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Validation of Two Variations of the QuEChERS Method for the Determination of Multiclass Pesticide Residues in Cereal-Based Infant Foods by LC–MS/MS

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Abstract Over the past years to ensure food safety and particular for food that intend to be consumed by infants and young children, the European Union has adopted specific legislation concerning the control of pesticide residue levels in that kind of food. In this paper, a liquid chromatography tandem quadrupole mass spectrometry (LC–MS/MS) multiresidue method for the simultaneous analysis of 23 pesticides and metabolites chosen according to the Commission Directives 2006/141/EC, 2006/125/EC, and 96 multiclass pesticides and metabolites chosen according to their physicochemical properties is presented and validated. The extraction procedure is based on three modifications of the quick, easy, cheap, effective, rugged, and safe method according to the analyte. The analytical performance was demonstrated by the analysis of extracts from cereal-based infant foods, spiked at two concentration levels for each pesticide or metabolite. Good sensitivity and selectivity of the method were obtained with limits of quantification at 10 or 3 μg/kg, depending on the analyte. All pesticides and metabolites, except six cases, gave recoveries in the range of 60.4–125.4%, with relative standard deviations less than 29.7%, for both validation levels.

Keywords QuEChERS · LC–MS–MS · Cereal-based infant foods · Validation · Pesticide residues

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Introduction

The protection of food crops against pests and diseases by various pesticides is a common approach in conventional farming. Because of the potential health risk for consumers resulting from acute and/or chronic dietary exposure, maximum residue levels for many pesticide/commodity combinations have been established in the European Union (Stepan et al. 2005).

Infants and children, comparing to adults, are heavily exposed to pesticides and biologically are more vulnerable to them, due to high food consumption rate per kilogram of body weight. Commission Directive 2006/125/EC codifies and replaces the previous Directive (European Commission 2006a, b) which had been amended a number of times. It sets the rules on the composition and labeling of processed cereal-based foods for particular nutritional use for infants and young children in good health and is intended for use by infants when they are being weaned and as a supplement to the diet of young children. The mentioned directive also establishes the principle on the prohibition of the use of certain pesticides for the production of agricultural products, intended for processed cereal-based food and baby food. However, this

prohibition does not necessarily guarantee that products are free from such pesticides, since some pesticides may contaminate the environment and their residues may be found in the products concerned. It also requires that processed cereal-based food and baby food must not contain residues of individual pesticides at levels exceeding 0.01 mg/kg, except for substances for which specific levels have been set in Annex VI and VII of the Directive, in which case-specific levels apply (0.003 mg/kg for disulfoton, terbufos, fensulfothion and their metabolites, fentin, haloxyfop and its esters, heptachlor, hexachlorobenzene, nitrofen, omethoate, aldrin, dieldrin, and endrin; 0.004 mg/kg for fipronil and fipronil-desulfinyl; 0.006 mg/kg for propineb/propylenethiourea, cadusafos, demeton-*S*-methyl, demeton-*S*-methyl sulfone, and oxydemeton-methyl; or 0.008 mg/kg for ethoprophos) (European Commission 2006a, b).

Since the 1st January 2008, Commission Directive 2006/141/EC replaced Directive 91/321/EEC and its successive amendments. This new Directive updated the requirements for the composition and labeling of infant formulae and follow-on formulae and also encompasses specific rules on the presence of pesticides residues in infant and follow-on formulae. It requires that baby food contains no detectable levels of pesticide residues, i.e., meaning less than 0.01 mg/kg. This Directive also prohibits the use of certain pesticides in the production of infant and follow-on formulae and establishes levels lower than the general maximum level of 0.01 mg/kg for a few pesticides.

Considering the low concentration levels needed for successful monitoring of pesticide residues in infant foods, sensitive and reliable confirmation and quantification methods are required (Hercegova et al. 2007). The progress in sample preparation of pesticide residue analysis in baby food follows the trends valid for methods for food analysis in general. Liquid extraction still represents the keystone extraction technique; alternative techniques such as supercritical fluid extraction (SFE), matrix solid-phase dispersion, solid-phase microextraction, and stir bar sorptive extraction, excellent in specific aspects, did not reach the widespread utilization in baby food analysis (Hercegova et al. 2007). A critical aspect of pesticide residues analysis is the cleanup of the crude extract which is required to reduce the matrix effect, and it is essential for the sufficient column sample capacity and the satisfactory long-term performance of the chromatographic system during the analysis of a range of samples (Hercegova et al. 2007). Among them, solid-phase extraction and gel permeation chromatography (GPC) are the most widely used cleanup techniques for baby food analysis. The differences in validation parameters, related to many sample preparation methods published in the last few years, are not significant; therefore, the trend is the employment of an easy and fast procedure (Hercegova et al. 2007).

Liquid chromatography–mass spectrometry (LC–MS) allows the rapid and efficient determination of many compounds that have been scarcely investigated in food until now, or determined with some difficulties by using laborious and time-consuming GC or conventional LC procedures (Hernandez et al. 2006). Selectivity and sensitivity are notably improved, the sample pre-treatment steps can be minimized, and reliable quantitation and confirmation can be easily achieved at the low concentration levels required (Hernandez et al. 2006). In the last years, some LC–MS/MS methods have been developed for the determination of more than 50 multiclass pesticides in baby foods. Ethyl acetate used as an extraction solvent combined with SFE (Hercegova et al. 2007) or GPC (Cajka and Hajslova 2004) has been shown to be applicable to pesticide residue analysis of baby food matrices and has been used to extract more than 98 multiclass pesticides. Acetonitrile as an extraction solvent has the disadvantage of being both more expensive and more toxic than ethyl acetate. However, because of its higher polarity, much less lipophilic material, such as oils and chlorophyll and to a lesser extent waxes, are co-extracted with the pesticides (Barcelo and Fernandez-Alba 2005). Quick, easy, cheap, effective, rugged, and safe (QuEChERS) method is an extraction procedure for pesticide multiresidue analysis that was developed between 2000 and 2002 and first reported in 2003 (Anastassiades et al. 2003). The QuEChERS procedure involves an initial extraction with acetonitrile followed by an extraction/partitioning step after the addition of a salt mixture. An aliquot of the raw extract is then cleaned up by dispersive solid-phase extraction. The final extract in acetonitrile is directly amenable to determinative analysis based on LC and/or GC (Patel et al. 2004). A lot of publications already deal with this method in its original form or variations of it for the determination of multiclass pesticides in several types of baby food matrices (fruit, potato and cereal-based baby foods, fruit and rice, fish and pasta, potato and pork-based baby foods, apple-based baby foods, romaine lettuce, and orange-based baby foods) (Wang et al. 2005).

The purpose of this paper is to present and validate two variations of the QuEChERS method for the determination of 120 pesticides and metabolites in cereal-based infant foods. Because of the different physicochemical properties of the compounds, several modifications either in the sample preparation step or in the determination step are required as to have better method performance. Therefore, a rapid multi-residue method by LC–MS/MS with electrospray interface (ESI), using the extraction method based on QuEChERS procedure for the determination of 97 multiclass pesticides and metabolites and 23 priority pesticides and metabolites included in the Commission Directives 2006/141/EC and 2006/125/EC, was validated. Also a similar method based on a QuEChERS

extraction procedure for the determination of fentin and another one for the determination of haloxyfop, haloxyfop-ethoxyethylester, and haloxyfop-methoxyethylester were also validated.

Materials and Methods

Chemicals and Apparatus

In this work, 120 pesticides and metabolites were used (obtained from Dr Ehrenstorfer Laboratories GmbH Germany), 23 of which are included in the Commission directives 2006/141 and 2006/125 and are marked with an asterisk: acephate, acetamiprid, aldicarb, aldicarb sulfone, aldicarb sulfoxide, ametryn, atrazine, azimsulfuron, azinphos methyl, azoxystrobin, benalaxyl, bensulfuron methyl, boscalid, bromuconazole, buprofezin, cadusafos*, carbaryl, carbofuran, carbofuran 3-hydroxy, carbosulfan, chlorotoluron, clofentezine, cyanazine, cymoxanil, demeton-*S*-methyl*, demeton-*S*-methyl sulfone*, demeton-*S*-methyl sulfoxide*, diazinon, dichlorvos, diethofencarb, difenoconazole, dimethoate, dimethomorph, disulfoton*, disulfoton-sulfone*, disulfoton-sulfoxide*, epoxiconazole, ethofumesate, ethoprophos*, etoxazole, famoxadone, fenamidone, fenbuconazole, fenhexamid, fenoxycarb, fenpropimorph, fenpyroximate, fensulfothion*, fensulfothion sulfone*, fensulfothion-oxon*, fensulfothion-oxon-sulfone*, fenthion, fentin*, fipronil*, fipronil-desulfinyl*, flutriafol, fosthiazate, furathiocarb, haloxyfop*, haloxyfop-ethoxyethylester*, haloxyfop-methoxyethylester*, hexaconazole, hexythiazox, imidacloprid, indoxacarb, iprovalicarb, kresoxim methyl, linuron, metalaxyl M, metconazole, methamidophos, methiocarb, methiocarb sulfone, methiocarb sulfoxide, methomyl, methoxyfenozide, metoxuron, monocrotophos, monolinuron, myclobutanil, nicosulfuron, omethoate*, oxamyl, phosalone, phosmet, pirimicarb, pirimiphos methyl, primisulfuron methyl, procloraz, profenofos, propamocarb, propargite, pymetrozine, pyraclostrobin, pyridaben, pyrifenox, pyrimethanil, pyriproxyfen, quinoxyfen, simazine, spinosad (A), spiroxamine, tebuconazole, tebufenozid, tebufenpyrad, terbufos*, terbufos-sulfone*, terbufos-sulfoxide*, terbuthylazine, tetraconazole, thiacloprid, thiamethoxam, thiodicarb, thiophanate methyl, tolylfluanid, triadimefon, triadimenol, triazophos, trifloxystrobin, and vamidothion.

