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Supplementary data

Experimental and theoretical determination of pesticide processing factors to model their behavior during virgin olive oil production

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Procedures

Sample treatment for pesticides with low or medium polarity. Briefly, a homogenized portion of 10 g of olives samples (in the case of olive oil, 3 g of oil + 7 mL H_2O Milli-Q) was weighed into 50 mL centrifuge tube; 10 mL MeCN (1% acetic acid) were added together with 4 g of $MgSO_4$ and 1 g of NaCl and immediately was shaken for 1 min. The mixture was centrifuged during 5 min at 2655 g. For the cleanup step, 1 g of EMR sorbent was activated with 5 mL of H_2O Milli-Q prior to use it. Afterwards, 5 mL of the upper layer (MeCN) was added into the centrifuge tube and shaken for 1 min and then centrifuged at 2655 × *g* for 5 min. A cleanup step was carried out transferring 5 mL of the centrifuged extract to a second centrifuge tube containing 1.6 g of $MgSO_4$ and 0.4 g of NaCl, which was shaken and centrifuged again. Then, this organic extract was diluted 1:10 by dissolving 100 µL of each extract with 900 µL of H_2O in the case of pesticides analyzed by LC, whereas 100 µL of each extract was dissolved with 200 µL of ethyl acetate and 700 µL of n-hexane for pesticides analyzed by GC. Finally, the final extract was filtered through 0.45 µm pore diameter filter.

Sample treatment for polar pesticides (QuPPe). A representative portion of olive oil sample of 10 g was weighed into a 50 mL centrifuge tube. Afterwards, 10 mL of MeOH (1% HCOOH) was added together with 10 mL of H_2O Milli-Q and immediately shaken for 1 min. An intermediate heating step was implemented to quantitatively extract diquat and paraquat (80°C during 15 min). Once the mixture reached the ambient temperature, was shaken for 1 min and thereupon it was centrifuged during 3 min at 1301 × *g*. Finally, an aliquot of 1 mL of aqueous phase (the highest density phase), was diluted with 4 mL MeCN (1% HCOOH), achieving a dilution factor of 10. Last, and prior the analysis, the final extract was filtered through 0.45 µm pore diameter filter.

Determination of pesticides by liquid chromatography tandem mass spectrometry. Two LC methods were used, one of them for polar pesticides (HILIC-MS/MS) and other for pesticides with lower or moderate polarity using a C18 column. LC-MS/MS were carried out with an UHPLC system (Dionex Ultimate 3000, Thermo Scientific, USA) instrument. The UHPLC system was coupled to a TSQ Quantiva triple quadrupole (QqQ) (Thermo, Scientific,

USA) equipped with a heated electrospray ionization probe (HESI) operating in positive and negative ion mode. The analytical parameters were the following: spray voltage, 3500 V; sheath gas, 45 arbitrary units (a.u.); aux gas, 13 a.u.; ion transfer tube temperature 342 °C; collision gas (CID gas) 1.5 mTorr. Xcalibur software 4.0.27.10 was used for method development, whereas Tracefinder software 3.2.5.12.0 (Thermo Scientific, USA) was employed for data analysis. Mass spectrometry conditions and multiple reaction monitoring (MRM) transitions were optimized individually for each pesticide.

Determination of pesticides by reversed phase LC-MS/MS. The separation of the nonpolar and low polarity analytes was carried out with an Agilent Zorbax Rapid Resolution High Definition (RRHD) Eclipse Plus C18 column (2.1 mm i.d. x 50 mm, 1.8 µm particle size). H_2O and MeCN were used as mobile phases both with 0.1% of formic acid at a flow rate of 0.3 $mL \cdot min^{-1}$. The temperature of column was 25 °C and the injection volume was 10 µL. The gradient profile held the initial mobile phase composition (5% MeCN) constant flow 3 min, followed by a linear gradient to 100% MeCN up to 18 min, holding this concentration for two additional minutes.

