Application of gas chromatography–(triple quadrupole) mass spectrometry with atmospheric pressure chemical ionization for the determination of multiclass pesticides in fruits and vegetables

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Abstract

A multi-residue method for the determination of 142 pesticide residues in fruits and vegetables has been developed using a new atmospheric pressure chemical ionization (APCI) source for coupling gas chromatography (GC) to tandem mass spectrometry (MS). Selected reaction monitoring (SRM) mode has been applied, acquiring three transitions for each compound. In contrast to the extensive fragmentation typically obtained in classical electron ionization (EI), the soft APCI ionization allowed the selection of highly abundant protonated molecules ($[M+H]^+$) as precursor ions for most compounds. This was favorable for both sensitivity and selectivity. Validation of the method was performed in which both quantitative and qualitative parameters were assessed using orange, tomato and carrot samples spiked at two levels, 0.01 and 0.1 mg/kg. The QuEChERS method was used for sample preparation, followed by a 10-fold dilution of the final acetonitrile extract with a mixture of hexane and acetone. Recovery and precision were satisfactory in the three matrices, at both concentration levels. Very low limits of detection (down 0.01 □g/kg for the most sensitive compounds) were achieved. Ion ratios were consistent and identification according to EU criteria was possible in 80% (0.01 mg/kg) to 96% (0.1 mg/kg) of the pesticide/matrix combinations. The method was applied to the analysis of various fruits and vegetables from the Mediterranean region of Spain.

Keywords

Atmospheric pressure chemical ionization; Gas chromatography; Tandem mass spectrometry; Triple quadrupole; Pesticides; Fruit and vegetable analysis, validation, identification

1. INTRODUCTION

The control of pesticide residues in food commodities is a requirement to verify compliance with regulatory limits set by the European Commission (EC 396/2005) to ensure good agricultural practice and food safety. Sensitive and robust analytical techniques are required that preferably cover various pesticide chemical classes with different physicochemical properties. A common analytical approach is to combine generic sample preparation techniques, with inherently low selectivity, with highly selective instrumental analysis.

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure is a popular generic sample preparation method for the extraction of pesticides from fruits and vegetables. It involves a rapid extraction using acetonitrile (MeCN) and a cleanup step based on dispersive-SPE (d-SPE) using a primary secondary amine (PSA) sorbent and anhydrous $MgSO_4$ to remove water [1] and [2]. Numerous applications have been successfully validated for a large number of pesticides in a variety of complex matrices [3], [4] and [5].

For a major part of the pesticides, liquid chromatography combined with mass spectrometry is considered as the method of choice [6]. However, many pesticides are also amenable to gas chromatography (GC) coupled to mass spectrometry (MS) which makes it a valuable complementary technique, especially because it is the only option for certain pesticide classes and therefore has to be used anyway. Several quantitative applications have been described in literature using GC–MS with a single quadrupole analyzer operating in selected ion monitoring (SIM) [7], [8] and [9], especially for multi-residue analysis with a limited number of compounds. However, the determination of a larger number of analytes usually requires more selective techniques, as tandem mass spectrometry (MS/MS). The use of triple quadrupole (QqQ) working under selected reaction monitoring (SRM) improves selectivity, as well as sensitivity [10], [11], [12], [13] and [14].

In GC–MS/MS, electron ionization (EI) is by far the most widely used ionization technique because of its capability of ionizing virtually any organic compound. A rather strong fragmentation is inherent to EI. This is a disadvantage in GC–MS/MS because in many cases fragments have to be used as precursor ions which are then further fragmented to smaller product ions. This compromises both sensitivity and selectivity compared to LC–

MS/MS where quasi molecular ions are obtained during ionization. Softer ionization modes such as chemical ionization (PCI, NCI) and supersonic molecular beam (cold EI) [15] are available for GC but these options have restrictions with respect to applicability and commercial availability, respectively. Atmospheric pressure chemical ionization (APCI), commonly used in LC–MS/MS, has been described as an alternative source for GC–MS and a way to couple GC to mass spectrometers initially developed for LC–MS [16] and [17]. Application studies including pharmaceutical development [18], profiling of phenolic compounds in oil [19], metabolic profiling [20] and pesticide residue analysis [21], most of them using GC–(APCI) TOF MS, can be found since 2009. Recently, we investigated the potential of APCI in GC–triple quadrupole MS for wide-scope pesticide residue analysis [22] and [23]. Compared to EI, little or no fragmentation occurs while compared to PCI/NCI the applicability to different classes of compounds was much wider. Besides the selectivity advantage arising from the ability to use the quasi-molecular ion as precursor ion, the sensitivity was also found to be substantially improved. This was partly due to the use of high-end MS/MS detectors normally used for LC–MS/MS (in fact, by changing the source, the same MS/MS could be coupled to either LC or GC).

In the previous papers the emphasis was on the potential, ionization mechanisms, and features of GC–(APCI) MS/MS. In this work the focus is on applicability for routine wide-scope multi-residue analysis of pesticides in fruits and vegetable matrices, with emphasis on quantitative and qualitative performance. A GC–(APCI) MS/MS method for simultaneous detection of 142 pesticides (around 48 of them non LC-amenable) was set up including three transitions for each compound. Using a QuEChERS method for sample preparation, the method was validated for three matrices (orange, tomato, carrot). Matrix effects, linearity, accuracy, limits of quantification (LOQs) and limits of detection (LODs) were established. With respect to identification, compliance of ion ratios with EU criteria was assessed. Real samples were analyzed to test the method applicability, including orange, tomato, carrot and also apple, lettuce and courgette.

2. EXPERIMENTAL

2.1. Reagents

Pesticide standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Stock standard solutions (around 500 □g/mL) were prepared by dissolving reference standards in acetone and stored in a freezer at □20 °C. Working standard mixtures were prepared by volume dilution of stock solutions in hexane for preparation of matrix-matched calibrants and in acetone for sample fortification.

Hexane, acetone, acetonitrile (MeCN), toluene, glacial acetic acid (HAc), anhydrous $MgSO_4$ and anhydrous sodium acetate (NaAc) were purchased from Scharlab (Barcelona, Spain). Solvents used were of pesticide residue analysis or HPLC grade. Two types of 2 mL micro-centrifuge tubes for QuEChERS d-SPE containing 50 mg PSA and 150 mg anhydrous $MgSO_4$, or 50 mg PSA, 150 mg anhydrous $MgSO_4$ and 50 mg C18, were obtained from Teknokroma (Barcelona, Spain).

2.2. Sample material

Three types of sample matrices were used in the validation study: orange, tomato and carrot. Blank samples, used for the matrix-matched calibration, sample fortification and quality control, were obtained from organic cultivars (pesticide free).

Three different varieties from each food commodity were analyzed to investigate the presence of pesticides, all of them purchased from local markets in the Castellón province (Spain).

Apple, lettuce and courgette samples, also purchased from local markets, were analyzed to extend the method applicability.

2.3. Instrumentation

Data were acquired using a GC system (Agilent 7890A, Palo Alto, CA, USA) equipped with an autosampler (Agilent 7693) and coupled to a triple quadrupole (QqQ) mass spectrometer (Xevo TQ-S, Waters Corporation, Manchester, UK), operating in APCI mode. A fused silica DB-5MS capillary column (length 30 m × I.D. 0.25 mm × film 0.25 □m)

(J&W Scientific, Folson, CA, USA) was used for GC separation. The injector was operated in splitless mode, injecting 1 ☐L at 280 °C. The oven temperature was programmed as follows: 70 °C (1 min), 15 °C/min to 150 °C and 10 °C/min to 300 °C (3 min). Helium was used as carrier gas in constant flow mode (2 mL/min). A pulsed splitless injection was carried out using an initial pressure of 240 kPa, maintained for 1 min, and then changed to a constant flow of 2 mL/min, which corresponded to a linear velocity of 52 cm/s. In the SRM method, automatic dwell time (values ranging from 3 to 63 ms) was applied in order to obtain 15 points per peak.

The interface temperature was set to 310 °C using N2 as auxiliary gas at 250 L/h, a make-up gas at 300 mL/min and cone gas at 170 L/h. The APCI corona discharge pin was operated at 1.8 ☐A. The water used as modifier when working under proton-transfer conditions was placed in an uncapped vial, which was located within a holder placed in the source door.

Targetlynx (a module of MassLynx) was used to handle and process the acquired data.

2.4. Sample treatment

The QuEChERS procedure applied was that proposed in the AOAC official method 2007.01 [2]: 15 g of sample (previously homogenized in a food chopper) were weighted in a 50 mL polypropylene centrifuge tube, mixed with 15 mL MeCN (with 1% HAc) and shaken by hand for 30 s. Then, 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous NaAc were added and immediately shaken vigorously by hand to prevent formation of $MgSO_4$ agglomerates. Then, the tube was centrifuged at 3000 rpm for 2 min.

For the cleanup step, 1 mL of the upper MeCN extract was transferred into a d-SPE tube containing 150 mg $MgSO_4$ and 50 mg PSA (or 150 mg $MgSO_4$, 50 mg PSA and 50 mg C18 when oranges were extracted). The tubes were shaken on a Vortex for 30 s and centrifuged at 3000 rpm for 2 min. Finally, 50 ☐L of the extract (acetonitrile) were transferred into a 2 mL vial and diluted with 300 ☐L of hexane and 150 ☐L of acetone.

Matrix-matched standards were prepared for each sample matrix as follows: after the cleanup step, 50 ☐L of the MeCN extract obtained from a blank sample were mixed with 250 ☐L of hexane, 150 ☐L of acetone, and 50 ☐L of the pesticide standard solution in

hexane at adequate concentration to obtain a calibration range of 0.1–100 ng/mL (corresponding to 1–1000 □g/kg in sample).

2.5. Validation study

The developed SRM method was validated using orange, carrot and tomato in order to evaluate linearity, recovery, precision, selectivity and LODs and LOQs.

Linearity was studied by injecting standards in hexane (n = 3) at eight concentration levels, 0.1, 0.5, 1, 5, 10, 25, 50 and 100 ng/mL, and was considered acceptable when regression coefficients were higher than 0.99 and residuals lower than 30%.

Accuracy was estimated from recovery experiments, by analyzing six replicates spiked at two levels (0.01 and 0.1 mg/kg). Precision was expressed as repeatability in terms of relative standard deviation (RSD, %) (n = 6) calculated for each fortification level.

The LOQ was defined as the lowest concentration level validated with satisfactory values of recovery (70–110%) and precision (RSD < 20%) [24].

The LOD, defined as the concentration corresponding to a signal-to-noise ratio of three, was estimated from the chromatogram of the matrix-matched standards at the lowest calibration level used for each compound.

The selectivity of the method was evaluated by verification of the absence of interfering peaks at the retention time of each compound in blank samples for the acquired MS/MS transitions.

The ratio of each of the two qualifier ions relative to the quantifier (calculated by dividing the lower by the higher response) were used to verify compliance with EU criteria [24] of the pesticides in the spiked samples and to confirm peak identity in real samples.

3. RESULTS AND DISCUSSION

3.1. GC–(APCI) MS/MS optimization

Optimization of the MS/MS conditions started by using pesticide standard solutions in hexane with the mass spectrometer operating in full scan mode to obtain the MS spectra. Experiments under N2 and proton transfer conditions (using water as modifier) were performed. The proton transfer mechanism revealed a notable tendency for the majority of the studied pesticides to be protonated, since the $[M+H]^+$ was present for most compounds and frequently as the peak base of the spectrum, with very low fragmentation. Thus the use of water as modifier was considered for further experiments.

The cone voltage was studied in the range 5–40 V for all compounds and those values which result in higher sensitivity were selected for each pesticide (**Table 1**). The helium flow rate was set at a relatively high flow rate of 2 mL/min since this was found to be beneficial for peak shape and analysis speed in an earlier work [23] using a GC–(APCI) MS system.

To continue with MS/MS optimization, the base peak of the spectrum for each compound ($[M+H]^+$ in most cases) was selected as precursor ion (in some cases, two different precursor ions were chosen). The main goal was to develop a SRM method with three MS/MS transitions (the most sensitive ones) in order to carry out a reliable quantification and identification of the pesticides detected in samples. The fragmentation pattern of the precursor ions was studied through product ion scan experiments at different collision energies (10, 20 and 30 eV) and again the most sensitive transitions were selected for the final SRM method. **Table 1** shows the SRM transitions corresponding to both quantifier and the qualifier transitions monitored.