LC–MS grade acetonitrile, methanol, and water were used. All solvents were obtained from Lab Scan (Ireland). Ammonium formate, magnesium sulfate anhydrous, and disodium hydrogencitrate sesquihydrate were obtained from Fluka (Buchs, Switzerland). Sodium chloride (ACS reagent grade ≥99.0%) and trisodium citrate dihydrate were obtained by Sigma-Aldrich (Madrid, Spain). Primary secondary amine (PSA; Bondesil-PSA 40 μm) were obtained by Varian Inc. Sulfuric acid and sodium hydroxide solutions concentrate 1.0 N TITRISOL Volumetric Solutions were obtained by Merck & Co. Inc.

Preparation of Standard Solutions

Stock standard solutions at 500 mg/L were prepared in methanol for fentin, haloxyfop, haloxyfop-ethoxyethylester, and haloxyfop-methoxyethylester and at 1,000 mg/L in acetone for the other 116 analytes. The stock standard solutions were stored at −20 °C. A single composite standard was prepared by combining aliquots of each stock solution and diluting in methanol to obtain a final concentration of 1 μg/mL. Matrix-matched calibration standard solutions for measurements were prepared in the extract of cereal-based infant food, previously analyzed for the absence of peaks interfering with the peaks of the analytes. The product used as blank composed of 19.7% of milk powder, 17.5% of wheat flour, sucrose, corn stark, whey in powdered form, prebiotic fibers, lactose, galactose, vanillin, glucose, and vitamins.

Preparation of Calibration Standards

The sample extraction procedure was followed for the preparation of matrix-matched standard solutions. At the final step, the blank extract was diluted in 3 mL of methanol. An aliquot of 2 mL is evaporated to dryness by a stream of N_2, and 1 mL of a standard solution, of the desired concentration, prepared in methanol/water (30:70, *v*/*v*) was added. Before the injection in the chromatographic system, the final solution was filtered through a disposable PTFE syringe filters, 0.45 μm.

Sample Preparation

Extraction of 116 Multiclass Pesticides and Metabolites

For the extraction 116 of the analytes (excluding fentin, haloxyfop, and its esters), the protocol of QuEChERS method concerning commodities with high fat content was followed (Anastassiades et al. 2007). For products with a water content <25% (e.g., wheat flour), water had to be added. The water should be at low temperature (<4 °C) to compensate the heat development caused by the addition of the salts (QuEChERS): According to this method, a 5-g portion of cereal-based infant food was weighted in a 50-mL PTFE centrifuge tube, and 10 mL of water (<4 °C) was added. A short vibration using a Vortex mixer (K-550-GE, Scientific Industries Inc., Bohemia, NY, USA) helped to disperse

solvent and pesticides well through the sample. For the extraction of the pesticides, 10 mL of acetonitrile was added and the tube was vigorously shaken for 1 min. A mixture of 1 g of NaCl, 4 g of $MgSO_4$, 1 g of trisodium citrate dehydrate, and 0.5 g of disodium hydrogencitrate sesquihydrate was added, and the tube was vigorously shaken for 1 or more minutes to prevent coagulation of $MgSO_4$. By adding the citrate buffering salts, most samples obtain pH values between 5 and 5.5. This pH range is a compromise, at which both the quantitative extraction of sour herbicides and the protection of alkali labile and acid labile compounds are sufficiently achieved (QuEChERS). The sample was then centrifuged at 4,000 rpm for 5 min. An aliquot of 8 mL of the supernatant acetonitrile phase was then taken and transferred to a 15-mL centrifuge tube and stored for at least 2 h in the freezer. Freezing out helps to partly remove some additional co-extractives with limited solubility in acetonitrile while the major part of fat and waxes solidify and precipitate. An aliquot of 6 mL of the still cold acetonitrile phase was transferred into a 15-mL centrifuge tube containing 150 mg of PSA and 900 mg of $MgSO_4$, and the tube was shaken vigorously for 30 s and centrifuged for 5 min at 4,000 rpm. An aliquot of 5 mL of the cleaned up extract was transferred into a screw cup storage vial, taking care to avoid sorbent particles of being carried over. The extract was slightly acidified by adding 50 μL of a 5% formic acid solution in acetonitrile. An aliquot of 2 mL of the extract was evaporated near to dryness and reconstituted in 1 mL of methanol/water (30:70, *v*/*v*), added in the following order: 0.3 mL of methanol was added in the flask, the extract was placed in an ultrasonic bath for 30 s, and then 0.7 mL of water was added. The final extract was placed again in an ultrasonic bath for 30 s. Before the injection in the chromatographic system, the final extract was filtered through a 0.45-μm disposable PTFE syringe filters. Following this extraction procedure, the concentration *C* (milligrams per kilogram) of the analytes in the sample corresponds to *C* (micrograms per milliliter) of the analytes in the extract.

Extraction of Fentin

The difference for the extraction procedure of fentin was at the final step of the procedure. An aliquot of 2 mL of the extract was evaporated near to dryness and reconstituted to 1 mL methanol. The final extract was placed in an ultrasonic bath for 30 s. Before the injection in the chromatographic system, the final extract was filtered through a 0.45-μm disposable PTFE syringe filters. Following this extraction procedure, the concentration *C* (milligrams per kilogram) of the analytes in the sample corresponds to *C* (micrograms per milliliter) of the analytes in the extract.

Extraction of Haloxyfop and Its Ethoxyethyl and Methoxyethyl Esters

For the extraction of haloxyfop and its ethoxyethyl and methoxyethyl esters, a different variation of the QuEChERS method for acidic pesticides was adopted (CRL for Single Residue Methods 2007): 5 g of cereal-based infant food was weighted in a 50-mL PTFE centrifuge tube, and 10 mL of water (<4 °C) was added. A short vibration using a Vortex mixer helped to disperse solvent and pesticides well through the sample. Then 300 μL of 5 mol/m^3 NaOH solution was added to adjust the pH to 12. The tube was shaken vigorously for 1 min, and the mixture was left to stand for 30 min, occasionally shaken every 10 min. Then 300 μL of 5 mol/m^3 H_2SO_4 solution and 10 mL of acetonitrile were added, and the tube was vigorously shaken for 1 min. A mixture of 1 g of NaCl, 4 g of $MgSO_4$, 1 g of trisodium citrate dehydrate, and 0.5 g of disodium hydrogencitrate sesquihydrate was added, and the tube was vigorously shaken for 1 or more minutes to prevent coagulation of $MgSO_4$. The sample was then centrifuged (4,000 rpm) for 5 min. An aliquot of 7 mL of the supernatant acetonitrile phase was then taken and transferred to a 15-mL centrifuge tube and stored for at least 2 h in the freezer. An aliquot of 5 mL of the still cold acetonitrile phase was transferred into a screw cup storage vial, taking care to avoid sorbent particles of being carried over. An aliquot of 2 mL of the extract was evaporated near to dryness and reconstituted in 1 mL of methanol. Before the injection in the chromatographic system, the final extract was filtered through a 0.45-μm disposable PTFE syringe filters. Following this extraction procedure, the concentration *C* (milligrams per kilogram) of the analytes in the sample corresponds to *C*(micrograms per milliliter) of the analytes in the extract.

Preparation of Fortification Samples

The recovery and repeatability experiments were conducted in two levels, the limit of quantification (LOQ) and 10× LOQ, with five replicates at each level. Working standard mixture solution was prepared in methanol at 100× LOQ; 5 g of cereal-based infant food, previously analyzed for the absence of pesticides, was weighted and spiked with 50 μL for the LOQ and 500 μL for the 10× LOQ of the working standard mixture solution.