Determination of polar pesticides by hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC-MS/MS. In order to carry out the separation of polar pesticides (amitrol, cyromazine, diquat, fosetyl, mepiquat, paraquat and trimesium) an Agilent Zorbax RRHD HILIC Plus column (2.1 mm i.d. x 100 mm, 1,8 µm particle size) was employed. Mobile phases were H_2O and MeCN; H_2O was adjusted with ammonium formate (100 mM) and pH of 2.85 using formic acid, and MeCN with 0.1% of formic acid (v/v). The initial conditions of the chromatographic method started with aqueous phase 90% and a flow rate of 0.2 $mL \cdot min^{-1}$. During the first 10 min a linear progression was held until to hit 80% of H_2O, then it was decreased until 40% and a flow or 0.3 $mL \cdot min^{-1}$ in 3 min. The last step was kept it during 3 min more being a total time of the analysis of 16 min.

Determination of pesticides by gas chromatography tandem mass spectrometry (GC-MS/MS). The rest of target pesticides (37) were analyzed by means of GC-MS technique. The optimum analytical parameters for each compound were evaluated individually. In order to choose the ideal voltage for each compound a fragmentation study was carried

out. A Focus gas chromatograph (Thermo Scientific, USA) equipped with a Varian FactorFour VF-5 ms capillary column of 30.0 m x 0.25 mm i.d. and 0.25 µm of film size (Varian Inc., Walnut Creek, CA, USA) was used for chromatographic separation. As carrier gas was employed helium with a flow rate of 1 $mL \cdot min^{-1}$. The injection volume of sample was of 4 µL in mode splitless with surge pressure (50 $mL \cdot min^{-1}$ during 1 min and 250 kPa during 0.5 min respectively). A splitless liner (5mm i.d.) covered with carbofrit was coupled in the injection port. A gradient of temperature was employed for the separation of the compounds, being the initial temperature established at 70 °C and hold during 2 min. Afterwards, a first ramp with a rate of 10 $°C \cdot min^{-1}$ was applied up to achieve 180 °C. Once it was remained during 5 min, the temperature was increased with a second raise up of 6 $°C \cdot min^{-1}$ until at 260 °C, straightaway a new ramp was applied with a rate of 4 $°C \cdot min^{-1}$ to achieve 300 °C, remaining constant during 2 min. The gas chromatograph was connected through a transfer line (at 300 °C) with a mass spectrometer Polaris Q-Ion Trap (Thermo Scientific, USA). The ionization of compounds was carried out by means of electron impact operating at 70 eV. A filament current of 250 µA and a multiplier voltage of 1638 V were used in MS mode. Specific MS/MS transitions were recorded for each pesticide analyzed with a scan event time of 0.39 s. Xcalibur software 2.0 (Thermo Scientific, USA) was used in order to development of the analytical method and data processed (Table SD3).

111 **Table SD1.** Features of all compounds studied and analytical technique employed.