Table 1. Experimental conditions of the optimized GC–(APCI)MS/MS method using water as modifier. Quantifier (Q) and qualifier (q) transitions.

t_R (min)	Window (min)	Compounds	SRM Transitions		Collision energy (eV)	Cone voltage (V)
6.2	6-7.75	Hexachlorobutadiene	Q	*258* > 223	20	30
			q_1	*258* > 141	20	
			q_2	*258* > 188	30	
6.45		Dichlorvos	Q	*221* > 109	20	10
			q_1	*221* > 127	20	
			q_2	*221* > 145	10	
6.95		Carbofuran	Q	165 > 123	10	20
			q_1	165 > 105	30	
			q_2	165 > 137	10	
8.19	7.75-8.8	Mevinphos	Q	193 >127	10	30
			q_1	*225* > 127	10	
			q_2	*225* > 193	10	
8.45		Propham	Q	138 > 120	10	30
			q_1	138 > 77	30	
			q_2	138 > 92	10	
9	8.8-9.95	Carbaryl	Q	145 > 117	20	10
			q_1	145 > 115	20	
			q_2	144 > 115	20	
9.1		2-Phenylphenol	Q	*171* > 153	20	10
			q_1	*171* > 151	30	
			q_2	*171* > 152	30	
9.21		Pentachlorobenzene	Q	*248* > 213	30	40
			q_1	*248* > 142	30	
			q_2	*248* > 178	30	
9.3		Molinate	Q	*188* > 126	10	20
			q_1	*188* > 98	20	
			q_2	*188* > 160	10	
10.02	9.95-10.3	Propoxur	Q	*210* > 111	10	10
			q_1	*210* > 168	10	
			q_2	*210* > 135	10	
10.05		Propachlor	Q	*212* > 170	20	30
			q_1	*212* > 94	30	
			q_2	*212* > 106	30	
10.12		Demeton-s-methyl	Q	143 > 111	10	20
			q_1	143 > 125	10	
			q_2	143 > 127	10	
10.14		Diphenylamine	Q	*170* > 93	30	40
			q_1	*170* > 152	20	
			q_2	*170* > 153	20	
10.39	10.2-10.8	Atrazine deisopropyl	Q	*174* > 132	20	30
			q_1	*174* > 104	30	
			q_2	*174* > 146	20	
10.4		Chlorpropham	Q	172 > 154	10	40
			q_1	172 > 111	20	
			q_2	172 > 126	20	
10.49		Ethalfluralin	Q	*334* > 232	10	20
			q_1	*334* > 186	30	
			q_2	*334* > 300	20	
10.5		Terbumeton desethyl	Q	*198* > 142	20	20
			q_1	*198* > 86	30	
			q_2	*198* > 100	30	
10.5		Atrazine desethyl	Q	*188* > 146	20	10
			q_1	*188* > 104	30	
			q_2	*188* > 111	10	
10.65		Trifluraline	Q	*336* > 232	20	30
			q_1	*336* > 186	30	
			q_2	*336* > 202	30	

Table 1 (continued).

t_R (min)	Window (min)	Compounds	SRM Transitions		Collision energy (eV)	Cone voltage (V)
10.7	10.5-11.3	Terbutylazine desethyl	Q	*202* > 146	20	20
			q_1	*202* > 104	30	
			q_2	*202* > 116	30	
10.79		Cadusafos	Q	*271* > 131	10	30
			q_1	*271* > 97	10	
			q_2	*271* > 125	20	
10.87		Phorate	Q	*261* > 97	30	20
			q_1	*261* > 171	10	
			q_2	*261* > 199	10	
10.97		alpha-HCH	Q	181 > 146	20	10
			q_1	181 > 109	30	
			q_2	217 > 181	10	
11.12		Hexachlorobenzene	Q	*282* > 247	30	40
			q_1	*282* > 177	30	
			q_2	*282* > 212	30	
11.2		Dichloran	Q	*207* > 190	20	10
			q_1	*207* > 124	30	
			q_2	*207* > 160	20	
11.2		Dimethoate	Q	*230* > 125	20	30
			q_1	*230* > 171	10	
			q_2	*230* > 199	10	
11.25	11-12	Ethoxyquin	Q	218 > 174	30	30
			q_1	218 > 160	30	
			q_2	218 > 202	20	
11.27		Simazine	Q	202 > 132	20	30
			q_1	202 > 104	30	
			q_2	202 > 124	20	
11.38		Atrazine	Q	*216* > 174	20	10
			q_1	*216* > 104	30	
			q_2	*216* > 132	30	
11.47		beta-HCH	Q	181 > 146	20	10
			q_1	181 > 109	30	
			q_2	217 > 181	10	
11.49		Terbumeton	Q	*226* > 170	20	30
			q_1	*226* > 114	30	
			q_2	*226* > 142	30	
11.6	11.5-11.85	Dioxathion	Q	271 > 97	30	20
			q_1	271 > 125	10	
			q_2	271 > 141	20	
11.6		gamma-HCH	Q	181 > 146	20	10
			q_1	181 > 109	30	
			q_2	217 > 181	10	
11.66		Terbutylazine	Q	*230* > 174	20	20
			q_1	*230* > 104	30	
			q_2	*230* > 132	30	
11.67		Propetamphos	Q	222 > 138	10	20
			q_1	222 > 110	20	
			q_2	*282* > 138	20	
11.68		Cyanophos	Q	*244* > 125	30	5
			q_1	*244* > 134	30	
			q_2	*244* > 150	20	
11.68		Terbufos	Q	187 > 97	10	5
			q_1	187 > 131	20	
			q_2	187 > 159	10	
11.72		Propyzamide	Q	*256* > 190	10	30
			q_1	*256* > 145	30	
			q_2	*256* > 173	20	
11.9	11.7-12.25	Diazinon	Q	*305* > 169	30	40
			q_1	*305* > 153	30	
			q_2	*305* > 249	20	

Table 1 (continued).

t_R (min)	Window (min)	Compounds	SRM Transitions		Collision energy (eV)	Cone voltage (V)
12.01		Terbacil	Q	161 > 144	20	20
			q_1	161 > 118	20	
			q_2	161 > 143	20	
12.05		delta-HCH	Q	181 > 146	20	30
			q_1	181 > 109	30	
			q_2	217 > 181	10	
12.08		Tefluthrin	Q	177 > 127	20	5
			q_1	*419* > 177	10	
			q_2	*419* > 325	10	
12.14		Chlorothalonil	Q	*265* > 230	20	30
			q_1	*265* > 133	30	
			q_2	*265* > 211	20	
12.4	12.25-12.7	Pirimicarb	Q	*239* > 182	20	10
			q_1	*239* > 109	30	
			q_2	*239* > 138	30	
12.43		Endosulfan ether	Q	*341* > 217	30	30
			q_1	*341* > 170	30	
			q_2	*341* > 205	20	
12.62		Phosphamidon	Q	*300* > 127	20	40
			q_1	*300* > 174	10	
			q_2	*300* > 227	10	
12.62		Dichlofenthion	Q	*315* > 259	20	30
			q_1	*315* > 179	20	
			q_2	*315* > 287	10	
12.66	12.4-13.15	Metribuzin	Q	*215* > 187	20	40
			q_1	*215* > 145	20	
			q_2	*215* > 171	20	
12.79		Vinclozolin	Q	*286* > 242	10	5
			q_1	*286* > 164	30	
			q_2	*286* > 172	20	
12.8		Parathion methyl	Q	*264* > 232	20	20
			q_1	*264* > 125	30	
			q_2	*264* > 155	30	
12.8		Chlorpyrifos methyl	Q	*322* > 125	30	40
			q_1	*322* > 212	30	
			q_2	*322* > 290	20	
12.94		Alachlor	Q	238 > 162	20	30
			q_1	238 > 132	30	
			q_2	*270* > 147	30	
12.96		Heptachlor	Q	335 > 264	20	40
			q_1	335 > 230	30	
			q_2	335 > 299	10	
13.03		Metalaxyl	Q	*280* > 220	10	30
			q_1	*280* > 160	20	
			q_2	*280* > 192	10	
13.1	12.8-13.6	Methiocarb sulfone	Q	201 > 122	10	20
			q_1	201 > 91	30	
			q_2	201 > 107	30	
13.15		Demeton-s-methylsulfone	Q	*263* > 125	20	30
			q_1	*263* > 169	10	
			q_2	*263* > 231	10	
13.25		Terbutryn	Q	*242* > 186	20	40
			q_1	*242* > 116	30	
			q_2	*242* > 138	30	
13.3		Methiocarb	Q	*226* > 169	10	30
			q_1	*226* > 121	20	
			q_2	*226* > 122	30	
13.3		Fenitrothion	Q	*278* > 125	30	40
			q_1	*278* > 169	30	
			q_2	*278* > 200	20	

Table 1 (continued).

t_R (min)	Window (min)	Compounds	SRM Transitions		Collision energy (eV)	Cone voltage (V)
13.34		Pirimiphos methyl	Q	*306* > 125	30	5
			q_1	*306* > 109	30	
			q_2	*306* > 164	30	
13.5		Malathion	Q	*331* > 125	30	20
			q_1	*331* > 117	20	
			q_2	*331* > 211	10	
13.61	13.3-13.85	Aldrin	Q	*363* > 159	20	30
			q_1	*363* > 215	20	
			q_2	*363* > 327	10	
13.63		Metolachlor	Q	*284* > 252	20	20
			q_1	*284* > 134	30	
			q_2	*284* > 176	30	
13.66		Fenthion	Q	*279* > 247	10	20
			q_1	*279* > 105	20	
			q_2	*279* > 169	30	
13.68		Cyanazine	Q	*241* > 214	20	30
			q_1	*241* > 132	30	
			q_2	*241* > 205	20	
13.71		Chlorpyrifos	Q	*350* > 198	20	20
			q_1	*350* > 294	10	
			q_2	*350* > 322	10	
13.72		Parathion-ethyl	Q	*292* > 236	20	20
			q_1	*292* > 110	30	
			q_2	*292* > 123	30	
13.76		Triadimefon	Q	*294* > 197	10	40
			q_1	*294* > 129	20	
			q_2	*294* > 141	20	
13.76		4,4'-Dichloronbenzophenone	Q	*251* > 139	20	20
			q_1	*251* > 111	30	
			q_2	*251* > 129	30	
14.04	13.85-14.4	Bromophos methyl	Q	*365* > 125	20	30
			q_1	*365* > 211	30	
			q_2	*365* > 239	30	
14.15		Isodrin	Q	*363* > 159	20	30
			q_1	*363* > 215	20	
			q_2	*363* > 327	10	
14.16		Cyprodinil	Q	*226* > 118	30	40
			q_1	*226* > 133	30	
			q_2	*226* > 210	30	
14.3		Pendimethalin	Q	*282* > 212	10	20
			q_1	264 > 147	30	
			q_2	264 > 201	20	
14.35	14.1-14.6	Heptachlor epoxide B	Q	351 > 251	30	20
			q_1	351 > 217	20	
			q_2	351 > 287	10	
14.37		Oxychlordane	Q	*421* > 151	20	10
			q_1	*421* > 115	20	
			q_2	*421* > 285	30	
14.41		Tolyfluanid	Q	238 > 137	20	5
			q_1	238 > 110	30	
			q_2	238 > 122	30	
14.43		Heptachlor epoxide A	Q	351 > 251	30	20
			q_1	351 > 217	20	
			q_2	351 > 287	10	
14.46		Chlorfenvinphos	Q	*359* > 170	30	30
			q_1	*359* > 99	10	
			q_2	*359* > 205	20	
14.47		Fipronil	Q	*437* > 368	20	30
			q_1	*437* > 255	30	
			q_2	*437* > 315	30	

Table 1 (continued).