Determination with Liquid Chromatography Tandem Mass Spectrometry

The LC system used consisted of two Varian Prostar 210 pumps. Detection was achieved using a triple quadrupole mass spectrometer (Varian model 1200 L) equipped with an

Table 1 Chromatographic and transition parameters for the 120 analytes for the LC–MS–MS determination using water/methanol 1 mmol/L ammonium formate gradient

Analyte	Chemical class	Precursor ion	Quantification transition *m/z*		Cone voltage (V)	Collision energy (eV)	Qualification transition m/z		Cone voltage (V)	Collision energy (eV)	RT (min)	Time segments
Acephate	Organophosphorous	[M+H]+	184	143	10	20	184	125	16	25	1.0	1+2+3
Acetamiprid	Neonicotinoid	[M+H]+	223	126	36	27	223	90	51	21	3.8	3+4+5
Aldicarb	Carbamate	[M+NH4]+	208	89	10	20	208	116	10	20	5.3	5+6+7
Aldicarb sulfone	Carbamate	[M+H]+	240	148	40	15	240	86	25	25	1.4	1+2+3
Aldicarb sulfoxide	Carbamate	[M+H]+	207	89	42	18	207	132	40	15	1.2	1+2+3
Ametryn	Triazines	[M+H]+	228.1	186.2	36	25	228.1	96.1	36	35	9.4	9+10+11
Atrazine	Triazines	[M+H]+	216.1	174	21	25	216.1	103.9	21	27	8.2	8+9+10
Azimsulfuron	Sulfonylurea	[M+H]+	425.1	182.1	31	23	425.1	156.1	31	43	7.4	7+8+9
Azinphos methyl	Strobilurin	[M+H]+	318	132.2	16	23	318	160.2	16	13	9.4	9+10+11
Azoxystrobin	Strobilurin	[M+H]+	404.1	371.9	36	19	404.1	343.9	36	29	9.8	9+10+11
Benalaxyl	Acylalanine	[M+H]+	326.2	148.2	26	27	326.2	208.2	26	21	12.0	11+12+13
Bensulfuron methyl	Sulfonylurea	[M+H]+	411.1	148.9	51	27	411.1	119	51	51	9.5	9+10+11
Boscalid	Pyridinecarboxamide	[M+H]+	343	307	71	27	343	140	76	27	10.2	10+11+12
Bromuconazole	Triazole	[M+H]+	378	159	46	30	378	70	46	15	10.5	10+11+12
Buprofezin	–	[M+H]+	306	116	40	21	306	201	40	17	14.2	14+15+16
Cadusafos	Organophosphorous	[M+H]+	271	215	35	15	271	159	45	25	10.0	10+11+12
Carbaryl	Carbamate	[M+H]+	202	145	40	15	202	127	40	50	7.6	7+8+9
Carbofuran	Carbamate	[M+H]+	222	123	25	40	222	165	25	25	6.8	6+7+8
Carbofuran 3-hydroxy	Carbamate	[M+H]+	238	163	25	15	238	181	21	15	3.8	3+4+5
Carbosulfan	Carbamate	[M+H]+	381	118	45	30	381	118	45	30	16.8	16+17+18
Chlorotoluron	Urea	[M+H]+	213.1	72	36	33	213.1	140	36	33	8.1	8+9+10
Clofentezine	Tetrazine	[M+H]+	303	138	56	15.5	303	102	56	47	13.2	13+14+15
Cyanazine	Triazines	[M+H]+	241.1	214.1	41	23	241.1	104.1	41	41	6.5	6+7+8
Cymoxanil	Acetamide	[M+H]+	199	128	46	13	199	111	41	25	4.4	4+5+6
Demeton-*S*-methyl*	Organophosphorous	[M+H]+	230.8	89	30	10.5	248	61	6	47	5.84	6+7+8
Demeton-*S*-methyl sulfone	Organophosphorous	[M+H]+	263	121	75	25	263	169	65	25	1.4	1+2+3
Demeton-*S*-methyl sulfoxide	Organophosphorous	[M+H]+	247	169	21	14	247	109	20	35	1.7	1+2+3
Diazinon	Organophosphorous	[M+H]+	305.1	169.1	21	29	305.1	96.6	21	41	12.1	12+13+14
Dichlorvos	Organophosphorous	[M+H]+	221	109	85	25	221	127	85	–	6.7	6+7+8
Diethofencarb	Carbamate	[M+H]+	268.1	226.1	31	15	268.1	180.1	31	23	9.6	9+10+11
Difenoconazole	Triazole	[M+H]+	406.1	250.9	41	37	406.1	337	41	23	13.2	13+14+15
Dimethoate	Organophosphorous	[M+H]+	230	125	11	29	230	199	11	13	2.9	3+4+5
Dimethomorph	Cinnamic acid	[M+H]+	388	301	60	17	388	165	60	28	9.9	9+10+11
Disulfoton	Organophosphorous	[M+H]+	275	89	9	5					10.1	10+11+12
Disulfoton-sulfone	Organophosphorous	[M+H]+	307	153	65	15	307	261	65	15	7	7+8+9
Disulfoton-sulfoxide	Organophosphorous	[M+H]+	291	185	45	25	291	213	45	15	6.7	6+7+8
Epoxiconazole	Triazole	[M+H]+	330.1	121	36	27	330.1	101.2	36	63	11.3	11+12+13
Ethofumesate	Benzofuran	[M+NH4]+	304	121.1	36	27	304.1	161.2	36	31	9.8	9+10+11

Table 1 (continued)

Analyte	Chemical class	Precursor ion	Quantification transition *m/z*		Cone voltage (V)	Collision energy (eV)	Qualification transition m/z		Cone voltage (V)	Collision energy (eV)	RT (min)	Time segments
Ethoprophos	Organophosphorous	[M+H]+	243	173	45	25	243	131	65	35	10.9	10+11+12+13
Etoxazole	–	[M+H]+	360	141	66	37	360	113	66	50	15.1	15+16+17
Famoxadone	Strobilurin	[M+NH4]+	392.2	238	16	23	392.2	330.9	16	15	12.9	12+13+14
Fenamidone	Strobilurin	[M+H]+	312	92	41	33	312	236	41	19	9.9	9+10+11
Fenbuconazole	Triazole	[M+H]+	337	125	41	37	337	70	41	33	11.7	11+12+13
Fenhexamid	Hydroxyanilide	[M+H]+	302	97	35	25	302	55	35	35	11.1	11+12+13
Fenoxycarb	Carbamate	[M+H]+	302	116	21	12	302	88	21	20	11.9	11+12+13
Fenpropimorph	Morpholine	[M+H]+	304	147.1	46	39	304.3	116.9	46	71	12.7	12+13+14
Fenpyroximate	Pyrazole	[M+H]+	422	366	15	20	422	135	16	41	15.5	15+16+17
Fensulfothion	Phenyl organophosphates	[M+H]+	309	281	85	25	309	253	35	35	7.2	7+8+9
Fensulfothion sulfone	Metabolite	[M+H]+	325	269	75	35	325	297	75	25	7.4	8+9+10
Fensulfothion-oxon	Metabolite	[M+H]+	293	265	65	35	293	237	75	35	4.8	5+6+7
Fensulfothion-oxon sulfone	Metabolite	[M+H]+	309	253	65	25	309	175	75	45	5.2	5+6+7
Fenthion	Organophosphorous	[M+H]+	279	169	21	23	279	247	41	10	13.0	12+13+14
Fentin[a]	Organotin	[M+H]+	347	193	115	45	347	116	115	45	10.2	
Fipronil[b]	Phenylpyrazole	[M−H]−	435	330	31	17	435	250	31	36	9.3	
Fipronil-desulfinyl[b]	metabolite	[M−H]−	386.9	350.7	45	35					9.1	
Flutriafol	Triazole	[M+H]+	302.1	122.9	41	39	302.1	109	41	43	8.6	8+9+10
Fosthiazate	Organophosphorous	[M+H]+	284	228	61	15	284	104	61	27	7.8	7+8+9
Furathiocarb	Carbamate	[M+H]+	383	195	51	23.5	383	252	51	19	13.9	13+14+15
Haloxyfop[c]	Aryloxyphenoxypropionate	[M−H]−	360	288	65	25	362	290	65	25	8.3	
Haloxyfop-ethoxyethylester [c]	Aryloxyphenoxypropionate	[M+H]+	434	316	75	35	434	288	75	35	12.5	
Haloxyfop methoxyethylester[c]	Aryloxyphenoxypropionate	[M+H]+	376	316	65	35	376	288	75	45	11.8	
Hexaconazole	Triazole	[M+H]+	314	70	30	20	314	159	26	30	12.7	12+13+14
Hexythiazox	Carboxamide	[M+H]+	353	228	60	20	353	168	60	30	14.8	14+15+16
Imidacloprid	Neonicotinoid	[M+H]+	256	209	45	20	256	175	45	25	3.3	3+4+5
Indoxacarb	Oxadiazine	[M+H]+	528	218	76	38	528	293	76	15	13.6	13+14+15
Iprovalicarb	Carbamate	[M+H]+	321	119	51	23	321	203	51	20	10.7	10+11+12
Kresoxim methyl	Strobilurin	[M+H]+	314.1	115.9	16	21	314.1	206.1	16	13	11.8	11+12+13
Linuron	Urea	[M+H]+	249	159.9	66	23	249	181.9	66	21	9.9	9+10+11
Metalaxyl M	Acylalanine	[M+H]+	280.1	220	46	19	280.1	159.9	46	31	8.4	8+9+10
Metconazole	Triazole	[M+H]+	320.1	69.4	48	23.5	320.1	125	48	45	12.6	12+13+14
Methamidophos	Organophosphorous	[M+H]+	142	94	30	20	142	125	50	15	0.9	1+2+3
Methiocarb	Carbamate	[M+H]+	243	169	15	25	226	121	60	25	10.0	9+10+11
Methiocarb sulfone	Carbamate	[M+H]+	275	122	25	25	258	122	25	45	4.7	4+5+6
Methiocarb sulfoxide	Carbamate	[M+H]+	242	185	25	15	242	168	25	30	3.7	3+4+5
Methomyl	Carbamate	[M+H]+	163	88	8	8	163	106	15	10	1.8	1+2+3
Methoxyfenozide	Diacylhydrazine	[M+H]+	369	149	36	23	369	133	41	31	10.7	10+11+12