Pesticide	Elemental composition	Pesticide class	Method selected
Acephate	$C_4H_{10}NO_3PS$	Organophosphorous	RP-LC
Acetamiprid	$C_{10}H_{11}ClN_4$	Neonicotinoide	RP-LC
Alachlor	$C_{14}H_{20}ClNO_2$	Chloroacetamide	RP-LC
Amitrol	$C_2H_4N_4$	Triazole	HILIC-LC
Atrazine	$C_8H_{14}ClN_5$	Triazine	RP-LC
Azinphos-ethyl	$C_{12}H_{16}N_3O_3PS_2$	Organophosphorous	GC
Azinphos-methyl	$C_{10}H_{12}N_3O_3PS_2$	Organophosphorous	RP-LC
Azoxystrobin	$C_{22}H_{17}N_3O_5$	Strobilurin	RP-LC
Benalaxyl	$C_{20}H_{23}NO_3$	Acylalanine	RP-LC
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	Pyrethroid	GC
Bromopropylate	$C_{17}H_{16}Br_2O_3$	Benzilate	GC
Buprofezin	$C_{16}H_{23}N_3OS$	-	RP-LC
Captan	$C_9H_8Cl_3NO_2S$	Phthalimide	GC
Carbaryl	$C_{12}H_{11}NO_2$	Carbamate	RP-LC
Carbendazim	$C_9H_9N_3O_2$	Benzimidazole	RP-LC
Carbofuran	$C_{12}H_{15}NO_3$	Carbamate	RP-LC
Carfentrazone-ethyl	$C_{15}H_{14}Cl_2F_3N_3O_3$	Triazolinone	RP-LC
Chlorfenvinphos	$C_{12}H_{14}Cl_3O_4P$	Organophosphorous	RP-LC
Chlorothalonil	$C_8Cl_4N_2$	Chloronitrile	GC
Chlorotoluron	$C_{10}H_{13}ClN_2O$	Urea	RP-LC
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	Organophosphorous	GC
Chlorpyrifos-methyl	$C_7H_7Cl_3NO_3PS$	Organophosphorous	GC
Cyhalothrin-λ	$C_{23}H_{19}ClF_3NO_3$	Pyrethroid	GC
Cyfluthrin-β	$C_{22}H_{18}Cl_2FNO_3$	Pyrethroid	GC
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	Pyrethroid	GC
Cyromazine	$C_6H_{10}N_6$	Triazine	HILIC-LC
Diazinon	$C_{12}H_{21}N_2O_3PS$	Organophosphorous	GC
Dichlobenil	$C_7H_3Cl_2N$	Benzonitrile	GC
Dicofol	$C_{14}H_9Cl_5O$	Organochlorine	GC
Difenoconazol	$C_{19}H_{17}Cl_2N_3O_3$	Triazole	RP-LC
Diflufenican	$C_{19}H_{11}F_5N_2O_2$	Pyridinecarboxamide	RP-LC
Dimethoate	$C_5H_{12}NO_3PS_2$	Organophosphorous	RP-LC
Dimethomorph	$C_{21}H_{22}ClNO_4$	Cinnamic acid	RP-LC
Diquat	$C_{12}H_{12}N_2^{+2}$	Bipyridyliu	HILIC-LC
Diuron	$C_9H_{10}Cl_2N_2O$	Urea	RP-LC