t_R (min)	Window (min)	Compounds	SRM Transitions		Collision energy (eV)	Cone voltage (V)
14.5	14.2-14.8	Captan	Q	264 > 236	10	30
			q_1	264 > 156	30	
			q_2	264 > 180	10	
14.52		Quinalphos	Q	*299* > 163	20	30
			q_1	*299* > 147	30	
			q_2	*299* > 271	10	
14.6		Folpet	Q	260 > 130	20	5
			q_1	260 > 102	30	
			q_2	260 > 232	10	
14.63		Procymidone	Q	*284* > 256	20	30
			q_1	*284* > 186	30	
			q_2	*284* > 228	20	
14.67		Triflumizole	Q	*346* > 278	10	10
			q_1	*346* > 206	20	
			q_2	*346* > 266	20	
14.79	14.4-15.3	Chinomethionate	Q	*235* > 175	20	30
			q_1	*235* > 104	30	
			q_2	*235* > 121	30	
14.8		Methidathion	Q	*303* > 145	10	10
			q_1	*303* > 125	20	
			q_2	*303* > 257	10	
14.8		trans-Chlordane	Q	371 > 264	30	10
			q_1	371 > 299	20	
			q_2	371 > 335	20	
14.82		Bromophos ethyl	Q	*393* > 337	20	10
			q_1	*393* > 162	30	
			q_2	*393* > 365	10	
15.01		Endosulfan I	Q	*405* > 323	10	5
			q_1	*405* > 217	30	
			q_2	*405* > 251	20	
15.14		Fenamiphos	Q	*304* > 217	20	40
			q_1	*304* > 202	30	
			q_2	*304* > 234	20	
15.17	15-15.8	Chlorfenson	Q	*303* > 159	10	5
			q_1	*303* > 111	10	
			q_2	*303* > 128	30	
15.32		Imazalil	Q	*297* > 159	20	10
			q_1	*297* > 109	20	
			q_2	*297* > 176	20	
15.36		Fludioxonil	Q	*248* > 127	30	30
			q_1	*248* > 154	20	
			q_2	*248* > 182	20	
15.43		*p,p'*-DDE	Q	*316* > 246	30	20
			q_1	*316* > 210	30	
			q_2	*316* > 281	20	
15.49		Dieldrin	Q	*379* > 325	10	20
			q_1	*379* > 254	30	
			q_2	*379* > 261	20	
15.57		Oxyfluorfen	Q	*362* > 316	10	30
			q_1	*362* > 237	20	
			q_2	*362* > 334	10	
15.61		Buprofezin	Q	*306* > 106	20	30
			q_1	*306* > 203	10	
			q_2	*306* > 250	10	
15.9	15.6-16.4	Endrin	Q	*379* > 343	10	30
			q_1	*379* > 243	20	
			q_2	*379* > 244	20	
16.05		Endosulfan II	Q	*405* > 323	10	30
			q_1	*405* > 217	30	
			q_2	*405* > 251	20	

Table 1 (continued).

t_R (min)	Window (min)	Compounds	SRM Transitions		Collision energy (eV)	Cone voltage (V)
16.18		*p,p'*-DDD	Q	235 > 165	20	5
			q_1	235 > 99	30	
			q_2	235 > 199	20	
16.25		Oxadixyl	Q	*279* > 219	10	5
			q_1	*279* > 117	30	
			q_2	*279* > 132	20	
16.27		Ethion	Q	*385* > 143	30	5
			q_1	*385* > 97	10	
			q_2	*385* > 125	20	
16.47	16.3-16.8	Sulprofos	Q	*323* > 139	20	10
			q_1	*323* > 155	30	
			q_2	*323* > 219	10	
16.62		Famphur	Q	*326* > 217	20	30
			q_1	*326* > 125	20	
			q_2	*326* > 152	30	
16.65		Carbofenothion	Q	*343* > 157	20	5
			q_1	*343* > 97	30	
			q_2	*343* > 121	30	
16.67		Carfentrazone ethyl	Q	*412* > 346	20	10
			q_1	*412* > 366	20	
			q_2	*412* > 384	10	
16.78	16.5-17	Propiconazole	Q	*342* > 159	20	30
			q_1	*342* > 187	20	
			q_2	*342* > 256	10	
16.8		Endosulfan sulfate	Q	323 > 217	30	10
			q_1	323 > 251	20	
			q_2	323 > 287	10	
16.84		Fenhexamid	Q	*302* > 143	30	30
			q_1	*302* > 142	30	
			q_2	*302* > 178	20	
16.85		*p,p'*-DDT	Q	235 > 165	20	5
			q_1	235 > 99	30	
			q_2	235 > 199	20	
17.17	16.9-17.9	Diflufenican	Q	*395* > 266	20	10
			q_1	*395* > 238	30	
			q_2	*395* > 246	30	
17.23		Captafol	Q	*348* > 312	10	10
			q_1	*348* > 117	30	
			q_2	*348* > 161	20	
17.27		Resmethrin	Q	*339* > 171	10	30
			q_1	*339* > 143	20	
			q_2	*339* > 293	10	
17.54		Iprodione	Q	*330* > 245	10	30
			q_1	*330* > 174	30	
			q_2	*330* > 288	10	
17.71		Fenoxycarb	Q	*302* > 256	10	40
			q_1	*302* > 183	20	
			q_2	*302* > 213	20	
17.71		Phosmet	Q	*318* > 160	10	20
			q_1	160 > 133	20	
			q_2	*318* > 133	30	
17.77		Bifenthrin	Q	181 > 165	20	10
			q_1	181 > 115	30	
			q_2	181 > 166	30	
17.86	17.6-18.7	Methoxychlor	Q	*345* > 213	20	10
			q_1	227 > 141	30	
			q_2	227 > 169	30	
18.22		Tetradifon	Q	*355* > 195	20	30
			q_1	*355* > 133	30	
			q_2	*355* > 167	20	

Table 1 (continued).

t_R (min)	Window (min)	Compounds	SRM Transitions		Collision energy (eV)	Cone voltage (V)
18.4		Azinphos methyl	Q	261 > 125	20	20
			q_1	261 > 167	10	
			q_2	261 > 183	10	
18.44		Leptophos	Q	*411* > 171	20	40
			q_1	*411* > 139	30	
			q_2	*411* > 379	20	
18.45		Pyriproxifen	Q	*322* > 185	20	10
			q_1	*322* > 129	30	
			q_2	*322* > 227	10	
18.55		lambda-Cyhalothrin	Q	*450* > 225	10	10
			q_1	*450* > 141	20	
			q_2	*450* > 157	30	
18.64	18.5-19.8	Mirex	Q	270 > 235	20	10
			q_1	270 > 117	30	
			q_2	270 > 141	30	
18.9		Acrinathrin	Q	428 > 401	20	10
			q_1	428 > 205	30	
			q_2	428 > 260	20	
18.88		Fenarimol	Q	*331* > 268	20	40
			q_1	*331* > 139	30	
			q_2	*331* > 259	20	
19.01		Azinphos ethyl	Q	289 > 137	20	20
			q_1	289 > 233	10	
			q_2	289 > 261	10	
19.43		Permethrin	Q	355 > 319	10	10
			q_1	391 > 183	30	
			q_2	391 > 355	10	
19.66		Coumaphos	Q	*363* > 227	30	30
			q_1	*363* > 211	30	
			q_2	*363* > 307	20	
20.09	19.7-20.35	Cyfluthrin	Q	*434* > 191	10	10
			q_1	*434* > 91	30	
			q_2	*434* > 127	30	
20.4	20.1-20.85	Cypermethrin	Q	*416* > 191	10	20
			q_1	*416* > 91	30	
			q_2	*416* > 127	30	
20.51		Flucythrinate	Q	412 > 219	30	5
			q_1	412 > 220	30	
			q_2	412 > 236	30	
20.59		Etofenprox	Q	359 > 183	20	10
			q_1	359 > 161	20	
			q_2	359 > 289	20	
21.21	20.85-21.6	Fenvalerate	Q	*419* > 225	10	10
			q_1	*420* > 125	10	
			q_2	*420* > 226	10	
21.38		tau-Fluvalinate	Q	*503* > 181	20	30
			q_1	*503* > 208	30	
			q_2	*503* > 250	20	
21.4		Esfenvalerate	Q	167 > 125	10	5
			q_1	167 > 99	30	
			q_2	167 > 139	10	
21.94	21.7-22.5	Deltamethrin	Q	*504* > 279	10	5
			q_1	*504* > 171	20	
			q_2	*504* > 200	30	
22.24		Azoxystrobin	Q	*404* > 372	10	20
			q_1	*404* > 329	30	
			q_2	*404* > 344	20	

Precursors corresponding to $M^{+\cdot}$ or $[M+H]^+$ are shown in italic.

3.2. Sample treatment optimization

With the QuEChERS sample preparation procedure, the final extract obtained is acetonitrile. The direct injection of the acetonitrile extract was considered less favorable. A (partial) solvent venting using a programmable temperature vaporizer injector could not be done with the GC system used, therefore a solvent exchange step was applied. Initially, in order to avoid evaporation until dryness, 1 mL of toluene was added to the 500 ☐L of the acetonitrile extract; evaporation until 300 ☐L using nitrogen stream was performed and then adjusted to 500 ☐L with toluene. In this way, no losses during the evaporation process were observed. However, the injection of the toluene extracts resulted in a dramatic loss of repeatability. Therefore, a solvent exchange into hexane was tested. In this case evaporation until dryness was unavoidable and the evaporation conditions had to be carefully optimized in order to avoid analyte losses. An evaporation system operating under vacuum was used, which allows a more controlled evaporation and at lower temperature compared with evaporation under nitrogen stream (miVac Modulator Concentrator, provided by Fisher Scientific S.A.S., Illkirch, France). The evaporation was carried out at 30 °C during approximately 30 minutes. However, no satisfactory results were obtained since some notable losses were observed in some analytes with low interday reproducibility.

Then, with the high sensitivity achieved in this GC–(APCI) MS/MS system in mind, the possibility of the direct dilution of the extract with hexane was considered. Standards in acetonitrile at 10 ng/mL were diluted with hexane (1/10), adding 20% of acetone to make the solution miscible. It is noteworthy that, in a multi-residue method that includes a large variety of compounds as in this work, the response of the most sensitive compounds are 1000 times higher than those ones with lower sensitivity. Consequently, dilution experiment led to a loss of some analytes that did not show enough sensitivity to be detected. A dilution of 1/5 with hexane (with 20% of acetone) was also tested but no considerable improvements with respect to the dilution 1/10 were observed for the less sensitive compounds, so this 1/10 dilution (with 20% of acetone) was selected for further experiments.

Then, experiments were performed by diluting acetonitrile sample extracts fortified at 10 ng/mL (dilution 1/10 with hexane) and it revealed a significant improvement in peak shapes and sensitivity. In presence of matrix, a higher amount of acetone had to be added

(30%) in order to keep the solution miscible. In conclusion, 50 ☐L of acetonitrile extract was mixed with 150 ☐L of acetone and 300 ☐L of hexane.

3.3. Matrix effect

Matrix effects for all matrices were checked comparing responses of standards in the mixture acetonitrile, hexane and acetone (in the proportions described above), at 10 ng/mL, with the response of matrix-matched standards (prepared as described in the section "Sample treatment"), at the same concentration. An enhancement of the signal was observed for most compounds except in a few cases such as pyrethroids where a slight suppression occurred, which was in agreement with earlier observations [22]. Matrix effects observed under GC–(APCI) MS are the result of that occurring in the GC inlet (normally enhancement) and in the APCI source (normally suppression). The signal enhancement observed for most compounds can be attributed to that occurring in the GC liner. The matrix shields active sites in the liner and column, which reduces interaction of the analytes on these sites, and leads to enhanced analyte peaks. This effect is most pronounced for polar analytes (typically those with strong hydrogen bonding potential) [25]. Looking at those compounds for which this enhancement is not expected (e.g. hexachlorobenzene, HCHs, etc.), no suppression coming from the APCI source is observed. Thus, it can be concluded that matrix effect observed in GC–(APCI) MS system are mainly arising from the GC inlet and to a lesser extend to suppression from APCI source. For optimum peak shape and sensitivity, as in any GC-based pesticide residue analysis, matrix-matched calibration curves were necessary to perform accurate quantitative analysis.

3.4. Validation results

Validation of the method was performed in terms of trueness (recovery) and precision, LODs and LOQs, and selectivity. These parameters were evaluated in three types of matrices, orange, tomato and carrot.

Linearity was studied in the range 0.1–100 ng/mL using pure solvent standard solutions and adjusted to quadratic curves. Each concentration level was injected in triplicate. The regression coefficients were higher than 0.99 for all compounds over the whole range tested. As mentioned above, matrix-matched calibration was used for quantification purposes. In this case, in order to quantify properly, shorter ranges were

selected depending on the concentration level to be quantified. In this way, residuals were better and lower than 30%.

Trueness and precision were evaluated by means of recovery experiments (n = 6) at two concentration levels (0.01 and 0.1 mg/kg) for each sample matrix. As can be observed in **Fig. 1**, the histograms show that most compounds presented satisfactory recoveries ranging between 70% and 120% for all the sample matrices at the two fortification levels, most of them between 70% and 110% (values are presented in **Table 2**). Thus, an LOQ of 0.01 mg/kg was demonstrated for most compounds. For the remaining compounds, acceptable results were obtained at 0.1 mg/kg (e.g. carbaryl in orange and carfentrazone-ethyl in carrot). For a limited number of compounds including molinate, propoxur and imazalil, the method was not suitable for the sample matrices and levels tested. Other compounds referred as problematic [26] and [27] as tolyfluanid, chlorothalonil and methiocarb sulfone, did not present satisfactory results in some matrices. RSDs lower than 10% were obtained for most analytes at both fortification levels, and even lower than 5%, as can be observed in **Fig. 2**.