Table 1 (continued)

Analyte	Chemical class	Precursor ion	Quantification transition *m/z*		Cone voltage (V)	Collision energy (eV)	Qualification transition m/z		Cone voltage (V)	Collision energy (eV)	RT (min)	Time segments
Metoxuron	Urea	[M+H]+	229	156.1	26	31	229	72.1	26	35	5.6	5+6+7
Monocrotophos	Organophosphorous	[M+H]+	224	127	40	20	224	98	46	17	2.4	2+3+4
Monolinuron	Urea	[M+H]+	215.1	125.9	61	25	215.1	148	61	19	7.7	7+8+9
Myclobutanil	Triazole	[M+H]+	289	125	36	41	289	70	36	33	10.7	10+11+12
Nicosulfuron	Sulfonylurea	[M+H]+	411.1	182.1	61	25	411.1	213	61	23	6.8	6+7+8
Omethoate	Organophosphorous	[M+H]+	214	109	50	35	214	125	50	30	0.9	1+2
Oxamyl	Oxime Carbamate	[M+H]+	237	72	36	15	237	90	36	15	1.4	1+2+3
Phosalone	Organophosphorous	[M+H]+	367.9	182	41	25.5	367.9	110.9	41	24	12.9	12+13+14
Phosmet	Carbamate	[M+H]+	317.9	133.1	31	49	317.9	160.1	31	19	9.4	9+10+11
Pirimicarb	Carbamate	[M+H]+	239.1	72.1	16	31	239.1	181.9	16	21	7.6	7+8+9
Pirimiphos methyl	Organophosphorous	[M+H]+	306.1	164.1	26	29	306.1	108.1	26	39	12.6	12+13+14
Primisulfuron methyl	Sulfonylurea	[M+H]+	376	308	16	17	376	266	31	23	12.7	12+13+14
Procloraz	Imidazole	[M+H]+	373	303	40	20	373	97	30	30	13.8	13+14+15
Profenofos	Organophosphorous	[M+H]+	373	303	40	20	373	97	30	30	13.8	13+14+15
Propamocarb	Carbamate	[M+H]+	189	102	16	23	189	144	16	17	1.2	1+2+3
Propargite	–	[M+H]+	368	231	30	10	368	175	1	21	15.2	15+16+17
Pymetrozine	Pyridine	[M+H]+	218	105	56	27	218	79	51	47	1.8	1+2+3
Pyraclostrobin	Strobilurin	[M+H]+	388	163	11	29	388	194	6	19	12.7	12+13+14
Pyridaben	–	[M+H]+	365	309	26	12.5	365	148	64	33.5	16.2	16+17+18
Pyrifenox	Pyridine	[M+H]+	295	93.1	16	31	295	263.1	16	25	11.3	11+12+13
Pyrimethanil	Anilinopyrimidine	[M+H]+	200	107	30	30	200	82	30	30	9.4	9+10+11
Pyriproxyfen	–	[M+H]+	322	96	15	21	322	185	11	29	14.6	14+15+16
Quinoxyfen	Quinoline	[M+H]+	308	197	50	40	308	162	25	50	14.7	14+15+16
Simazine	Triazines	[M+H]+	202.1	124.2	26	25	202.1	132.2	26	27	6.7	6+7+8
Spinosad (A)	Spinosin	[M+H]+	733	142	65	20	746	132	65	23	13.7	13+14+15
Spiroxamine	Morpholine	[M+H]+	298	144	41	27	298	100	41	35	10.7	10+11+12
Tebuconazole	Triazole	[M+H]+	308	70	30	20	308	125	30	35	12.1	12+13+14
Tebufenozid	Diacylhydrazine	[M+H]+	353	133	40	20	353	297	40	16	11.9	11+12+13
Tebufenpyrad	Pyrazole	[M+H]+	334	145	25	30	334	117	51	47	14.0	13+14+15
Terbufos	Organophosphorous	[M+H]+	289	103.3	31	15	289	57	31	15	10.9	11+12+13+14
Terbufos-sulfone	Metabolite	[M+H]+	321	171	65	15	321	265	65	15	7.7	8+9+10
Terbufos-sulfoxide	Metabolite	[M+H]+	305	187	45	15	305	131	35	35	7.7	8+9+10
Terbuthylazine	Triazines	[M+H]+	230.1	174.1	44	23	230.1	103.9	44	43.5	9.8	9+10+11
Tetraconazole	Triazole	[M+H]+	372	159	40	40	372	70	36	45	11.5	11+12+13
Thiacloprid	Carbamate	[M+H]+	253	126	81	29	253	186	76	19	5.2	5+6+7
Thiamethoxam	Neonicotinoid	[M+H]+	292	211	51	17	292	181	56	31	2.2	2+3+4
Thiodicarb	Carbamate	[M+H]+	355	108	30	25	355	88	30	25	8.1	8+9+10
Thiophanate methyl	Benzimidazole	[M+H]+	343	151	40	25	343	192	40	21	6.9	6+7+8

Table 1 (continued)

Analyte	Chemical class	Precursor ion	Quantification transition *m/z*		Cone voltage (V)	Collision energy (eV)	Qualification transition m/z		Cone voltage (V)	Collision energy (eV)	RT (min)	Time segments
Tolylfluanid	Sulfamide	[M+NH4]+	364	237.9	6	19	364	137.1	6	37	12.2	12+13+14
Triadimefon	Triazole	[M+H]+	294	197	28	21	294	225	28	21	10.5	10+11+12
Triadimenol	Triazole	[M+H]+	296	70	11	11	296	227	11	9	10.9	10+11+12
Triazophos	Organophosphorous	[M+H]+	314	119.1	36	47	314	162.1	36	25	10.9	10+11+12
Trifloxystrobin	Strobilurin	[M+H]+	409	186	11	23	409	206	6	21	13.4	13+14+15
Vamidothion	Organophosphorous	[M+H]+	288	146	10	30	288	118	10	40	3.9	3+4+5

[a] Fentin requires special LC conditions; therefore, a different injection is performed for its detection

[b] Product ion mass spectra for fipronil and fipronil-desulfinyl were obtained in the negative mode electrospray ionization; therefore, a second injection of the sample extract is performed by using a method for the detection of these two analytes

[c] Haloxyfop, haloxyfop-methoxyethylester, and haloxyfop-ethoxyethylester were determined by performing negative and positive mode electrospray ionization during the same run

electrospray ionization interface operating in the positive or negative mode. Typical source parameters were as follows: Cone voltage and collision energy varied depending on the precursor ion as shown in Table 1, and source temperature was set at 250 °C and drying gas temperature at 250 °C. Drying gas and nebulizing gas was nitrogen generated from a high purity generator, and their pressures were set at 18 and 55 psi, respectively. For the operation in MS/MS mode, Argon 99.999% was used as collision gas with a pressure of 0.2 Pa. The multiple reaction monitoring experiments were conducted with a dwell time of 100–250 ms, depending at the analyte. For instrument control, data acquisition, and processing, Varian MS Workstation software version 6.8 was used.