Dodine	$C_{15}H_{33}N_3O_2$	Guanidine	RP-LC
Endosulfan-sulfate	$C_9H_6Cl_6O_4S$	Organochlorine	GC
Endosulfan-α	$C_9H_6Cl_6O_3S$	Organochlorine	GC
Endosulfan-β	$C_9H_6Cl_6O_3S$	Organochlorine	GC
Ethion	$C_9H_{22}O_4P_2S_4$	Organophosphorous	GC
Fenamiphos	$C_{13}H_{22}NO_3PS$	Organophosphorous	RP-LC
Fenamiphos-sulfone	$C_{13}H_{22}NO_4PS$	Organophosphorous	RP-LC
Fenhexamid	$C_{14}H_{17}Cl_2NO_2$	Hydroxyanilide	RP-LC
Fenitrothion	$C_9H_{12}NO_5PS$	Organophosphorous	GC
Fenpropathrin	$C_{22}H_{23}NO_3$	Pyrethroid	GC
Fenthion	$C_{10}H_{15}O_3PS_2$	Organophosphorous	RP-LC
Fenthion-sulfoxide	$C_{10}H_{15}O_4PS_2$	Organophosphorous	RP-LC
Fenvalerate	$C_{25}H_{22}ClNO_3$	Pyrethroid	GC
Fipronil	$C_{12}H_4Cl_2F_6N_4OS$	Phenylpyrazole	GC
Fluroxypyr	$C_7H_5Cl_2FN_2O_3$	Pyridinecarboxylic acids	RP-LC
Fluvalinate-τ	$C_{26}H_{22}ClF_3N_2O_3$	Pyrethroid	GC
Folpet	$C_9H_4Cl_3NO_2S$	Phthalimide	GC
Fosetyl	$C_2H_6O_3P^-$	-	HILIC-LC
Gibberellic Acid	$C_{19}H_{22}O_6$	-	RP-LC
Imidacloprid	$C_9H_{10}ClN_5O_2$	Neonicotinoide	RP-LC
Iprodione	$C_{13}H_{13}N_3Cl_2O_3$	Dicarboximide	GC
Isoproturon	$C_{12}H_{18}N_2O$	Urea	RP-LC
Kresoxim-methyl	$C_{18}H_{19}NO_4$	Strobilurin	RP-LC
Lindane	$C_6H_6Cl_6$	Organochlorine	GC
Malaoxon	$C_{10}H_{19}O_7PS$	Organophosphorous	RP-LC
Malathion	$C_{10}H_{19}O_6PS_2$	Organophosphorous	RP-LC
Mepiquat	$C_7H_{16}N^+$	Quaternary ammonium	HILIC-LC
Metalaxyl	$C_{15}H_{21}NO_4$	Acylalanine	RP-LC
Methamidophos	$C_2H_8NO_2PS$	Organophosphorous	RP-LC
Methidathion	$C_6H_{11}N_2O_4PS_3$	Organophosphorous	GC
Methomyl	$C_5H_{10}N_2O_2S$	Oxime carbamate	RP-LC
Monocrotophos	$C_7H_{14}NO_5P$	Organophosphorous	RP-LC
Norflurazon	$C_{12}H_9ClF_3N_3O$	Pyridazinone	RP-LC
Omethoate	$C_5H_{12}NO_4PS$	Organophosphorous	RP-LC
Oxyfluorfen	$C_{15}H_{11}ClF_3NO_4$	Diphenyl ether	GC
Paclobutrazol	$C_{15}H_{20}ClN_3O$	Triazole	RP-LC

Paraquat	$C_{12}H_{14}N_2^{+2}$	Bipyridylium	HILIC-LC
Parathion	$C_{10}H_{14}NO_5PS$	Organophosphorous	GC
Parathion-methyl	$C_8H_{10}NO_5PS$	Organophosphorous	GC
Penconazol	$C_{13}H_{15}Cl_2N_3$	Triazole	RP-LC
Permethrin	$C_{21}H_{20}Cl_2O_3$	Pyrethroid	GC
Phosmet	$C_{11}H_{12}NO_4PS_2$	Organophosphorous	RP-LC
Pirimiphos-methyl	$C_{11}H_{20}N_3O_3PS$	Organophosphorous	RP-LC
Procymidone	$C_{13}H_{11}Cl_2NO_2$	Dicarboximide	GC
Profenofos	$C_{11}H_{15}BrClO_3PS$	Organophosphorous	GC
Pyrazophos	$C_{14}H_{20}N_3O_5PS$	Phosphorothiolate	GC
Pyrimethanil	$C_{12}H_{13}N_3$	Anilinopyrimidine	RP-LC
Pyriproxifen	$C_{20}H_{19}NO_3$	Juvenile hormon mimic	GC
Quinalphos	$C_{12}H_{15}N_2O_3PS$	Organophosphorous	RP-LC
Quinmerac	$C_{11}H_8ClNO_2$	Quinolinecarboxylic acid	RP-LC
Quizalofop-p-ethyl	$C_{19}H_{17}ClN_2O_4$	Ariloxifenoxi propionato	RP-LC
Rotenone	$C_{23}H_{22}O_6$	Rotenoide	RP-LC
Simazine	$C_7H_{12}ClN_5$	Triazine	RP-LC
Spinosyn A	$C_{41}H_{65}NO_{10}$	Spynosin	RP-LC
Spinosyn D	$C_{41}H_{65}NO_{10}$	Spynosin	RP-LC
Tebuconazole	$C_{16}H_{22}ClN_3O$	Triazole	RP-LC
Teflubenzuron	$C_{14}H_6Cl_2F_4N_2O_2$	Benzoylurea	RP-LC
Terbuthylazine	$C_9H_{16}ClN_5$	Triazine	RP-LC
Terbuthylazine-desethyl	$C_7H_{12}ClN_5$	Triazine	RP-LC
Tetraconazole	$C_{13}H_{11}Cl_2F_4N_3O$	Triazole	RP-LC
Tetradifon	$C_{12}H_6Cl_4O_2S$	-	GC
Thiacloprid	$C_{10}H_9ClN_4S$	Neonicotinoide	RP-LC
Thiamethoxam	$C_8H_{10}ClN_5O_3S$	Neonicotinoide	RP-LC
Thiophanate-methyl	$C_{12}H_{14}N_4O_4S_2$	Benzimidazole	RP-LC
Tolclofos-methyl	$C_9H_{11}Cl_2O_3PS$	Aromatic hydrocarbon	GC
Trichlorfon	$C_4H_8Cl_3O_4P$	Organophosphorous	RP-LC
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	Dinitroaniline	GC
Trimethyl-sulfonium	$C_3H_9S^+$	Glyphosate counterion	HILIC-LC
Vinclozolin	$C_{12}H_9Cl_2NO_3$	Dicarboximide	GC