Table 2. Average recovery (percent), R.S.D. (in parenthesis) and limits of quantification (LOQ) obtained after the application of the developed method to orange, tomato and carrot samples (n=6) fortified at two concentration levels.

Compounds	Orange				Tomato				Carrot			
	Fortification levels (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)	Fortification levels (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)	Fortification levels (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)
	10	100			10	100			10	100		
Acrinathrin	97 (7)	101 (1)	10	0.3	90 (3)	101 (14)	10	0.1	99 (9)	107 (8)	10	0.09
Alachlor	92 (3)	99 (4)	10	0.09	100 (3)	85 (3)	10	0.07	97 (4)	103 (4)	10	0.05
Aldrin	56 (14)	78 (5)	100	1.2	91 (7)	81 (6)	10	4.3	80 (10)	77 (5)	10	3
Atrazine	93 (2)	100 (5)	10	0.2	96 (4)	75 (3)	10	0.17	112 (2)	97 (4)	10	0.1
Atrazine deisopropyl	104 (5)	117 (4)	10	1.8	118 (9)	92 (10)	10	7.5	120 (14)	113 (5)	10	10
Atrazine desethyl	100 (4)	99 (4)	10	0.25	95 (3)	76 (3)	10	0.52	118 (3)	92 (5)	10	0.43
Azinphos ethyl	-	91 (3)	100	1.2	-	83 (8)	100	4	-	120 (1)	100	5.5
Azinphos methyl	74 (15)	95 (2)	10	2.5	73 (2)	81 (7)	10	1.5	86 (11)	110 (7)	10	2.9
Azoxystrobin	110 (4)	109 (7)	10	0.03	106 (4)	83 (11)	10	0.06	100 (14)	88 (11)	10	0.14
Bifenthrin	87 (6)	86 (2)	10	0.2	85 (4)	75 (4)	10	0.38	101 (4)	70 (3)	10	0.43
Bromohos ethyl	120 (4)	120 (2)	10	0.02	108 (3)	102 (3)	10	0.05	105 (3)	107 (5)	10	0.04
Bromophos methyl	120 (1)	116 (1)	10	0.03	105 (2)	101 (2)	10	0.09	117 (4)	106 (4)	10	0.09
Buprofezin	91 (7)	86 (4)	10	0.33	83 (3)	74 (5)	10	0.3	98 (5)	93 (5)	10	0.42
Cadusafos	120 (8)	119 (6)	10	1.4	113 (10)	100 (5)	10	5	118 (7)	149 (4)	10	3.6
Captafol	87 (21)	109 (4)	10	1.15	70 (10)	72 (10)	10	1.5	98 (16)	113 (14)	10	1.5
Captan	67 (7)	72 (5)	100	1.3	106 (4)	73 (5)	10	1.2	89 (9)	87 (4)	10	6
Carbaryl	43 (51)	78 (8)	100	30	87 (21)	77 (8)	10	30	99 (12)	70 (5)	10	6
Carbofenothion	119 (8)	134 (3)	10	0.2	104 (6)	101 (5)	10	0.43	112 (3)	117 (7)	10	0.25
Carbofuran	91 (13)	98 (6)	10	4.3	105 (8)	113 (13)	10	1.7	117 (14)	95 (6)	10	0.88
Carfentrazone ethyl	120 (2)	113 (2)	10	0.02	117 (2)	113 (3)	10	0.06	136 (5)	118 (4)	100	0.2
Chinomethionate	80 (13)	94 (10)	10	0.88	85 (3)	67 (6)	10	0.6	104 (2)	81 (5)	10	0.47
trans-Chlordane	97 (14)	89 (10)	10	1	99 (4)	81 (5)	10	1	104 (14)	79 (8)	10	1.5
Chlorfenson	111 (7)	102 (8)	10	0.3	92 (2)	81 84)	10	0.25	82 (2)	96 (3)	10	0.3
Chlorfenvinphos	87 (7)	89 (2)	10	0.11	92 (4)	80 (3)	10	0.09	100 (6)	115 (6)	10	0.06
Chlorothalonil	113 (8)	95 (9)	10	2.5	>150 (6)	96 (14)	100	6.3	>150 (4)	>150 (5)	n.e	3.2
Chlorpropham	94 (7)	88 (8)	10	0.08	87 (3)	70 (5)	10	0.14	106 (3)	94 (4)	10	0.13
Chlorpyrifos	95 (5)	100 (4)	10	0.01	105 (11)	85 (2)	10	0.01	101 (4)	100 (3)	10	0.32
Chlorpyrifos methyl	91 (1)	96 (2)	10	0.01	96 (2)	81 (4)	10	0.03	98 (3)	106 (1)	10	0.08
Coumaphos	98 (4)	97 (1)	10	0.01	82 (3)	75 (6)	10	0.04	105 (9)	97 (5)	10	0.04
Cyanazine	96 (7)	101 (2)	10	0.02	91 (3)	85 (4)	10	0.06	113 (6)	119 (4)	10	0.19
Cyanophos	96 (5)	99 (3)	10	0.14	100 (3)	84 (4)	10	0.23	107 (4)	106 (3)	10	0.09
Cyfluthrin	92 (2)	101 (3)	10	0.08	96 (4)	87 (4)	10	0.03	109 (4)	111 (6)	10	0.02
lambda-Cyhalothrin	70 (7)	96 (2)	10	0.04	89 (3)	85 (3)	10	0.14	107 (5)	106 (2)	10	0.14
Cypermethrin	97 (3)	105 (4)	10	0.14	94 (4)	87 (6)	10	0.07	109 (3)	109 (6)	10	0.39
Cyprodinil	82 (13)	88 (8)	10	0.44	82 (7)	82 (6)	10	0.39	100 (3)	84 (5)	10	0.65
p,p'-DDD	107 (10)	118 (2)	10	0.21	80 (5)	74 (5)	10	0.54	105 (5)	96 (5)	10	0.5
p,p'-DDE	80 (3)	88 (6)	10	0.11	83 (4)	70 (3)	10	0.14	94 (5)	75 (4)	10	0.2
p,p'-DDT	90 (6)	109 (2)	10	3	74 (8)	71 (6)	10	2.2	88 (15)	80 (9)	10	4.3
Deltamethrin	94 (7)	101 (4)	10	0.19	117 (5)	87 (10)	10	0.79	73 (4)	70 (8)	10	4.6
Demeton-s-methyl	84 (4)	66 (4)	10	1.3	140 (7)	104 (5)	100	6.3	119 (7)	103 (4)	10	10
Demeton-s-methylsulfone	107 (5)	104 (3)	10	0.31	88 (7)	70 (9)	10	0.83	114 (12)	128 (9)	10	3
Diazinon	98 (4)	104 (3)	10	0.02	98 (2)	86 (4)	10	0.07	101 (3)	100 (4)	10	0.02
Dichlofenthion	93 (3)	95 (3)	10	0.02	87 (2)	79 (4)	10	0.05	113 (2)	81 (6)	10	0.3
Dichloran	95 (4)	97 (4)	10	0.08	96 (3)	78 (5)	10	0.14	115 (3)	95 (2)	10	0.17
4,4'-Dichlorobenzophenone	92 (4)	89 (2)	10	0.07	92 (2)	87 (4)	10	0.08	111 (2)	108 (3)	10	0.1
Dichlorvos	88 (4)	93 (2)	10	0.08	103 (2)	83 (4)	10	0.13	117 (4)	101 (3)	10	0.08
Dieldrin	91 (4)	94 (3)	10	0.3	91 (3)	76 (3)	10	1	106 (5)	88 (4)	10	1

Table 2 (continued).

Compounds	Orange				Tomato				Carrot			
	Fortification levels (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)	Fortification levels (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)	Fortification levels (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)
	10	100			10	100			10	100		
Diflufenican	104 (4)	96 (2)	10	0.01	86 (4)	72 (1)	10	0.02	117 (2)	81 (5)	10	0.05
Dimethoate	100 (4)	102 (4)	10	0.04	97 (2)	74 (5)	10	0.07	121 (7)	133 (6)	10	0.21
Dioxathion	99 (4)	99 (1)	10	0.15	83 (2)	77 (6)	10	0.47	118 (4)	103 (5)	10	0.1
Diphenylamine	106 (6)	100 (3)	10	0.13	86 (6)	71 85)	10	0.3	117 (4)	93 (3)	10	0.21
Endosulfan eher	98 (4)	92 (3)	10	0.33	102 (4)	78 (3)	10	0.3	108 (4)	87 (6)	10	0.37
Endosulfan I	99 (5)	91 (5)	10	0.25	102 (6)	81 (4)	10	0.25	104 (6)	76 (4)	10	1.9
Endosulfan II	94 (12)	101 (17)	10	0.25	100 (10)	83 (2)	10	0.25	107 (3)	95 (4)	10	0.2
Endosulfan sulfate	93 (4)	90 (2)	10	0.13	90 (4)	83 (4)	10	0.19	101 (5)	114 (3)	10	0.17
Endrin	90 (7)	95 (4)	10	1.1	98 (4)	83 (2)	10	0.27	106 (5)	112 (6)	10	0.15
Esfenvalerate	114 (2)	116 (6)	10	0.5	79 (1)	87 (5)	10	0.13	97 (5)	109 (7)	10	0.2
Ethalfluralin	98 (6)	92 (10)	10	0.01	92 (4)	81 (4)	10	0.01	119(4)	88 (5)	10	0.02
Ethion	106 (8)	118 (10)	10	0.06	93 (4)	87 (3)	10	0.1	102 (4)	106 (7)	10	0.05
Ethoxiquin	116 (6)	118 (3)	10	0.71	84 (16)	71 (19)	10	0.24	-	3 (7)	n.e	0.54
Etofenprox	91 (4)	89 (2)	10	0.08	89 (3)	75 (4)	10	0.04	104 (3)	80 (4)	10	0.08
Famphur	99 (4)	103 (5)	10	0.01	93 (1)	88 (4)	10	0.02	115 (6)	105 (6)	10	0.02
Fenamiphos	105 (4)	98 (1)	10	0.02	89 (2)	70 (4)	10	0.03	119 (7)	113 (5)	10	0.1
Fenarimol	99 (5)	99 (3)	10	0.02	88 (2)	80 (4)	10	0.06	117 (19)	94 (4)	10	0.04
Fenhexamid	107 (8)	107 (3)	10	1.2	94 (13)	83 (7)	10	1.4	>150 (33)	>150 (4)	n.e	2.7
Fenitrothion	97 (3)	102 (2)	10	0.03	93 (2)	84 (5)	10	0.03	103 (6)	100 (2)	10	0.44
Fenoxycarb	103 (5)	106 (2)	10	0.65	97 (3)	85 (5)	10	1.4	114 (9)	119 (4)	10	1.9
Fenthion	103 (3)	109 (2)	10	0.02	83 (3)	80 (4)	10	0.03	102 (5)	100 (4)	10	0.06
Fenvalerate	102 (9)	114 (6)	10	0.94	70 (6)	76 (4)	10	0.48	94 (6)	120 (5)	10	1
Fipronil	103 (5)	114 (2)	10	0.01	94 (2)	93 (3)	10	0.01	120 (3)	108 (5)	10	0.01
Flucythrinate	104 (4)	102 (3)	10	0.05	88 (3)	82 (5)	10	0.17	116 (7)	106 (5)	10	0.12
Fludioxonil	87 (13)	82 (15)	10	0.17	120 (5)	76 (4)	10	0.41	109 (4)	89 (6)	10	0.83
Fluvalinate	125 (4)	124 (5)	10	0.11	93 (4)	83 (6)	10	0.09	110 (5)	117 (10)	10	0.08
Folpet	101 (5)	93 (5)	10	0.38	70 (7)	70 (3)	10	0.34	104 (7)	87 (4)	10	1.2
alpha-HCH	95 (16)	117 (3)	10	0.94	79 (5)	70 (9)	10	3	99 (13)	95 (9)	10	3
beta-HCH	108 (12)	113 (7)	10	3	70 (10)	81 (4)	10	10	98 (18)	86 (13)	10	3.9
delta-HCH	121 (8)	112 (7)	10	3	91 (6)	69(9)	10	5	90 (9)	94 (6)	10	4.9
gamma-HCH	99 (13)	117 (7)	10	3	96 (8)	76 (9)	10	5	103 (10)	90 (8)	10	5
Heptachlor	79 (7)	92 (4)	10	0.3	103 (5)	76 (7)	10	0.5	118 (6)	97 (3)	10	0.6
Heptachlor epoxide A	75 (12)	96 (3)	10	1.2	119 (9)	84 (10)	10	2.5	113 (15)	102 (7)	10	0.6
Heptachlor epoxide B	91 (15)	95 (3)	10	0.6	108 (6)	78 (5)	10	0.35	102 (8)	93 (5)	10	0.6
Hexachlorobenzene	95 (6)	76 (7)	10	2	83 (10)	86 (5)	10	1.8	88 (9)	70 (9)	10	3.3
Hexachlorobutadiene	72 (3)	98 (12)	10	0.22	73 (3)	78 (5)	10	0.39	92 (4)	89 (11)	10	0.68
Imazalil	57 (16)	18 (8)	n.e	1.3	58 (11)	8 (10)	n.e	2.2	60 (22)	21 (3)	n.e	3
Iprodione	100 (4)	103 (2)	10	0.1	100 (3)	83 (4)	10	0.17	104 (9)	112 (4)	10	0.17
Isodrin	75 (13)	80 (3)	10	0.68	89 (4)	74 (3)	10	2.1	103 (7)	75 (6)	10	0.5
Leptophos	89 (5)	92 (3)	10	0.04	81(2)	76 (5)	10	0.07	115 (3)	87 (7)	10	0.1
Malathion	101 (6)	111 (6)	10	0.07	99 (2)	91 (4)	10	0.08	105 (11)	110 (5)	10	0.07
Metalaxyl	98 (4)	101 (2)	10	0.04	93 (1)	72 (2)	10	0.04	120 (5)	108 (4)	10	0.08
Methidathion	84 (9)	107 (6)	10	0.56	102 (4)	96 (6)	10	0.71	110 (9)	120 (5)	10	1.5
Methiocarb	102 (4)	109 (3)	10	0.3	93 (2)	86 (7)	10	0.14	120 (7)	111 (6)	10	0.27
Methiocarb sulfone	100 (6)	98 (4)	10	0.06	102 (13)	83 (6)	10	0.2	>150 (54)	135 (4)	n.e	1.3
Methoxychlor	94 (2)	93 (3)	10	0.27	108 (2)	97 (3)	10	0.27	118 (4)	115 (5)	10	0.24
Metolachlor	95 (4)	106 (8)	10	0.63	93 (5)	84 (3)	10	0.58	103 (5)	108 (5)	10	1
Metribuzin	98 (3)	89 (4)	10	1.5	86 (5)	77 (5)	10	1.4	114 (3)	85 (5)	10	1.3