Determination of 119 Multiclass Pesticides and Metabolites Included Haloxyfop and Its Ethoxyethyl and Methoxyethyl Esters

Chromatographic separation was achieved using a Polaris C_{18} 5-μm particle size, 50×2-mm analytical column from Varian, at a flow rate of 250 μL/min with a mobile phase consisting of water/methanol (90:10, *v*/*v*)–1 mmol/L ammonium formate (solvent A) and methanol/water (90:10, *v*/*v*)–1 mmol/L ammonium formate (solvent B). A gradient program was used consisting of 90% of solvent A and 10% of solvent B, ramped linearly over the course of 14 min to 100% of solvent B. This composition was held for a further 6 min before returning to the initial condition. The column was re-equilibrated for 10 min at the initial mobile phase composition. The total run time was 30 min. The injection volume was 20 μL. In order to avoid carry-over, the autosampler was purged with a mixture of methanol/water (50:50, *v*/*v*) before sample injection.

Determination of Fentin

Chromatographic separation was achieved using a Zorbax Eclipse XDB-C_{18}, 50×2.1 mm, 5 μm analytical column at a flow rate of 250 μL/min with a mobile phases consisting of water 5 mmol ammonium formate with 1% formic acid and methanol 5 mmol ammonium formate with 1% formic acid

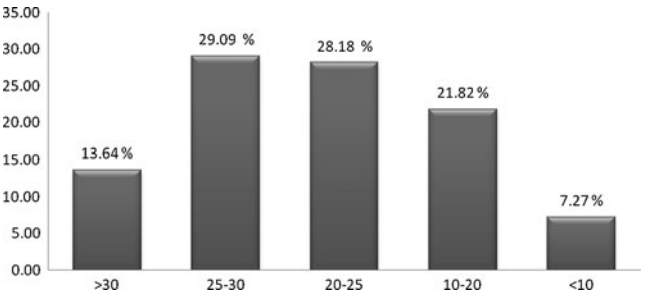


Fig. 1 Time distribution of the 116 analytes divided in five time clusters

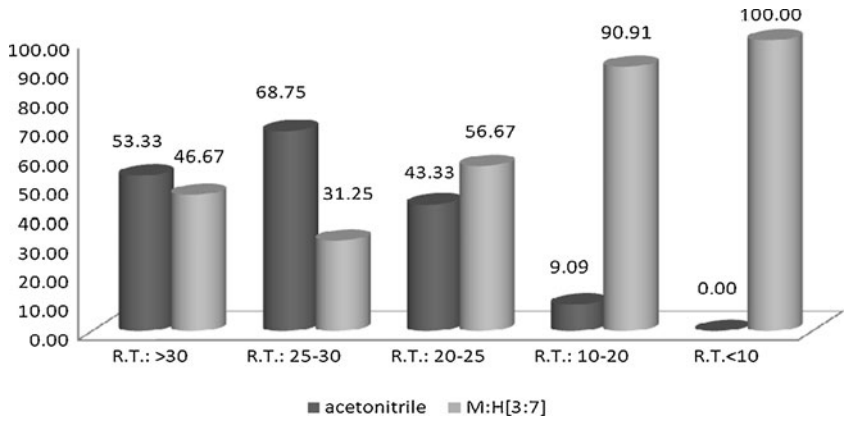


Fig. 2 Comparison of signal-to-noise ratios of the 116 analytes at 10 μg/mL in acetonitrile and methanol/water (3:7, *v*/*v*) for each time cluster. The *red column* represents the percentage of the analytes with higher signal-to-noise ratio with methanol/water (3:7, *v*/*v*) as final solvent and the *blue one* with the acetonitrile

(Michelangelo Anastassiades, personal communication, 2009). The gradient program and the injection volume were the same with the one described in the previous section.

Confirmation

For screening purposes, a first injection was performed by using a method with only one transition of each compound. The chosen transition for screening was that of the quantifier because it is more sensitive than that of the qualifier and minimizes the possibility of false negative results.

Individual MS/MS methods including two transitions for each analyte were developed and used for confirmation purposes. Then, an independent confirmation injection is performed for every positive sample. Confirmation includes comparison of retention times and acceptable tolerances of the ion ratios of qualifier and quantifier ions of the analyte in the matrix-matched calibration standard and the sample extract. The retention time of the analyte in the sample extract must match that of the matrix-matched calibration standard with a tolerance of ±2.5%, and the acceptable tolerances of the ion ratios must match those mentioned in Document SANCO/10684/2009 (European Commission 2009).

Results and Discussion

Selection of Final Solvent

The evaporation of acetonitrile and the reconstruction in methanol/water (30:70, *v*/*v*) is a one extra step in the method,

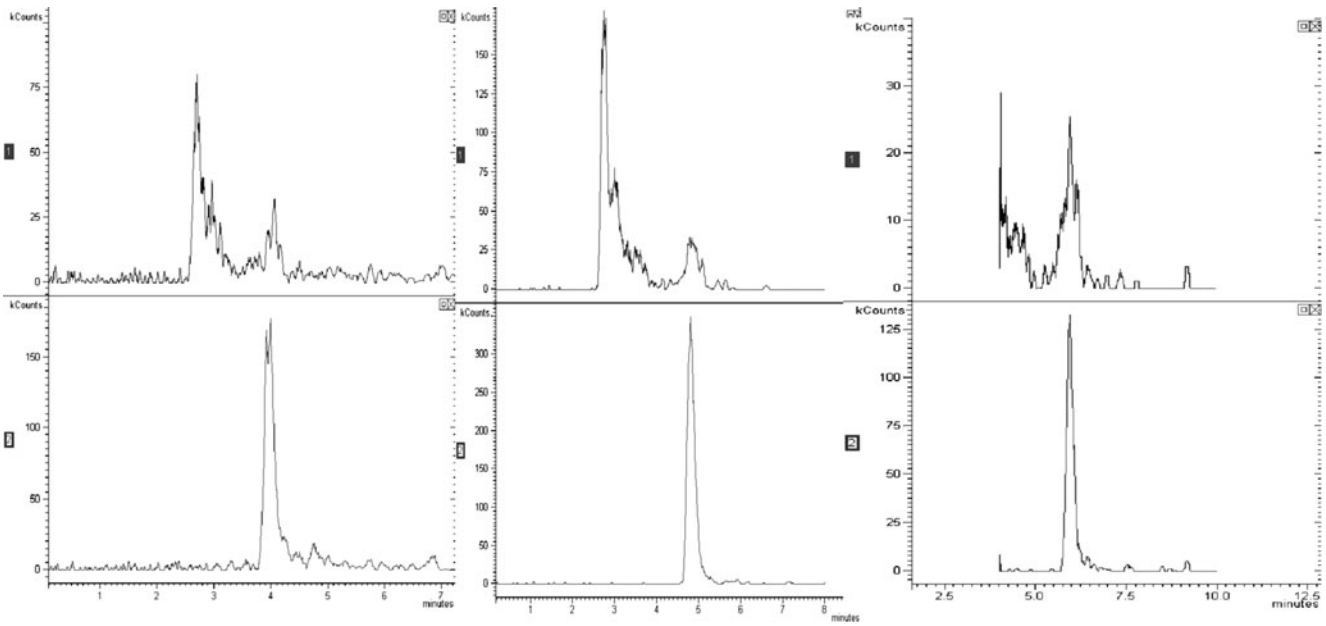


Fig. 3 Chromatograms of standard solutions at 10 μg/mL of the analytes **a** methamidophos, **b** acephate, and **c** omethoate at acetonitrile (upper chromatogram) and methanol/water (30:70, *v*/*v*) (lower chromatogram)

Table 2 Summary of calibration line parameters for the 120 pesticides (correlation coefficient r^2, slope of the regression line b, mean standard deviation of the slope of the regression line S_b, mean of the population that corresponds to $x=0$ a, mean standard deviation of the mean of the population that corresponds to $x=0$ S_a, standard uncertainty of the concentration S_u)

