT**
or CUMPSC1875CB2CT
- b) Mix for 20 s
- c) Centrifuge @ 3450 rcf for 1 minute
- d) Transfer 400µL of supernatant to an autosampler vial
- e) Add 25 µL of TPP solution (10 g/mL triphenylphosphate on MeCN with 1.6% formic acid)
- f) Shake for 5 s
- g) Extract is ready for analysis

3. Automated DSI-GC-MS Analysis

GC-MS was performed using an Agilent (Little Falls, DE, USA) 5890 Series II GC and 5972 MS instrument. Injection was performed using a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland) using second generation automated DSI accessory (Linex) in combination with an Optic 3 PTV (Atas-GL International BV, Veldhoven, NL)

Note: Equivalent instrumentation and analytical columns can be used

Analyte Protectant Solution

(95% or better purity, prepare at 10:1:1 mg/mL in 7:3 water/MeCN, Sigma or Fluka)

- 3-ethoxy-1,2-propanediol
- D-sorbitol
- L-gulonic acid
- c-lactone

A quality check standard solution of 16 µg/mL triphenylphosphate (TPP) is prepared in MeCN containing 1.6% formic acid (FA)

For analysis by DSI-GC-MS, 20 μL of the analyte protectant solution was added to all the final extracts and matrix-matched calibration standards by transfer of 400 μL of extract into an autosampler vial and adding 25 μL of TPP solution

Conditions:

- Injection volume 10 μL
- 100° C (held 3.5 min with 50:1 split ratio)
- Ramp at 5° C/s to 280°C (use splitless for 3.5 min, then 50:1 split until 9 min, then change split flow to 20:1 and cool injector temperature to 150° C)

GC Separation:

- Varian VF-5 EZ-guard column (30 m x 0.25 mm id x 0.25 μm film thickness) with an integrated retention gap (5 m x 0.25 mm) at the inlet and an additional 1 m of uncoated capillary at the MS entrance
- He carrier gas @ 1 mL/ min

Oven temperature program:

- Start at 3.5 min (after sample introduction)
- 80° C hold for 3.5 min
- Ramp to 230° C at 108° C/min
- Then ramp to 300° C at 45° C/min, hold for 10 min.
- MS transfer line temperature at 290° C
- Electron ionization (EI) at -70 eV in SIM and full-scan (50–600 m/z) modes in different experiments

Agilent Chemstation for data acquisition/processing and GC-MS control, and Cycle Composer and Atas Evolution software are used to control the automated DSI process and PTV. The pesticide analytes in GC-MS and SIM ions are shown in the table below.

GC-MS SIM Conditions for the Monitored Pesticides

Pesticide	Start time (min)	t _R (min)	m/z (% relative abundance)	
			Quantitation ion	Qualifier ions
Dimethoate	4.5	15.89	87 (100)	125 (45), 93 (54), 58 (19)
Simazine		16.00	201 (78)	173 (41), 186 (51), 158 (25)
Diazinon	16.09	16.18	179 (100)	137 (98), 304 (47), 152 (70)
Diuron		16.52	72 (100)	232 (38), 234 (26), 187 (11)
Carbaryl	17.49	17.70	144 (100)	115 (33), 116 (26), 145 (15)
Malathion		18.03	173 (94)	125 (100), 93 (93), 127 (75)
Fenthion	18.1	18.27	278 (100)	125 (37), 109 (33), 79 (19)
Methidathion	19.05	19.29	145 (88)	93 (40), 125 (27), 302 (19)
Napropamide	13.39	19.58	271 (26)	72 (100), 128 (63)
p,p'-DDE		19.367	318 (64)	246 (100), 248 (64), 316 (56)
Oxyfluorfen		19.71	361 (38)	252 (100), 300 (35), 280 (14)
Carfentrazone-ethyl	20	20.28	312 (100)	330 (65), 340 (63), 376 (31)
TPP	20.38	20.96	326 (100)	325 (87), 77 (88), 215 (20)
Phosmet		21.17	160 (100)	133 (15), 104 (15), 193 (4)
Pyriproxyfen	21.30	21.50	136 (100)	226 (12), 185 (6)
Deltamethrin	22.8	23.59	253 (85)	181 (100), 251 (44), 152 (20)

4. LC-MS/MS Analysis

Suggested Instrumentation: Agilent 1100 HPLC (consisting of vacuum degasser, autosampler Model WPALS, and a binary pump) equipped with a Prodigy ODS-3 (150 mm x 3 mm) and 5 µ particle size analytical column coupled to a ODS-C18 (4 mm x 2 mm and 5 µ particle size) guard column from Phenomenex (Torrance, CA, USA).

- Column temperature: 30° C
- Injection volume 5 µl.
- Mobile phase A water, B MeCN, both with 0.1% FA
- Gradient program:
 - Flow rate 0.3 mL/min
 - 25% solvent B linear gradient to 100% over the first 5 min
 - Hold for 7 min until 12 min
 - 11-min post run column wash

The LC system is connected to an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Toronto, Canada) operated in ESI positive mode. Optimizations of the mass analyzer parameters were done by infusion of 1 µg/mL analyte solutions at 10 µL/min with a

syringe pump (Harvard Apparatus, Holliston, MA, USA) using the autotune function.

Note: Equivalent instrumentation and analytical columns can be used

Final MS/MS conditions include:

- N₂ pressure 55 psi
- nebulizer gas setting 14
- curtain gas setting 11
- collision gas setting 12
- 4200 V ionspray voltage
- ESI temperature 525° C
- focusing potential 100 V
- entrance potential 10 V
- 0.15 s dwell time

The pesticide analytes by LC-MS/MS are shown in the table below with respective analytical ions

LC-MS/MS Conditions for the Monitored Pesticides

(Quantitation ion is shown as first mass)

Pesticide	Start time (min)	t _R (min)	Precursor ion (m/z)	Product ions (m/z)
Omethoate	2.5	2.68	214.0	183.2, 125.2
Dimethoate	5	6.83	230.0	199.1, 125.1
Simazine	7.6	7.98	202.0	124.2, 132.2
Carbaryl		8.48	202.2	145.1, 127.1
Diuron		8.67	233.1	72.2, 160.1
Phosmet	9	9.27	318.0	160.2, 133.2
Methodathion		9.28	303.0	145.1, 85.1
Malathion		9.64	331.0	127.2, 285.2
TPP	9.8	10.18	327.0	77.2, 152.0

*Adapted and used by permission from Cunha, Sara C., Lehotay, Steven J., Mastovska, Katerina, Fernandes, Jos O., Beatriz, Maria, Oliveira, P. P., Sep. Sci. 2007, 30, 620 – 632, DOI 10.1002/jssc.200600410

Listing of instrument manufacturers and standards suppliers does not constitute endorsement by UCT. Equivalent systems may be used