Table 2 (continued).

Compounds	Orange				Tomato				Carrot			
	Fortification levels (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)	Fortification levels (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)	Fortification levels (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)
	10	100			10	100			10	100		
Mevinphos	96 (3)	84 (2)	10	0.04	92 (4)	80 (4)	10	0.07	119 (7)	119 (6)	10	0.11
Mirex	72 (9)	77 (10)	10	3	79 (11)	54 (7)	10	1.1	71 (11)	50 (8)	10	1.8
Molinate	>150 (2)	140 (1)	n.e	0.01	>150 (1)	127 (1)	n.e	0.01	>150 (3)	129 (2)	n.e	0.01
Oxadixyl	118 (19)	117 (17)	10	0.71	99 (2)	93 (2)	10	0.32	118 (8)	98 (5)	10	0.71
Oxychlordane	73 (8)	n. a.	10	0.68	90 (4)	n. a.	10	0.3	99 (5)	n. a.	10	1
Oxyfluorfen	93 (4)	103 (18)	10	0.01	97 (6)	80 (3)	10	0.02	111 (3)	98 (3)	10	0.02
Parathion ethyl	97 (5)	105 (5)	10	0.03	92 (2)	83 (4)	10	0.03	118 (3)	103 (3)	10	0.06
Parathion methyl	99 (3)	101 (3)	10	0.01	91 (3)	82 (5)	10	0.04	97 (5)	107 (3)	10	0.33
Pendimethalin	93 (4)	94 (4)	10	0.13	92 (2)	80 (2)	10	0.1	109 (4)	94 (3)	10	0.05
Pentachlorobenzene	87 (5)	100 (3)	10	0.09	83 (4)	70 (3)	10	0.18	85 (4)	86 (5)	10	0.09
Permethrin	103 (4)	94 (2)	10	1	89 (3)	108 (3)	10	1	86 (10)	70 (3)	10	0.79
2-Phenylphenol	102 (7)	120 (4)	10	0.05	115 (6)	114 (4)	10	0.08	105 (4)	95 (4)	10	0.03
Phorate	94 (10)	107 (4)	10	1.4	102 (12)	72 (5)	10	2.3	113 (11)	107 (3)	10	2.2
Phosmet	87 (13)	103 (3)	10	0.04	106 (3)	81 (10)	10	0.16	113 (7)	117 (9)	10	0.83
Phosphamidon	99 (4)	93 (1)	10	0.04	100 (2)	87 (2)	10	0.1	110 (5)	120 (7)	10	0.68
Pirimicarb	95 (6)	104 (6)	10	0.71	97 (5)	86(2)	10	0.6	120 (4)	104 (4)	10	0.75
Pirimiphos methyl	96 (3)	105 (2)	10	0.07	86 (3)	82 (5)	10	0.04	101 (1)	106 (4)	10	0.04
Procymidone	94 (5)	101 (8)	10	0.05	96 (2)	82 (4)	10	0.1	107 (2)	108 (3)	10	0.05
Propachlor	101 (2)	98 (3)	10	0.33	89 (2)	80 (3)	10	0.21	119 (3)	103 (4)	10	0.17
Propetamphos	95 (7)	96 (3)	10	0.44	86 (5)	70 (7)	10	0.56	102 (24)	102 (3)	10	0.16
Propham	80 (5)	82 (5)	10	0.19	86 (10)	81 (9)	10	0.6	110 (2)	99 (2)	10	0.1
Propiconazole	98 (2)	102 (7)	10	0.21	98 (6)	82 (3)	10	0.28	109 (3)	104 (4)	10	0.26
Propoxur	>150 (3)	>150 (3)	n.e	0.13	>150 (4)	>150 (2)	n.e	0.25	>150 (10)	>150 (7)	n.e	0.43
Propyzamide	95 (4)	95 (4)	10	0.03	98 (1)	85 (4)	10	0.1	100 (5)	94 (3)	10	0.27
Pyriproxifen	94 (3)	92 (3)	10	0.06	93 (3)	83 (3)	10	0.08	120 (5)	96 (3)	10	0.08
Quinalphos	98 (7)	105 (5)	10	0.04	94 (1)	85 (2)	10	0.11	104 (4)	108 (3)	10	0.08
Resmethrin	89 (3)	92 (2)	10	0.17	87 (3)	77 (3)	10	0.07	103 (6)	104 (5)	10	0.09
Simazine	112 (10)	105 (6)	10	0.13	88 (7)	72 (5)	10	0.79	116 (5)	100 (4)	10	0.83
Sulprofos	94 (4)	97 (3)	10	0.07	79 (3)	71 (3)	10	0.07	113 (3)	88 (5)	10	0.06
Tefluthrin	86 (4)	91 (1)	10	0.05	91 (4)	78 (3)	10	0.04	106 (2)	85 (3)	10	0.04
Terbacil	83 (8)	86 (8)	10	0.25	95 (5)	85 (4)	10	0.79	90 (7)	109 (8)	10	0.83
Terbufos	98 (9)	93 (3)	10	1.6	88 (11)	78 (5)	10	1.1	95 (7)	100 (4)	10	1.5
Terbumeton	93 (4)	86 (3)	10	0.19	93 (4)	71 (4)	10	0.15	112 (2)	104 (3)	10	0.12
Terbumeton desethyl	112 (4)	88 (2)	10	0.48	94 (3)	72 (2)	10	0.71	112 (2)	93 (5)	10	0.42
Terbutryn	126 (3)	123 (3)	10	0.07	107 (2)	90 (3)	10	0.04	114 (3)	103 (3)	10	0.06
Terbutylazine	94 (3)	107 (10)	10	0.06	90 (1)	73 (4)	10	0.07	109 (4)	114 (2)	10	0.06
Terbutylazine desethyl	103 (3)	97 (3)	10	0.01	94 (2)	71 (3)	10	0.13	117 (2)	105 (3)	10	0.13
Tetradifon	98 (5)	102 (3)	10	0.02	84 (4)	82 (4)	10	0.05	120 (4)	93 (6)	10	0.13
Tolyfluanid	77 (12)	66 (8)	10	0.25	12 (32)	49 (15)	n.e	0.21	60 (22)	110 (11)	100	0.23
Triadimefon	95 (3)	97 (2)	10	0.02	89 (2)	81 (3)	10	0.03	97 (2)	104 (3)	10	0.09
Triflumizole	97 (9)	96 (9)	10	0.06	90 (4)	76 (3)	10	0.11	104 (3)	105 (3)	10	0.21
Trifluraline	98 (2)	93 (3)	10	0.02	91 (2)	73 (5)	10	0.01	115 (2)	90 (2)	10	0.01
Vinclozolin	92 (3)	95 (5)	10	0.02	92 (3)	81 (3)	10	0.02	112 (2)	102 (2)	10	0.02

n.a. not available
n.e. LOQ not estimated as validation parameters at both fortification levels were not satisfactory
Underlined, not acceptable results

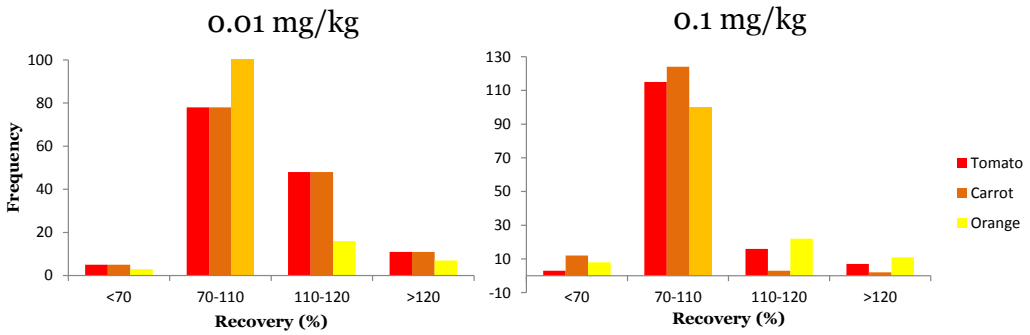

Fig. 1. Histograms obtained from the recovery experiments of the three sample matrices fortified at (a) 0.01 mg/kg and (b) 0.1 mg/kg.

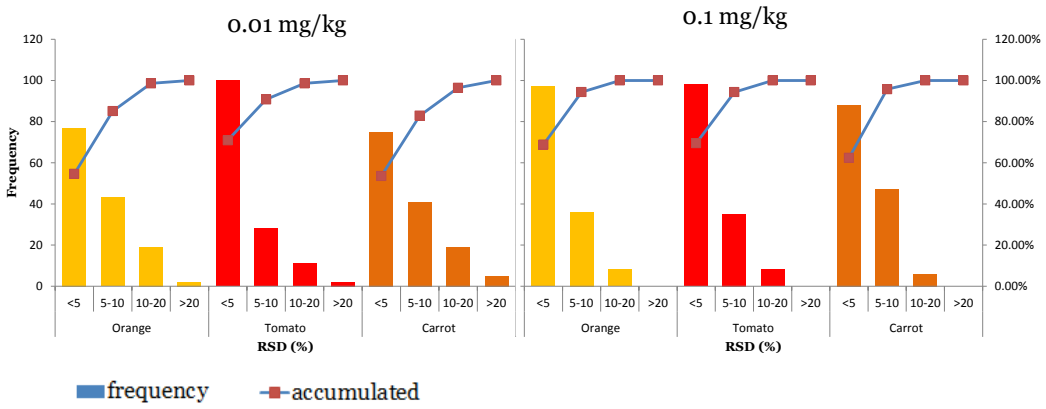

Fig. 2. Histograms obtained from the RSD values of the three sample matrices fortified at (a) 0.01 mg/kg and (b) 0.1 mg/kg.

Low LODs were obtained for all compounds since most of them ranged between 0.01 and 1 □g/kg in the three matrices (see **Fig. 3**). Only few values were higher than 1 □g/kg. **Fig. 4** shows four examples (selected from different LOD ranges showed in **Fig. 3**) for which signal-to-noise ratios were calculated from the lowest matrix-matched standard in orange samples and where LODs can be estimated by extrapolation.