Analytes	r	r^2	b	S_b	a	S_a	S_u
Acephate	0.999	0.998	2E+08	3E+06	4E+05	2E+05	0.0018
Acetamiprid	0.997	0.994	3E+08	1E+07	1E+05	5E+05	0.0030
Aldicarb	0.999	0.999	4E+08	6E+06	−2E+05	3E+05	0.0013
Aldicarb sulfone	1.000	0.999	2E+08	3E+06	−2E+05	2E+05	0.0012
Aldicarb sulfoxide	0.999	0.999	3E+08	6E+06	−1E+05	3E+05	0.0015
Ametryn	1.000	0.999	2E+09	2E+07	−4E+06	1E+06	0.0012
Atrazine	0.998	0.996	1E+08	4E+06	1E+05	2E+05	0.0024
Azimsulfuron	0.997	0.993	2E+08	8E+06	6E+05	4E+05	0.0032
Azinphos methyl	0.997	0.994	8E+07	3E+06	−3E+05	2E+05	0.0032
Azoxystrobin	0.999	0.998	8E+08	1E+07	2E+05	8E+05	0.0016
Benalaxyl	0.998	0.997	4E+08	1E+07	−1E+05	6E+05	0.0022
Bensulfuron methyl	0.999	0.998	6E+08	1E+07	−2E+06	7E+05	0.0019
Boscalid	0.994	0.988	6E+07	3E+06	−2E+05	2E+05	0.0044
Bromuconazole	0.995	0.989	4E+07	2E+06	−2E+05	1E+05	0.0041
Buprofezin	0.994	0.988	2E+08	1E+07	−6E+05	6E+05	0.0044
Cadusafos[a]	0.988	0.976	6.1E+06	3.9E+05	−1.4E+07	8.1E+06	2.53
Carbaryl	0.998	0.995	7E+08	2E+07	2E+06	1E+06	0.0028
Carbofuran	0.997	0.993	3E+09	1E+08	9E+06	7E+06	0.0033
Carbofuran 3-hydroxy	0.999	0.999	5E+08	7E+06	1E+06	4E+05	0.0013
Carbosulfan	0.995	0.990	1E+09	4E+07	−2E+06	2E+06	0.0040
Chlorotoluron	0.998	0.995	6E+08	2E+07	4E+05	1E+06	0.0028
Clofentezine	0.996	0.992	3E+07	1E+06	−2E+05	6E+04	0.0035
Cyanazine	0.998	0.997	3E+08	7E+06	−7E+05	4E+05	0.0022
Cymoxanil	0.996	0.992	9E+07	3E+06	2E+05	2E+05	0.0036
Demeton-*S*-methyl sulfone[a]	0.990	0.981	1.2E+06	7.3E+04	−1.6E+06	1.5E+06	2.44
Demeton-*S*-methyl sulfoxide[a]	0.994	0.988	2.5E+06	1.3E+05	−4.5E+06	2.6E+06	1.98
Demeton-*S*-methyl[a]	0.994	0.987	4.5E+06	2.4E+05	−7.8E+06	4.9E+06	2.04
Diazinon	0.999	0.998	3E+08	6E+06	−8E+05	3E+05	0.0017
Dichlorvos	0.997	0.994	8E+07	3E+06	−6E+04	2E+05	0.0031
Diethofencarb	0.982	0.964	2E+09	2E+08	−2E+07	1E+07	0.0077
Difenoconazole	0.986	0.972	5E+07	4E+06	−4E+05	2E+05	0.0067
Dimethoate[a]	0.994	0.988	1.8E+06	8.9E+04	−3.4E+06	1.8E+06	1.97
Dimethomorph	0.997	0.994	1E+07	5E+05	−5E+04	3E+04	0.0031
Disulfoton[a]	0.993	0.986	4.4E+05	2.2E+04	−1.0E+06	4.6E+05	2.00
Disulfoton-sulfone[a]	0.994	0.988	2.9E+06	1.5E+05	−5.3E+06	3.1E+06	1.98
Disulfoton-sulfoxide[a]	0.993	0.986	4.5E+06	2.4E+05	−7.3E+06	5.0E+06	2.11
Epoxiconazole	0.998	0.997	4E+08	1E+07	−3E+04	6E+05	0.0022
Ethofumesate	0.996	0.992	1E+08	5E+06	1E+05	3E+05	0.0036
Ethoprophos[a]	0.988	0.976	1.1E+06	7.7E+04	−1.2E+06	1.6E+06	2.70
Etoxazole	0.998	0.995	2E+09	7E+07	−5E+06	4E+06	0.0028
Famoxadone	0.996	0.991	4E+07	2E+06	−1E+05	9E+04	0.0037
Fenamidone	0.999	0.998	4E+08	8E+06	2E+05	5E+05	0.0019
Fenbuconazole	0.998	0.997	1E+08	4E+06	−5E+05	2E+05	0.0023
Fenhexamid	0.986	0.973	2E+07	2E+06	8E+04	9E+04	0.0066
Fenoxycarb	0.999	0.997	7E+08	2E+07	−1E+06	9E+05	0.0021
Fenpropimorph	0.996	0.991	7E+08	3E+07	−3E+06	2E+06	0.0037
Fenpyroximate	0.997	0.993	5E+08	2E+07	−2E+06	1E+06	0.0033

Table 2 (continued)

Analytes	r	r^2	b	S_b	a	S_a	S_u
Fensulfothion sulfone[a]	0.994	0.987	4.8E+06	2.5E+05	−8.9E+06	5.0E+06	2.01
Fensulfothion[a]	0.994	0.988	1.8E+06	9.1E+04	−4.0E+06	1.9E+06	1.93
Fensulfothion-oxon[a]	0.994	0.988	3.4E+06	1.7E+05	−6.2E+06	3.5E+06	1.98
Fensulfothion-oxon-sulfone[a]	0.992	0.984	5.8E+06	3.3E+05	−1.1E+07	6.7E+06	2.22
Fenthion	0.999	0.998	2E+09	4E+07	−2E+06	2E+06	0.0020
Fentin[a]	0.988	0.975	7.1E+05	4.6E+04	−2.0E+06	9.4E+05	2.52
Fipronil[a]	0.993	0.985	1.3E+06	7.1E+04	−1.8E+06	1.4E+06	2.20
Fipronil-desulfinyl[a]	0.994	0.989	1.3E+07	6.6E+05	−2.8E+07	1.3E+07	1.90
Flutriafol	0.999	0.998	8E+07	2E+06	−1E+05	1E+05	0.0020
Fosthiazate	0.999	0.997	5E+08	1E+07	1E+06	6E+05	0.0021
Furathiocarb	0.999	0.997	2E+09	4E+07	−4E+06	2E+06	0.0021
Haloxyfop[a]	0.994	0.987	8.6E+05	4.4E+04	−1.7E+06	8.9E+05	1.98
Haloxyfop-ethoxyethylester[a]	0.984	0.969	1.9E+05	1.4E+04	−2.7E+05	2.9E+05	2.94
Haloxyfop-methoxyethylester[a]	0.984	0.968	2.0E+04	1.6E+03	−1.7E+04	3.2E+04	3.02
Hexaconazole	0.948	0.899	1E+07	2E+06	−9E+04	1E+05	0.0163
Hexythiazox	0.993	0.987	1E+08	8E+06	−6E+05	4E+05	0.0046
Imidacloprid	0.999	0.998	2E+08	5E+06	−3E+05	3E+05	0.0020
Indoxacarb	0.995	0.989	3E+07	1E+06	−1E+05	7E+04	0.0041
Iprovalicarb	0.999	0.998	3E+09	6E+07	5E+06	4E+06	0.0018
Kresoxim methyl	1.000	0.999	1E+08	1E+06	1E+05	8E+04	0.0013
Linuron	0.994	0.989	2E+08	1E+07	6E+05	6E+05	0.0043
Metalaxyl M	0.999	0.999	6E+08	8E+06	9E+05	5E+05	0.0013
Metconazole	0.999	0.998	6E+07	1E+06	−1E+04	6E+04	0.0017
Methamidophos	0.994	0.989	2E+08	8E+06	9E+05	4E+05	0.0043
Methiocarb	1.000	0.999	2E+08	2E+06	−3E+05	1E+05	0.0011
Methiocarb sulfone	0.980	0.958	6E+07	6E+06	7E+05	3E+05	0.0084
Methiocarb sulfoxide	0.999	0.999	1E+09	2E+07	3E+05	1E+06	0.0014
Methomyl	0.999	0.998	5E+08	9E+06	−6E+05	5E+05	0.0016
Methoxyfenozide	0.998	0.997	3E+09	7E+07	5E+06	4E+06	0.0023
Metoxuron	0.999	0.997	7E+07	1E+06	−7E+04	8E+04	0.0020
Monocrotophos	0.998	0.996	2E+08	6E+06	−2E+05	3E+05	0.0027
Monolinuron	0.992	0.984	5E+08	3E+07	1E+06	2E+06	0.0051
Myclobutanil	0.995	0.990	2E+07	1E+06	2E+05	6E+04	0.0041
Nicosulfuron	0.998	0.996	3E+08	9E+06	−1E+06	5E+05	0.0026
Omethoate[a]	0.995	0.989	6.3E+05	3.0E+04	−1.3E+06	6.1E+05	1.87
Oxamyl	1.000	0.999	8E+08	1E+07	−2E+05	6E+05	0.0012
Phosalone	0.999	0.998	1E+08	3E+06	−5E+05	2E+05	0.0018
Phosmet	0.994	0.988	3E+07	2E+06	1E+05	9E+04	0.0044
Pirimicarb	0.999	0.998	2E+08	5E+06	9E+05	3E+05	0.0018
Pirimiphos methyl	0.995	0.989	2E+08	9E+06	−4E+05	5E+05	0.0042
Primisulfuron methyl	0.998	0.996	1E+08	4E+06	−4E+05	2E+05	0.0026
Procloraz	0.996	0.991	1E+08	5E+06	−6E+05	3E+05	0.0037
Profenofos	0.989	0.979	2E+07	2E+06	−5E+04	9E+04	0.0058
Propamocarb	0.998	0.996	1E+09	4E+07	3E+06	2E+06	0.0026
Propargite	0.998	0.997	1E+09	3E+07	−2E+06	2E+06	0.0023
Pymetrozine	0.994	0.989	2E+08	1E+07	−1E+06	6E+05	0.0043
Pyraclostrobin	1.000	0.999	9E+08	9E+06	−8E+05	5E+05	0.0009
Pyridaben	0.990	0.981	8E+08	5E+07	−2E+06	3E+06	0.0056
Pyrifenox	0.995	0.991	4E+08	2E+07	−2E+06	9E+05	0.0039