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Table SD2. Recoveries (%) and relative standard deviation obtained in the preliminary analysis by GC-MS/MS (n=6).

Pesticide	R (%)	RSD (n=6)
Azinphos-ethyl	109.53	10.86
Bifenthrin	31.12	2.02
Bromopropylate	65.85	10.12
Captan	99.32	8.47
Chlorothalonil	76.96	5.68
Chlorpyrifos	60.65	6.48
Chlorpyrifos-methyl	73.68	4.30
Cyfluthrin-beta_(Sum)	70.53	7.40
Cyhalothrin-lambda	77.71	27.44
Cypermethrin_(Sum)	53.31	5.88
Diazinon	83.22	29.95
Dichlobenil	99.73	2.30
Dicofol	51.02	10.44
Endosulfan-alfa	29.17	16.49
Endosulfan-beta	63.23	8.28
Endosulfan-sulfate	101.50	10.42
Ethion	81.24	7.10
Fenitrothion	120.67	14.58
Fenpropathrin	45.79	1.99
Fenvalerate_(Sum)	56.77	3.84
Fipronil	118.80	7.63
Fluvalinate-tau (Sum)	57.54	3.38
Folpet	90.39	5.73
Iprodione	106.48	11.51
Lindane	61.37	11.18
Methidathion	120.74	5.68
Oxyfluorfen	83.99	5.64
Parathion	100.54	4.96
Parathion-methyl	112.97	2.09
Permethrin (Sum)	36.31	3.15
Procymidone	95.44	10.65
Profenophos	65.99	9.76
Pyrazophos	98.01	11.86
Tetradifon	54.35	5.08
Thiometon	78.99	8.18
Tolclofos-methyl	80.71	9.35
Trifluralin	66.85	5.43
Vinclozolin	103.89	7.46

119 **Table SD3.** Analytical parameters of method development for GC-MS/MS analysis