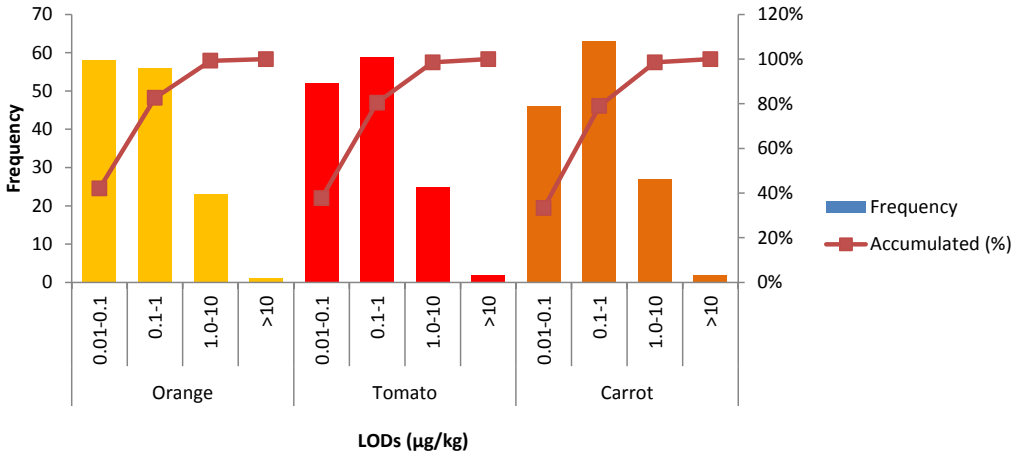

Fig. 3. Histograms obtained from the LOD values of the three sample matrices.

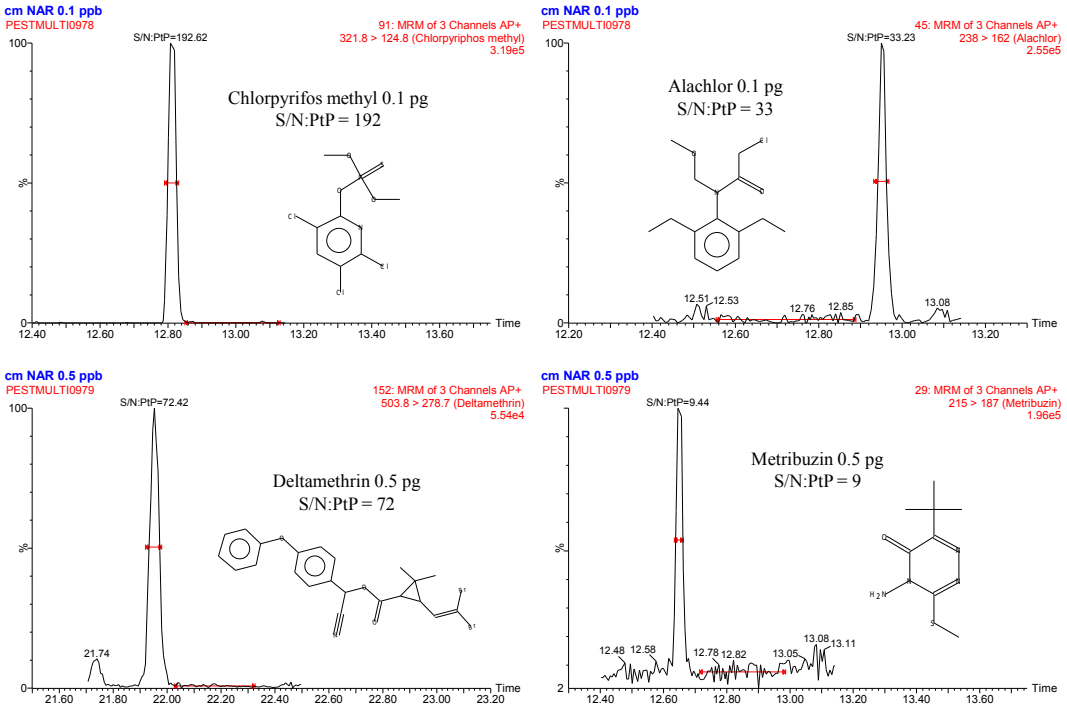

Fig. 4. GC–(APCI) MS/MS chromatogram of four pesticides from the lowest matrix-matched standard (0.1–0.5 ng/mL, corresponding to 0.1–0.5 pg on column) in orange samples. S/N:PtP: peak-to-peak signal-to-noise ratio.

The selectivity, as evaluated for each of the three specific SRM transitions measured, was satisfactory. GC–MS/MS chromatograms did not show interfering peaks at the analyte retention time for any of the pesticides investigated in this work.

3.5. Qualitative aspects: consistency of ion ratios and identification

With respect to the identification of pesticides in samples, criteria have been set for the ratio of the response obtained for the transitions measured [24]. Depending on the relative abundance of the two transitions, the ion ratio should be within 20–50% of the reference value. This aspect was evaluated in the validation for all pesticides, in each of the three matrices, at the two concentration levels. For each pesticide, two ion ratios were calculated: the first qualifier/quantifier (q1/Q) and the second qualifier/quantifier (q2/Q). The average ion ratio obtained for up to eight matrix-matched standards in the range of 0.1–100 ng/mL was used as reference ion ratio (values are included in **Table 3**). For the calculation of the average, signals with poor S/N and saturated signals were excluded. In general, the ion ratios for the different concentrations of the standards were very consistent (RSD <10% in most cases), even when the ion ratio was very unfavorable (<0.10).

For the spiked samples, the deviation of the individual ion ratios were calculated against the reference value and then compared with the maximum tolerable deviations according to the SANCO guideline [24]. In **Table 3**, for each pesticide, in each matrix and for each level (with n = 6), the number of ion-ratio compliances is given. Overall, the percentage of pesticides that met the ion ratio criterion for one ratio was 77–81% at 0.01 mg/kg, and 95–97% at 0.1 mg/kg, with not many differences between the three matrices tested. For 60–65% of the pesticides, the criterion was met for both ratios determined. The reason for not meeting the criteria generally was a too low sensitivity of one of the qualifier transitions measured. For the pesticide methidathion, no suitable qualifier transitions could be obtained and no adequate identification was possible.

Table 3. Study of the q/Q ratios and compliance with EU criteriaa for the three matrices studied at 0.01 and 0.1 mg/kg.

Compound	Orange						Tomato						Carrot					
	q1/Q	q2/Q	ion ratio compliance (# out of 6)				q1/Q	q2/Q	ion ratio compliance (# out of 6)				q1/Q	q2/Q	ion ratio compliance (# out of 6)			
			0.01	0.1	0.01	0.1			0.01	0.1	0.01	0.1			0.01	0.1	0.01	0.1
			q1/Q	q1/Q	q2/Q	q2/Q			q1/Q	q1/Q	q2/Q	q2/Q			q1/Q	q1/Q	q2/Q	q2/Q
Acrinathrin	0.951	0.63	6	6	6	6	0.831	0.598	5	6	6	6	0.844	0.651	5	6	6	6
Alachlor	0.999	0.057	6	6	6	6	0.987	0.045	6	6	6	6	0.999	0.046	6	6	6	6
Aldrin	0.949	0.836	4	6	4	6	0.998	0.867	3	6	4	5	1	0.835	3	6	3	6
Atrazine	0.397	0.099	6	6	6	6	0.339	0.099	6	6	6	6	0.329	0.089	6	6	6	6
Atrazine deisopropyl	0.534	0.548	5	6	5	6	0.529	0.481	5	6	6	6	0.518	0.47	6	6	6	6
Atrazine desethyl	0.2	0.002	6	6	0	6	0.178	0.001	6	6	0	5	0.195	0.001	6	6	0	6
Azinphos ethyl	0.552	0.534	0	6	0	6	0.597	0.469	0	6	0	6	0.595	0.471	0	4	0	6
Azinphos methyl	0.337	0.096	6	6	0	6	0.256	0.067	0	6	0	6	0.248	0.072	0	6	0	6
Azoxystrobin	0.344	0.31	6	6	6	6	0.391	0.304	6	6	6	6	0.406	0.289	6	6	6	6
Bifenthrin	0.932	0.056	6	6	6	6	0.973	0.055	6	6	6	6	0.982	0.054	6	6	6	6
Bromophos ethyl	0.654	0.445	6	6	6	6	0.597	0.376	6	6	6	6	0.564	0.358	6	6	6	6
Bromophos methyl	0.233	0.228	6	6	6	6	0.194	0.229	6	6	6	6	0.19	0.232	6	6	6	6
Buprofezin	0.16	0.316	5	6	3	0	0.112	0.176	5	6	4	6	0.126	0.149	6	5	6	0
Cadusafos	0.23	0.069	5	6	0	6	0.313	0.057	5	6	0	5	0.343	0.057	6	6	0	6
Captafol	0.492	0.452	6	6	6	6	0.461	0.409	5	6	6	6	0.447	0.393	4	6	5	6
Captan	0.483	0.271	0	6	5	6	0.335	0.227	0	6	2	6	-	0.216	-	-	2	6
Carbaryl	0.926	0.71	2	6	0	0	0.953	0.795	0	6	0	5	0.913	0.842	4	4	5	1
Carbofenothion	0.049	0.007	6	6	0	6	0.045	0.006	6	6	0	6	0.045	0.006	6	6	5	6
Carbofuran	0.054	0.006	0	6	0	5	0.061	0.007	0	6	0	4	0.058	0.006	1	6	5	6
Carfentrazone ethyl	0.329	0.305	6	6	6	6	0.471	0.356	6	6	6	6	0.457	0.337	6	6	6	6
Chinomethionate	0.602	0.412	6	5	6	5	0.566	0.375	5	6	6	6	0.563	0.354	6	6	6	6
trans-Chlordane	0.324	0.105	1	6	5	5	0.363	0.141	5	6	6	6	0.347	0.138	5	6	0	6
Chlorfenson	0.459	0.119	6	6	6	6	0.283	0.104	6	6	6	6	0.252	0.097	6	6	6	6
Chlorfenvinphos	0.853	0.284	6	6	6	6	0.941	0.228	6	6	6	6	0.93	0.23	6	6	6	6
Chlorothalonil	0.718	0.004	6	6	0	6	0.645	0.004	2	6	0	0	0.677	0.003	6	6	4	6
Chlorpropham	0.404	0.285	6	6	6	6	0.449	0.229	6	6	6	6	0.453	0.252	6	6	6	6
Chlorpyrifos	0.352	0.148	6	6	6	6	0.416	0.13	6	6	6	6	0.426	0.126	6	6	6	6
Chlorpyrifos methyl	0.44	0.155	6	6	6	6	0.459	0.146	6	6	6	6	0.438	0.137	6	6	6	6
Coumaphos	0.371	0.181	6	6	6	6	0.41	0.184	6	6	6	6	0.407	0.184	6	6	6	6
Cyanazine	0.117	0.093	6	6	6	6	0.101	0.091	6	6	6	6	0.099	0.096	6	6	6	6
Cyanophos	0.64	0.133	6	6	6	6	0.658	0.14	6	6	6	6	0.658	0.143	6	6	6	6
Cyfluthrin	0.335	0.307	6	6	6	6	0.346	0.315	6	6	3	6	0.344	0.304	6	6	6	6
lambda-Cyhalothrin	0.079	0.023	6	6	6	6	0.062	0.022	6	6	6	6	0.06	0.022	6	6	6	6
Cypermethrin	0.334	0.314	6	6	6	6	0.325	0.314	6	6	6	6	0.329	0.312	6	6	6	6
Cyprodinil	0.548	0.732	6	6	5	6	0.501	0.669	5	6	4	6	0.516	0.614	6	6	3	6
p,p'-DDD	0.214	0.028	6	6	0	6	0.276	0.02	5	6	6	6	0.268	0.02	5	6	0	6
p,p'-DDE	0.148	0.055	6	6	6	6	0.163	0.047	6	6	6	6	0.177	0.045	6	6	0	6
p,p'-DDT	0.29	0.017	6	6	0	6	0.258	0.018	0	6	0	6	0.267	0.017	0	6	0	6
Deltamethrin	0.131	0.142	6	6	6	6	0.131	0.132	4	6	0	6	0.132	0.128	4	6	2	6
Demeton-s-methyl	0.321	0.009	5	5	0	0	0.398	0.013	0	6	0	0	0.393	-	0	5	-	-
Demeton-s-methylsulfone	0.947	0.391	6	6	6	6	0.904	0.371	6	6	6	6	0.9	0.378	6	6	5	6
Diazinon	0.773	0.514	6	6	6	6	0.723	0.589	6	6	6	6	0.698	0.571	6	6	6	6
Dichlofenthion	0.462	0.917	6	6	6	6	0.362	0.898	6	6	6	6	0.341	0.9	6	6	6	6
Dichloran	0.842	0.912	6	6	6	6	0.906	0.781	6	6	6	6	0.874	0.729	6	6	6	6
4,4'-Dichlorobenzophenone	0.554	-	6	6	-	-	0.518	-	6	6	-	-	0.5	-	6	6	-	-
Dichlorvos	0.091	0.061	6	6	6	6	0.088	0.061	6	6	6	6	0.089	0.062	6	6	6	6
Dieldrin	0.774	0.689	6	6	6	6	0.761	0.748	6	6	6	6	0.789	0.721	5	6	6	6

Table 3 (continued).