Table 2 (continued)

Analytes	r	r^2	b	S_b	a	S_a	S_u
Pyrimethanil	0.992	0.983	4E+07	2E+06	2E+05	1E+05	0.0052
Pyriproxyfen	0.997	0.994	9E+08	3E+07	−3E+06	2E+06	0.0032
Quinoxyfen	0.988	0.977	1E+07	7E+05	−1E+04	4E+04	0.0061
Simazine	0.982	0.965	2E+07	1E+06	2E+05	8E+04	0.0076
Spinosad (A)	0.994	0.989	4E+08	2E+07	−2E+06	1E+06	0.0043
Spiroxamine	0.999	0.999	2E+09	3E+07	−6E+06	2E+06	0.0015
Tebuconazole	0.955	0.911	1E+07	2E+06	2E+04	9E+04	0.0124
Tebufenozid	0.999	0.998	6E+08	1E+07	7E+05	6E+05	0.0016
Tebufenpyrad	0.999	0.999	9E+07	1E+06	−3E+05	8E+04	0.0014
Terbufos[a]	0.988	0.976	8.4E+05	5.3E+04	−2.3E+06	1.1E+06	2.47
Terbufos-sulfone[a]	0.993	0.986	2.9E+06	1.6E+05	−4.5E+06	3.2E+06	2.13
Terbufos-sulfoxide[a]	0.993	0.986	7.8E+06	4.3E+05	−1.2E+07	8.7E+06	2.14
Terbuthylazine	0.998	0.996	1E+09	4E+07	2E+06	2E+06	0.0027
Tetraconazole	0.974	0.949	3E+07	4E+06	−3E+05	2E+05	0.0092
Thiacloprid	0.998	0.995	1E+09	4E+07	2E+06	2E+06	0.0027
Thiamethoxam	0.996	0.993	3E+08	1E+07	5E+05	7E+05	0.0034
Thiodicarb	0.998	0.997	3E+08	6E+06	−1E+04	4E+05	0.0022
Thiophanate methyl	0.997	0.995	8E+08	3E+07	−6E+05	2E+06	0.0029
Tolylfluanid	0.949	0.900	8E+06	1E+06	4E+03	7E+04	0.0133
Triadimefon	0.988	0.977	6E+07	4E+06	−7E+04	2E+05	0.0061
Triadimenol	0.988	0.977	8E+07	5E+06	−6E+05	3E+05	0.0062
Triazophos	0.993	0.986	6E+07	3E+06	1E+05	2E+05	0.0048
Trifloxystrobin	0.996	0.993	3E+08	1E+07	−2E+06	6E+05	0.0034
Vamidothion	0.999	0.997	5E+08	1E+07	5E+05	7E+05	0.0020

[a] The study was performed at the following concentration levels ranging from 10 to 110 ng/mL except for the analytes marked with the asterisk for which the concentrations ranged from 1.5 to 30 ng/mL

but the use of methanol/water (30:70, *v*/*v*) as the final solvent proved to have its advantages, mostly for the more polar compounds that elute early. In order to determine the differences between acetonitrile and methanol/water (30:70, *v*/*v*), a standard solution of the 116 compounds at 10 μg/mL was prepared in acetonitrile and one in methanol/water (30:70, *v*/*v*). Five injections of each standard solution were performed at the same day as to have statistical results. The time distribution of the compounds is shown in Fig. 1. The 57.3% of the analytes elute between the range of 0 and 25 min, and the largest number of analytes is distributed between two 5-min time periods from 20 to 25 and 25 to 30, at the middle of the chromatographic program. As shown in Fig. 2, the standard solution in methanol/water (30:70, *v*/*v*) gave higher signal-to-noise (S/N) ratio for the compounds that elute at the early. This percentage was at 100% for the compounds that elute before the first 10 min, 90.9% for those that elute at the next 10 min, and 56.7% for those that elute between 20 and 25 min. The percentages are altered in favor of the acetonitrile for the compounds that elute after the 25 min. Also a main advantage of the methanol/water (30:70, *v*/*v*) as final solvent is that it mixes better with the mobile phase because they are prepared from the same solvents, methanol, and water, and the ratio between the mobile phases at the starting point of the chromatographic program (80% water, 20% methanol) and the final solvent of methanol/water (30:70, *v*/*v*) are almost the same; therefore, we achieve better chromatographic peaks, especially for the more polar compounds that elute early, as shown in Fig. 3.

MS Optimization

The ionization of the pesticides and metabolites in positive and negative electrospray ion source was studied. Table 1 shows the precursor ions used for data acquisition, the transition used for quantification and confirmation (Federal Institutor of Risk Assessment 2009), the cone voltage and collision energy for each transition, the retention times of the analytes, and the time segment in which the transitions are monitored. Pesticides are ionized in the forms of $[M+H]^+$or $[M+NH_4]^+$ions. Tandem mass spectrometry provides a powerful confirmatory tool for pesticide residue analysis

Table 3 Recovery data (n=5), obtained for the 120 analytes at two concentration levels in the cereal-based infant food matrix

Analyte	Mean recovery (%) 1st level	SD_r 1st level	Mean recovery (%) 2nd level	SD_r 2nd level	S/N at the 1st level
Acephate	69.2	21.0	79.2	5.6	13
Acetamiprid	86.9	16.9	105.9	11.3	320
Aldicarb	64.2	8.4	97.3	11.9	15
Aldicarb sulfone	59.4	6.9	88.2	18.5	45
Aldicarb sulfoxide	64.5	26.1	73.8	11.6	20
Ametryn	81.3	17.3	87.1	12.8	200
Atrazine	102.4	22.4	88.8	19.6	20
Azimsulfuron	87.7	18.9	95.8	16.9	25
Azinphos methyl	62.9	15.2	100.5	15.8	70
Azoxystrobin	87.7	14.5	97.2	13.3	500
Benalaxyl	82.8	14.3	88.5	12.3	25
Bensulfuron methyl	56.4	16.8	97.9	18.1	100
Boscalid	108.3	15.9	74.9	14.7	250
Bromuconazole	79.4	25.6	82.5	20.8	20
Buprofezin	90.0	19.4	102.1	16.8	84
Cadusafos[a]	122.7	3.2	63.5	7.4	24
Carbaryl	82.1	15.8	96.3	17.1	600
Carbofuran	86.8	10.3	107.0	12.5	1,000
Carbofuran 3-hydroxy	72.9	13.7	95.6	18.1	13
Carbosulfan	68.4	17.5	85.2	20.1	495
Chlorotoluron	78.6	20.1	99.9	14.3	110
Clofentezine	81.8	24.4	89.3	15.2	30
Cyanazine	63.2	19.8	106.9	21.0	75
Cymoxanil	107.9	11.0	105.8	17.5	140
Demeton-*S*-methyl[a]	84.4	7.6	84.4	7.6	17
Demeton-*S*-methyl sulfone[a]	107.9	7.7	83.3	9.1	28
Demeton-*S*-methyl sulfoxide[a]	111.6	11.7	72.3	3.3	50
Diazinon	88.1	20.9	84.3	16.5	250
Dichlorvos	45.1	14.3	95.9	18.7	15
Diethofencarb	83.7	12.7	104.1	15.5	540
Difenoconazole	102.9	18.3	84.5	21.2	55
Dimethoate[a]	108.9	5.5	97.4	4.5	30
Dimethomorph	100.2	20.7	84.3	18.0	13
Disulfoton[a]	62.8	9.3	57.6	22.1	30
Disulfoton-sulfone[a]	89.9	15.4	84.3	3.7	50
Disulfoton-sulfoxide[a]	124.3	13.9	114	2	24
Epoxiconazole	71.5	18.3	88.2	14.6	500
Ethofumesate	75.3	19.5	94.7	19.7	40
Ethoprophos[a]	116.5	6.6	94.6	5.03	150
Etoxazole	108.2	16.7	70.0	19.4	751
Famoxadone	70.0	19.4	72.4	14.9	40
Fenamidone	105.8	18.0	94.9	21.7	75
Fenbuconazole	72.6	18.7	75.0	18.6	30
Fenhexamid	78.4	7.2	90.9	20.7	20
Fenoxycarb	99.0	15.3	76.9	8.2	25
Fenpropimorph	113.5	11.7	109.4	21.2	25
Fenpyroximate	111.9	3.2	72.3	12.1	175
Fensulfothion[a]	101.8	12.8	113.9	11.3	31