Pesticide	Retention time (min)	Q	q	CE
Methamidophos	8.79	140.9 > 126.0	140.9 > 95.1	0.60
Dichlobenil	10.30	170.9 > 136.1	170.9 > 100.2	1.50
Trifluralin	14.41	263.9 > 206.0	263.9 > 160.1	1.00
Thiometon	15.43	125.1 > 79.0	125.1 > 62.0	1.00
Lindane	16.53	218.7 > 184.0	180.9 > 145.1	1.00
Diazinon	16.91	179.0 > 137.2	179.0 > 164.1	1.50
Chlorothalonil	17.32	265.9 > 231.0	265.9 > 205.1	1.50
Chlorpyriphos-methyl	19.31	285.8 > 271.0	285.8 > 241.0	1.25
Vinclozolin	19.54	211.9 > 172.0	211.9 > 145.1	1.25
Parathion-methyl	19.65	232.9 > 124.1	232.9 > 201.1	1.25
Tolclofos-methyl	19.65	264.9 > 250.0	264.9 > 220.1	1.25
Fenitrothion	20.82	246.9 > 138.1	246.9 > 106.2	1.00
Chlorpyrifos	21.45	313.8 > 285.9	313.8 > 258.0	0.75
Parathion	21.83	124.9 > 97.0	124.9 > 93.1	1.00
Diclofop	22.40	279.9 > 251.1	279.9 > 182.2	1.00
Fipronil	23.13	367.0 > 245.1	367.0 > 255.1	1.25
Captan	23.58	148.9 > 105.1	148.9 > 79.2	0.75
Procymidone	23.67	282.8 > 255.1	282.8 > 220.2	1.00
Folpet	23.80	259.8 > 232.0	259.8 > 130.1	0.75
Methidathion	24.05	144.8 > 85.0	144.8 > 58.1	0.50
Endosulfan-α	24.54	206.9 > 172.1	206.9 > 136.1	2.00
Profenofos	25.31	336.8 > 309.0	336.8 > 266.9	0.75
Oxyfluorfen	25.76	252.2 > 196.1	252.2 > 224.1	1.75
Endosulfan-β	26.73	194.8 > 159.1	194.8 > 123.1	1.75
Ethion	26.94	230.8 > 202.9	230.8 > 174.9	0.75
Endosulfan sulfate	28.06	387.0 > 289.0	387.0 > 253.0	1.25
Iprodione	29.61	313.9 > 245.1	313.9 > 271.0	1.00
Bifenthrin	29.82	180.9 > 166.2	180.9 > 153.3	1.00
Bromopropylate	29.89	340.8 > 185.1	340.8 > 155.1	1.00
Fenpropathrin	30.16	265.0 > 210.1	265.0 > 172.1	1.00
Dicofol	30.19	138.9 > 111.1	138.9 > 93.0	1.25
Tetradifon	30.76	226.8 > 199.0	226.8 > 164.0	1.25
Cyhalothrin-lambda	31.53	208.9 > 141.2	208.9 > 169.2	1.00
Pyrazophos	31.85	221.0 > 193.1	221.0 > 149.2	1.00
Azinphos-ethyl	32.09	132.0 > 77.0	132.0 > 104.1	1.00
Permethrin 1	32.94	183.0 > 168.1	183.0 > 153.3	1.25
Permethrin 2	33.19	183.0 > 168.1	183.0 > 153.3	1.25
Cyfluthrin-β1	34.28	206.0 > 151.1	206.0 > 179.1	1.50
Cyfluthrin-β2	34.37	206.0 > 151.1	206.0 > 179.1	1.50
Cypermethrin 1	34.59	208.9 > 193.1	208.9 > 141.1	1.25
Cypermethrin 2	34.81	208.9 > 193.1	208.9 > 141.1	1.25
Cypermethrin 3	34.90	208.9 > 193.1	208.9 > 141.1	1.25
Cypermethrin 4	34.99	208.9 > 193.1	208.9 > 141.1	1.25
Fenvalerate 1	36.32	224.9 > 147.0	224.9 > 119.1	1.00
τ-Fluvalinate 1	36.55	250.0 > 200.1	250.0 > 215.1	1.25
τ-Fluvalinate 2	36.71	250.0 > 200.1	250.0 > 215.1	1.25
Fenvalerate 2	36.74	224.9 > 147.0	224.9 > 119.1	1.00
Esfenvalerate	36.75	224.9 > 147.0	224.9 > 119.1	0.90

120 Q: Transition used for quantification; q: Transition selected for confirmation; CE: Collision energy.