Compound	Orange						Tomato						Carrot					
	q1/Q	q2/Q	ion ratio compliance (# out of 6)				q1/Q	q2/Q	ion ratio compliance (# out of 6)				q1/Q	q2/Q	ion ratio compliance (# out of 6)			
			0.01	0.1	0.01	0.1			0.01	0.1	0.01	0.1			0.01	0.1	0.01	0.1
			q1/Q	q1/Q	q2/Q	q2/Q			q1/Q	q1/Q	q2/Q	q2/Q			q1/Q	q1/Q	q2/Q	q2/Q
Diflufenican	0.177	0.136	6	6	6	6	0.156	0.107	6	6	6	6	0.164	0.106	6	6	6	6
Dimethoate	0.771	0.331	6	6	6	6	0.865	0.249	6	6	6	6	0.881	0.249	6	6	6	6
Dioxathion	0.341	0.125	6	6	6	6	0.305	0.146	6	6	5	6	0.281	0.131	6	6	6	6
Diphenylamine	0.09	0.071	6	6	6	6	0.076	0.079	6	6	6	6	0.076	0.079	6	6	6	6
Endosulfan ether	0.775	0.794	6	6	6	6	0.779	0.791	6	6	6	6	0.835	0.754	6	6	6	6
Endosulfan I	0.832	0.751	6	6	5	6	0.745	0.698	6	6	6	6	0.704	0.7	5	6	5	6
Endosulfan II	0.794	0.648	0	5	5	6	0.601	0.509	6	6	6	6	0.569	0.528	6	6	6	6
Endosulfan sulfate	0.733	0.554	6	6	6	6	0.757	0.61	6	6	6	6	0.751	0.592	6	6	6	6
Endrin	0.917	0.991	5	6	6	6	1.007	0.885	5	6	6	6	0.996	0.888	5	6	6	6
Esfenvalerate	-	-	-	-	-	-	0.047	0.032	3	6	4	6	0.062	0.031	6	6	6	6
Ethalfluralin	0.712	0.275	6	6	6	6	0.589	0.344	6	6	6	6	0.544	0.361	6	6	6	6
Ethion	0.327	0.041	6	6	6	6	0.285	0.024	6	6	6	6	0.289	0.022	6	6	6	6
Ethoxyquin	0.649	0.552	6	6	6	6	0.657	0.522	6	6	6	6	0.646	0.53	0	1	0	1
Etofenprox	0.305	0.109	6	6	6	6	0.334	0.109	6	6	6	6	0.345	0.115	6	6	6	6
Famphur	0.364	0.068	6	6	6	6	0.305	0.056	6	6	6	6	0.304	0.056	6	6	6	6
Fenamiphos	0.4	0.16	6	6	6	6	0.361	0.198	6	6	6	6	0.352	0.22	6	6	6	6
Fenarimol	0.736	0.343	6	6	6	6	0.623	0.305	6	6	6	6	0.643	0.304	6	6	6	6
Fenhexamid	0.615	0.498	6	6	6	6	0.658	0.478	6	6	6	6	0.656	0.481	6	6	6	6
Fenitrothion	0.371	0.27	6	6	6	6	0.388	0.259	6	6	6	6	0.372	0.253	6	6	6	6
Fenoxycarb	0.106	0.068	5	6	6	6	0.103	0.063	6	6	5	6	0.111	0.064	6	6	6	6
Fenthion	0.285	0.83	6	6	6	6	0.294	0.79	6	6	6	6	0.3	0.783	6	6	6	6
Fenvalerate	0.304	0.252	6	6	6	6	0.436	0.331	6	6	2	6	0.466	0.366	6	6	5	6
Fipronil	0.253	0.199	6	6	6	6	0.209	0.174	6	6	6	6	0.208	0.181	6	6	6	6
Flucythrinate	0.394	0.358	6	6	6	6	0.425	0.42	6	6	6	6	0.41	0.423	6	6	6	6
Fludioxonil	0.539	0.793	5	5	6	5	0.712	0.787	6	6	6	6	0.71	0.775	6	6	6	6
Fluvalinate	0.095	0.059	6	6	6	6	0.106	0.052	6	6	6	6	0.108	0.052	6	6	6	6
Folpet	0.45	0.488	6	6	6	6	0.456	0.422	6	6	6	6	0.447	0.407	6	6	6	6
alpha-HCH	0.578	0.597	4	6	4	6	0.675	0.61	4	6	0	5	0.744	0.561	4	6	2	6
beta-HCH	0.649	0.759	0	6	3	6	0.695	0.678	2	6	0	6	0.725	0.688	2	6	2	4
delta-HCH	0.579	0.617	3	5	1	6	0.6	0.586	0	6	0	4	0.627	0.596	0	6	0	6
gamma-HCH	0.628	0.485	3	6	5	6	0.707	0.411	4	6	0	6	0.722	0.391	3	6	0	5
Heptachlor	0.864	0.377	5	6	4	6	0.833	0.344	6	6	5	6	0.793	0.331	4	6	5	6
Heptachlor epoxide A	0.874	0.773	3	6	4	6	0.939	0.835	3	6	4	6	0.997	0.735	5	6	4	6
Heptachlor epoxide B	0.914	0.825	4	6	5	6	0.988	0.789	6	6	6	6	0.973	0.782	5	6	6	6
Hexachlorobenzene	0.951	0.084	4	6	0	6	0.881	0.052	5	6	0	0	0.807	0.049	5	6	0	5
Hexachlorobutadiene	0.923	0.446	6	6	6	6	0.977	0.359	6	6	6	6	0.994	0.336	6	6	5	6
Imazalil	0.33	0.288	2	6	0	6	0.315	0.349	0	3	0	6	0.308	0.352	0	6	0	0
Iprodione	0.149	0.097	6	6	6	6	0.169	0.099	6	6	6	6	0.161	0.089	6	6	6	6
Isodrin	0.956	0.875	4	6	5	6	0.922	0.874	6	6	6	6	0.828	0.81	5	6	6	6
Leptophos	0.252	0.12	6	6	6	6	0.255	0.109	6	6	6	6	0.255	0.109	6	6	6	6
Malathion	0.061	0.064	6	6	6	6	0.043	0.058	6	6	6	6	0.04	0.057	6	6	5	6
Metalaxyl	0.688	0.198	6	6	6	6	0.711	0.131	6	6	6	6	0.738	0.127	6	6	6	6
Methidathion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methiocarb	0.894	0.139	6	6	6	6	0.917	0.155	6	6	6	6	0.864	0.155	6	6	6	6
Methiocarb sulfone	0.168	0.046	6	6	6	6	0.208	0.078	6	6	0	6	0.217	0.071	6	6	6	6
Methoxychlor	0.367	0.303	6	6	6	6	0.344	0.216	4	3	3	1	0.239	0.145	6	6	4	6
Metolachlor	0.397	0.244	6	6	6	6	0.366	0.196	6	6	6	6	0.357	0.185	6	6	6	6

Table 3 (continued).

Compound	Orange						Tomato						Carrot					
	q1/Q	q2/Q	ion ratio compliance (# out of 6)				q1/Q	q2/Q	ion ratio compliance (# out of 6)				q1/Q	q2/Q	ion ratio compliance (# out of 6)			
			0.01	0.1	0.01	0.1			0.01	0.1	0.01	0.1			0.01	0.1	0.01	0.1
			q1/Q	q1/Q	q2/Q	q2/Q			q1/Q	q1/Q	q2/Q	q2/Q			q1/Q	q1/Q	q2/Q	q2/Q
Metribuzin	0.25	0.153	3	6	0	6	0.287	0.131	6	6	6	6	0.305	0.136	4	6	5	6
Mevinphos	0.589	0.27	6	6	6	6	0.451	0.29	6	6	6	6	0.488	0.287	6	6	0	6
Mirex	0.265	0.283	3	6	0	6	0.258	0.248	0	5	0	4	0.238	0.23	0	5	0	4
Molinate	0.17	0.026	6	6	6	6	0.161	0.026	6	0	6	6	0.167	0.024	6	0	6	0
Oxadixyl	0.311	0.107	6	6	6	6	0.283	0.091	6	6	6	6	0.284	0.087	6	6	6	6
Oxychlordane	0.307	-	6	0	0	0	0.262	0.07	5	0	0	0	0.237	0.069	5	0	6	0
Oxyfluorfen	0.33	0.002	6	6	6	6	0.354	0.002	6	6	6	6	0.365	0.002	6	6	0	6
Parathion ethyl	0.521	0.463	6	6	6	6	0.451	0.37	6	6	6	6	0.434	0.382	6	6	6	6
Parathion methyl	0.86	0.288	6	6	6	6	0.755	0.248	6	6	6	6	0.716	0.232	6	6	6	6
Pendimethalin	0.263	0.199	6	6	6	6	0.222	0.174	6	6	6	6	0.223	0.158	6	6	6	6
Pentachlorobenzene	0.559	0.47	5	6	6	6	0.428	0.326	4	6	6	6	0.401	0.321	3	6	5	6
Permethrin	0.126	0.101	5	6	4	6	0.158	0.117	5	6	5	6	0.168	0.126	6	6	6	6
2-Phenylphenol	0.885	0.152	6	6	6	6	0.774	0.114	6	6	6	6	0.746	0.117	6	6	6	6
Phorate	0.847	0.538	5	6	3	6	0.893	0.862	5	6	3	6	0.893	0.856	5	6	4	6
Phosmet	0.232	0.255	6	5	5	6	0.213	0.231	5	6	5	6	0.236	0.207	6	6	5	6
Phosphamidon	0.565	0.684	6	6	6	6	0.532	0.575	6	6	6	6	0.492	0.552	6	6	6	6
Pirimicarb	0.179	0.067	6	6	6	6	0.204	0.063	6	6	6	6	0.202	0.062	6	6	6	6
Pirimiphos methyl	0.916	0.613	6	6	6	6	0.96	0.539	6	6	6	6	0.931	0.593	6	6	6	6
Procymidone	0.338	0.215	6	6	6	6	0.282	0.169	6	6	6	6	0.278	0.163	6	6	6	6
Propachlor	0.666	0.247	6	6	6	6	0.574	0.237	6	6	6	6	0.513	0.222	6	6	6	6
Propetamphos	0.264	0.251	6	6	6	6	0.245	0.213	6	6	6	6	0.247	0.215	4	6	5	6
Propham	0.318	0.12	3	6	6	6	0.272	0.144	0	6	6	6	0.223	0.129	6	6	6	6
Propiconazole	0.699	0.214	6	6	6	6	0.753	0.197	6	6	6	6	0.785	0.206	6	6	6	6
Propoxur	0.223	0.003	6	6	0	6	0.324	0.002	6	6	6	6	0.345	0.003	6	6	6	6
Propyzamide	0.716	0.428	6	6	6	6	0.764	0.444	6	6	6	6	0.783	0.447	6	6	6	6
Pyriproxifen	0.803	0.696	6	6	6	6	0.785	0.611	6	6	6	6	0.75	0.611	6	6	6	6
Quinalphos	0.619	0.617	6	6	6	6	0.654	0.559	6	6	6	6	0.636	0.576	6	6	6	6
Resmethrin	0.595	0.163	6	6	6	6	0.562	0.163	6	6	6	6	0.553	0.165	6	6	6	6
Simazine	0.832	0.481	5	6	5	6	0.838	0.452	6	6	6	6	0.811	0.41	6	6	6	6
Sulprofos	0.901	0.936	6	6	6	6	0.93	0.804	6	6	6	6	0.997	0.872	6	6	6	6
Tefluthrin	0.439	0.231	6	6	5	6	0.212	0.115	2	2	5	4	0.226	0.124	2	6	6	6
Terbacil	0.335	0.179	3	6	6	6	0.357	0.168	4	6	4	6	0.379	0.177	5	6	0	6
Terbufos	0.855	0.288	4	6	0	5	0.936	0.394	4	6	1	6	0.856	0.433	5	6	5	6
Terbumeton	0.066	0.06	6	6	6	6	0.063	0.06	6	6	0	6	0.062	0.053	6	6	5	6
Terbumeton desethyl	0.224	0.092	6	6	6	6	0.221	0.087	6	6	6	6	0.199	0.077	6	6	6	6
Terbutryn	0.071	0.05	6	6	6	6	0.068	0.046	6	6	6	6	0.064	0.047	6	6	6	6
Terbutylazine	0.192	0.08	6	6	6	6	0.176	0.083	6	6	6	6	0.173	0.081	6	6	6	6
Terbutylazine desethyl	0.161	-	6	6	-	-	0.142	-	6	6	-	-	0.141	-	6	6	-	-
Tetradifon	0.064	0.02	6	6	0	6	0.047	0.022	6	6	0	6	0.049	0.017	6	6	0	6
Tolyfluanid	0.055	0.041	6	6	5	6	0.05	0.041	5	6	5	6	0.057	0.041	6	6	6	6
Triadimefon	0.263	0.198	6	6	6	6	0.312	0.236	6	6	6	6	0.315	0.243	6	6	6	6
Triflumizole	0.065	0.028	6	6	6	6	0.054	0.022	6	6	6	6	0.056	0.022	6	6	6	6
Trifluraline	0.392	0.594	6	6	6	6	0.366	0.476	6	6	6	6	0.358	0.448	6	6	6	6
Vinclozolin	0.306	0.124	6	6	6	6	0.27	0.108	6	6	6	6	0.275	0.105	6	6	6	6

* ion ratio criteria according to SANCO/12495/2011

3.6. Application to real samples

In order to test the applicability of the developed method, three types of orange, tomato and carrot samples collected from local markets were analyzed. Moreover, the method was expanded for the analysis of three types of apple, lettuce and courgette, including a matrix-matched calibration for each sample matrix and a quality control at 0.05 mg/kg.

A total of 43 different pesticides were identified in the analyzed samples, most of them at levels well below 0.01 mg/kg and all under their corresponding MRLs. An overview of the detected pesticides is shown in **Table 4**.

Orange was the most contaminated sample and several positive findings were present in all the varieties analyzed. In tomato and carrot samples, pesticides were frequently detected but most of them below the LOQ. The different varieties of apple, lettuce and courgette did not present many positive findings, although those in apple samples were the most abundant. Among positive findings, only a small number were found above the LOQ (see **Table 5**). A concentration level around 1 mg/kg of the fungicide folpet was the most significant finding, detected in one of the apple varieties, although not exceeding its MRL (3 mg/kg). Captan and bifenthrin, which are commonly used in agricultural crops, were also detected at high levels in apple samples, between 0.1 and 0.5 mg/kg. The OP insecticide chlorpyrifos is also frequently used in apple and orange crops, for which concentrations between 0.03 and 0.1 mg/kg were found. The maximum positive findings in tomato samples were for the fungicide iprodione (around 0.1 mg/kg), whose presence is common in vegetable crops. The higher concentrations levels of pesticides found in carrot samples occurred for metalaxyl and cypermethrin around 0.1 mg/kg. Regarding courgette samples analyzed, no pesticides above 0.01 mg/kg were found.

Table 4. List of detected pesticides in the different samples analyzed. Red color indicates the presence of the pesticide in the three varieties of the studied matrix and purple and green, the presence in two and one varieties, respectively.

Pesticide	Orange	Tomato	Carrot	Apple	Lettuce	Courgette
Diphenylamine						
Chlorpropham						
Terbumeton desethyl						
Terbutylazine desethyl						
Dimethoate						
Terbutylazine						
Chlorothalonil						
Phosphamidon						
Chlorpyrifos methyl						
Metalaxyl						
Methiocarb sulfone						
Methiocarb						
Chlorpyrifos						
Triadimefon						
4,4-Dichlorobenzophenone						
Cyprodinil						
Pendimethalin						
Fipronil						
Captan						
Folpet						
Procymidone						
Trifumizole						
Fenamiphos						
Fludioxonil						
p,p'-DDE						
Oxadixyl						
Sulprofos						
Famphur						
Propiconazole I						
Endosulfan sulfate						
Fenhexamid						
Propiconazole II						
Diflufenican						
Iprodione						
Phosmet						
Bifenthrin						
Pyriproxifen						
Fenarimol						
Coumaphos						
Cypermethrin						
Deltamethrin						
Azoxystrobin						

Table 5. Concentrations of pesticides above the LOQ (mg/kg) detected in analyzed samples.

Pesticide	Orange			Tomato			Carrot			Apple			Lettuce		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Azoxystrobin								0.023							
Bifenthrin										0.11					
Captan										0.12	0.44				
Chlorpyrifos		0.11	0.035							0.028	0.042	0.059			
Chlorpyrifos methyl			0.013												
Cypermethrin									0.14						
Cyprodinil				0.015											
p,p'-DDE								0.035							
Fenhexamid					0.013										
Fludioxonil				0.011											
Folpet											1.3				
Iprodione				0.13	0.048	0.055									
Metalaxyl									0.13				0.013		
Pyriproxifen				0.024											

As regards identification, all detected pesticides were identified by the use of three transitions and the compliance of at least one q/Q ratio. Identification was problematic at low levels in a few compounds due to unfavorable q/Q ratios.

As an illustrative example, **Fig. 5** shows GC–(APCI) MS/MS chromatograms corresponding to three of the positive findings detected in analyzed samples: chlorpyriphos in apple (0.04 mg/kg), pyriproxyfen in tomato (0.02 mg/kg) and triadimefon in lettuce (below LOQ). A reliable identification of analytes in these samples was feasible by means of the experimental q/Q intensity ratios, even at those low concentration levels.

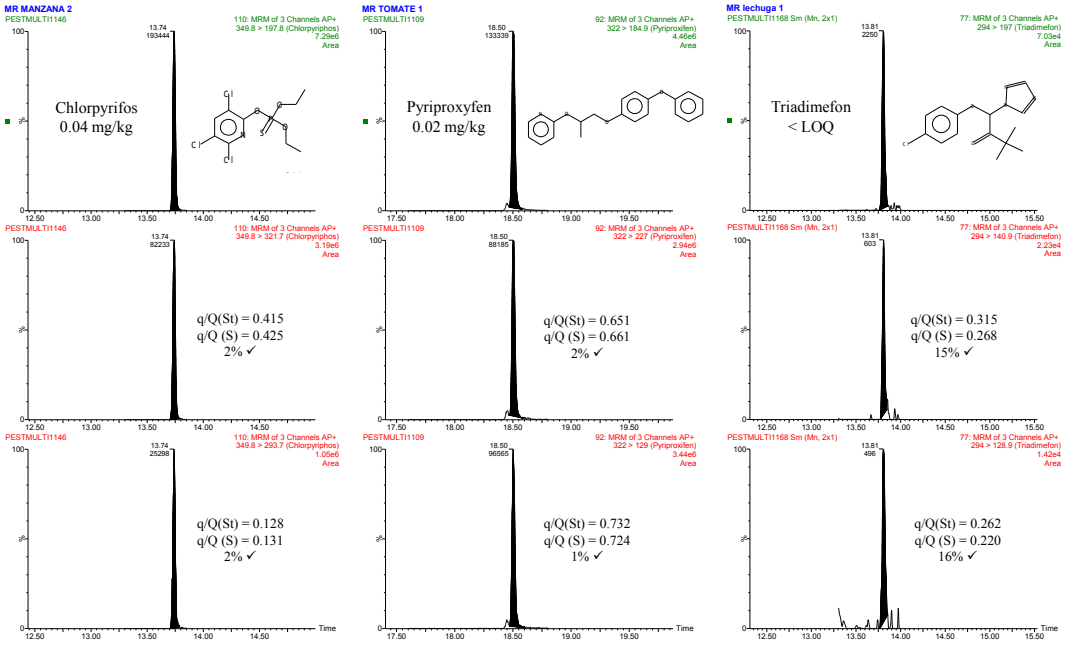

Fig. 5. GC–(APCI) MS/MS chromatograms for pesticides detected in apple, tomato and lettuce. (Q) quantification transition; (q) qualifier transition; (St) standard; (S) sample.

4. CONCLUSIONS

A multi-residue method for the determination of pesticide residues in fruit and vegetables was developed with satisfactory results using an innovative system based on an APCI source coupled to GC–(QqQ) MS/MS. The soft ionization allowed the use of the quasi-molecular ion as precursor in most cases contributing to an excellent selectivity and sensitivity. The high sensitivity (LODs of 1–100 fg on-column for most compounds) allowed dilution of QuEChERS extract by a factor of 10, without compromising method detection limits for most of the pesticides studied. The method was successfully validated for the simultaneous quantification and identification of 142 pesticides (three transitions each) in orange, tomato and carrot matrices at 0.01 and 0.1 mg/kg. This demonstrates the suitability of GC–(APCI) MS/MS for quantitative routine residue analysis. Analysis of fruit and vegetable samples allowed identifying and quantifying several pesticides like folpet, captan, bifenthrin, chlorpyrifos, iprodione and chlorothalonil. In all cases, the concentration levels were below the MRLs set by the EU.

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References

[1] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, J. AOAC Int., 86 (2003), p. 412

[2] S.J. Lehotay, K. Maštovská, A.R. Lightfield, J. AOAC Int., 88 (2005), p. 615

[3] S.C. Cunha, J.O. Fernandes, J. Chromatogr. A, 1218 (2011), p. 7748

[4] S. Walorczyk, J. Chromatogr. A, 1208 (2008), p. 202

[5] L. Cherta, J. Beltran, F. López, F. Hernández, Food Anal. Methods, 6 (2013), p. 1170

[6] L. Alder, K. Greulich, G. Kempe, B. Vieth, Mass Spectrom. Rev., 25 (2006), p. 838

[7] L. Cherta, J. Beltran, T. Portolés, F. Hernández, Anal. Bioanal. Chem., 402 (2012), p. 2301

[8] P. Zhao, L. Wang, L. Zhou, F. Zhang, S. Kang, C. Pan, J. Chromatogr. A, 1225 (2012), p. 17

[9] D.I. Kolberg, O.D. Prestes, M.B. Adaime, R. Zanella, Food Chem., 125 (2011), p. 1436

[10] M.I. Cervera, C. Medina, T. Portolés, E. Pitarch, J. Beltrán, E. Serrahima, L. Pineda, G. Muñoz, F. Centrich, F. Hernández, Anal. Bioanal. Chem., 397 (2010), p. 2873

[11] C.M. Medina, E. Pitarch, T. Portolés, F.J. López, F. Hernández, J. Sep. Sci., 32 (2009), p. 2090

[12] P.P. Bolaños, J.L.F. Moreno, D.D. Shtereva, A.G. Frenich, J.L.M. Vidal, Rapid Commun. Mass Spectrom., 21 (2007), p. 2282

[13] J. Robles-Molina, B. Gilbert-López, J.F. García-Reyes, N.R. Martos, A. Molina-Díaz, Anal. Methods, 3 (2011), p. 2221

[14] F.J. Camino-Sánchez, A. Zafra-Gómez, S. Cantarero-Malagón, J.L. Vílchez, Talanta, 89 (2012), p. 322

[15] A. Amirav, A. Gordin, M. Poliak, A.B. Fialkov, J. Mass Spectrom., 43 (2008), p. 141

[16] E.C. Horning, D.I. Carroll, I. Dzidic, Clin. Chem., 23 (1977), p. 13

[17] R. Schiewek, M. Lorenz, R. Giese, K. Brockmann, T. Benter, S. Gäb, O.J. Schmitz, Anal. Bioanal. Chem., 392 (2008), p. 87

[18] T. Bristow, M. Harrison, M. Sims, Rapid Commun. Mass Spectrom., 24 (2010), p. 1673

[19] R. García-Villalba, T. Pacchiarotta, A. Carrasco-Pancorbo, A. Segura-Carretero, A. Fernández-Gutiérrez, A.M. Deelder, O.A. Mayboroda, J. Chromatogr. A, 1218 (2011), p. 959

[20] A. Carrasco-Pancorbo, E. Nevedomskaya, T. Arthen-Engeland, T. Zey, G. Zurek, C. Baessmann, A.M. Deelder, O.A. Mayboroda, Anal. Chem., 81 (2009), p. 10071

[21] T. Portolés, J.V. Sancho, F. Hernández, A. Newton, P. Hancock, J. Mass Spectrom., 45 (2010), p. 926

[22] T. Portolés, J.G.J. Mol, J.V. Sancho, F. Hernández, Anal. Chem., 84 (2012), p. 9802

[23] T. Portolés, L. Cherta, J. Beltran, F. Hernández, J. Chromatogr. A, 1260 (2012), p. 183

[24] European Control Guidelines SANCO/12495/2011.

[25] T. Cajka, K. Maštovská, S.J. Lehotay, J. Hajšlová, J. Sep. Sci., 28 (2005), p. 1048

[26] S.J. Lehotay, A. De Kok, M. Hiemstra, P. Van Bodegraven, J. AOAC Int., 88 (2005), p. 595

[27] A. Peruga, M. Barreda, J. Beltrán, F. Hernández, Food Addit. Contam. Part A: Chem. Anal. Control Expo. Risk Assess., 30 (2013), p. 298