Table 3 (continued)

Analyte	Mean recovery (%) 1st level	SD_r 1st level	Mean recovery (%) 2nd level	SD_r 2nd level	S/N at the 1st level
Fensulfothion sulfone[a]	100.4	12.8	80.4	4.9	20
Fensulfothion-oxon[a]	83.9	3.8	123.1	11	233
Fensulfothion-oxon-sulfone[a]	118.7	8.9	120.3	18.4	342
Fenthion	99.5	10.0	109.6	14.9	75
Fentin[a]	67.5	16.1	73.8	16.5	23
Fipronil[a]	112	6.1	100.1	8.5	67
Fipronil-desulfinyl[a]	82.7	21.1	110.6	9.9	417
Flutriafol	99.9	18.8	107.6	21.8	13
Fosthiazate	83.5	10.6	108.6	13.3	600
Furathiocarb	108.1	15.4	76.1	12.3	300
Haloxyfop[a]	94.4	10.3	122.8	5.7	18
Haloxyfop-ethoxyethylester[a]	80.9	11.8	81.9	11.2	24
Haloxyfop-methoxyethylester[a]	80.4	24.9	76.8	10	30
Hexaconazole	60.5	14.3	108.9	19.0	50
Hexythiazox	74.9	10.4	87.4	24.6	10
Imidacloprid	96.2	18.5	98.5	22.8	60
Indoxacarb	108.0	13.5	70.7	19.1	46
Iprovalicarb	86.1	18.6	87.1	11.6	850
Kresoxim methyl	123.8	19.8	75.2	19.7	100
Linuron	102.4	10.8	89.7	11.9	13
Metalaxyl M	79.7	11.0	103.8	14.8	450
Metconazole	67.2	7.6	101.7	20.4	25
Methamidophos	58.8	15.3	81.5	23.7	25
Methiocarb	79.2	11.4	87.5	20.6	32
Methiocarb sulfone	105.3	23.5	117.1	18.4	13
Methiocarb sulfoxide	68.9	3.4	102.4	21.3	320
Methomyl	84.9	3.6	89.5	9.9	13
Methoxyfenozide	93.3	17.1	88.7	15.1	700
Metoxuron	68.3	21.2	107.1	19.8	20
Monocrotophos	86.2	18.6	120.3	11.9	40
Monolinuron	114.4	7.3	99.1	12.1	210
Myclobutanil	72.0	14.3	96.7	21.0	25
Nicosulfuron	61.1	12.9	105.6	20.9	30
Omethoate[a]	30.2	5.6	36.8	7.8	35
Oxamyl	114.3	21.2	95.2	12.4	100
Phosalone	76.8	8.9	65.8	26.1	80
Phosmet	56.0	21.4	99.4	27.4	20
Pirimicarb	73.7	18.6	95.6	14.0	300
Pirimiphos methyl	82.7	14.2	75.0	20.5	127
Primisulfuron methyl	83.8	21.6	91.5	14.1	13
Procloraz	108.2	14.1	69.3	17.7	130
Profenofos	90.8	23.6	77.4	20.3	41
Propamocarb	98.0	20.3	78.6	16.8	15
Propargite	118.9	9.9	80.6	24.4	410
Pymetrozine	75.8	20.7	90.6	16.0	13
Pyraclostrobin	100.3	13.6	83.2	22.4	195
Pyridaben	115.5	8.6	66.5	6.8	300
Pyrifenox	91.1	18.0	93.9	16.7	48

Table 3 (continued)

Analyte	Mean recovery (%) 1st level	SD_r 1st level	Mean recovery (%) 2nd level	SD_r 2nd level	S/N at the 1st level
Pyrimethanil	123.5	14.7	86.3	20.1	13
Pyriproxyfen	114.0	11.5	65.0	15.1	125
Quinoxyfen	102.7	14.8	56.2	8.4	30
Simazine	85.3	25.5	80.8	18.0	10
Spinosad (A)	120.0	29.7	67.1	11.4	30
Spiroxamine	107.6	17.0	68.8	8.9	500
Tebuconazole	88.7	19.0	82.4	24.3	13
Tebufenozid	95.4	19.8	86.2	10.0	350
Tebufenpyrad	88.4	24.0	68.5	24.3	19
Terbufos[a]	91.3	20.6	95.5	4.7	13
Terbufos-sulfone[a]	122.4	12.1	85.5	4.4	55
Terbufos-sulfoxide[a]	121.4	9.4	87.7	3.3	50
Terbuthylazine	115.3	7.3	66.6	3.1	250
Tetraconazole	66.2	20.5	71.0	10.8	40
Thiacloprid	86.6	14.5	103.9	10.1	400
Thiamethoxam	68.8	18.7	109.4	5.4	80
Thiodicarb	100.1	16.0	102.0	5.6	45
Thiophanate methyl	94.9	20.4	90.9	21.9	150
Tolylfluanid	64.7	20.1	72.5	9.1	40
Triadimefon	125.4	14.6	75.6	21.3	12
Triadimenol	86.1	20.3	101.7	20.2	33
Triazophos	76.8	20.9	94.5	11.3	11
Trifloxystrobin	118.8	5.7	69.8	12.3	295
Vamidothion	76.5	9.6	106.4	10.7	750

The first level was 10 μg/kg (except for the analytes marked with an asterisk for which it was 3 μg/kg) and the second ten times higher

[a] The recovery was estimated at 3 and 30 μg/kg

because it discriminates efficiently between the analyte and the matrix signal. Individual standard solutions at 100 μg/mL were prepared in methanol/water (30:70, *v*/*v*) for optimization of the system by multiple injections at different cone voltage and collision energy.

The source optimization of each pesticide was tuned by introducing each analyte into the mass spectrometer through direct infusion via a syringe pump at a flow rate of 250 μL/min. MS–MS spectra were acquired to obtain information about the maximum number of transitions available for each compound. Typically, the quantification transition was selected to achieve maximum sensitivity. The optimum cone voltage varies between 6 and 85 kV depending on the analyte. Product ion mass spectra for the pesticides were obtained in the positive and negative mode of electrospray ionization using collision-induced dissociation. Variation of the collision energy influences both sensitivity and fragmentation. The collision energy was optimized for two selective product ions of each precursor ion. The optimized values acquired are listed in Table 1.

The time-scheduled data acquisition sequence involved 18 overlapping segments of 1 min each. In each segment, 2 to 35 transitions are monitored. By this technique, we create an artificial window maximum± 2 min from the retention time of each compound. Therefore, the instrument consumes the ideal amount of time as to have a successful acquisition with less time shifts that can easily cause loss of a peak and sufficient dwell and scan time, without stacking a lot of transitions in one time segment. In our study, 116 transitions are acquired in each run, but considering the low detection levels and the different sensitivities of the analytes, this technique was proved to be very useful. This technique was also proved useful in the development of multi-residue methods with a large number of transitions in one run. With dwell times of 0.1 s, the average scan cycle time for the segments varies between 0.2 and 1 s.

Validation

The validation study was performed according to the European SANCO guidelines 10684/2009 (European Commission 2009). Analytical characteristics evaluated were sensitivity, mean recovery (as a measure of trueness), and repeatability (as a measure of precision).

Linearity

Calibration curves were constructed from injections of matrix-matched calibration standards in blank cereal-based infant food extract in methanol/water (30:70, *v*/*v*) for the 116 analytes and in blank cereal-based infant food extract in methanol for fentin, haloxyfop, and its esters. The linearity was estimated at eight concentrations levels 1.5–3.0–5.0–7.5–10–15–20 and 30 μg/mL for the 23 analytes of Appendix IV of the Commission Directives 2006/141/EC and 2006/125/EC and 10–15–23–34–50–76–110 μg/kg for the rest 97 analytes. These calibration curves are used to obtain the predicted concentration C (milligrams per kilogram) of the analyte from a sample which produces an observed response y by the equation:

$$C = (y - a)/b$$

In Table 2, the basic calibration line parameters for the analytes are presented, including the uncertainty S_u on the estimated concentration C. According to EURACHEM/CITAC Guide (2000), there are four sources of uncertainty to consider in arriving at an uncertainty on the estimated concentration C. The most significant of them for normal practice are due to variability in the peak area y. The uncertainty S_u of C due to variability in y can be estimated from the calibration data, by the following equation:

$$S_u = \frac{S_{y/C}}{b}$$

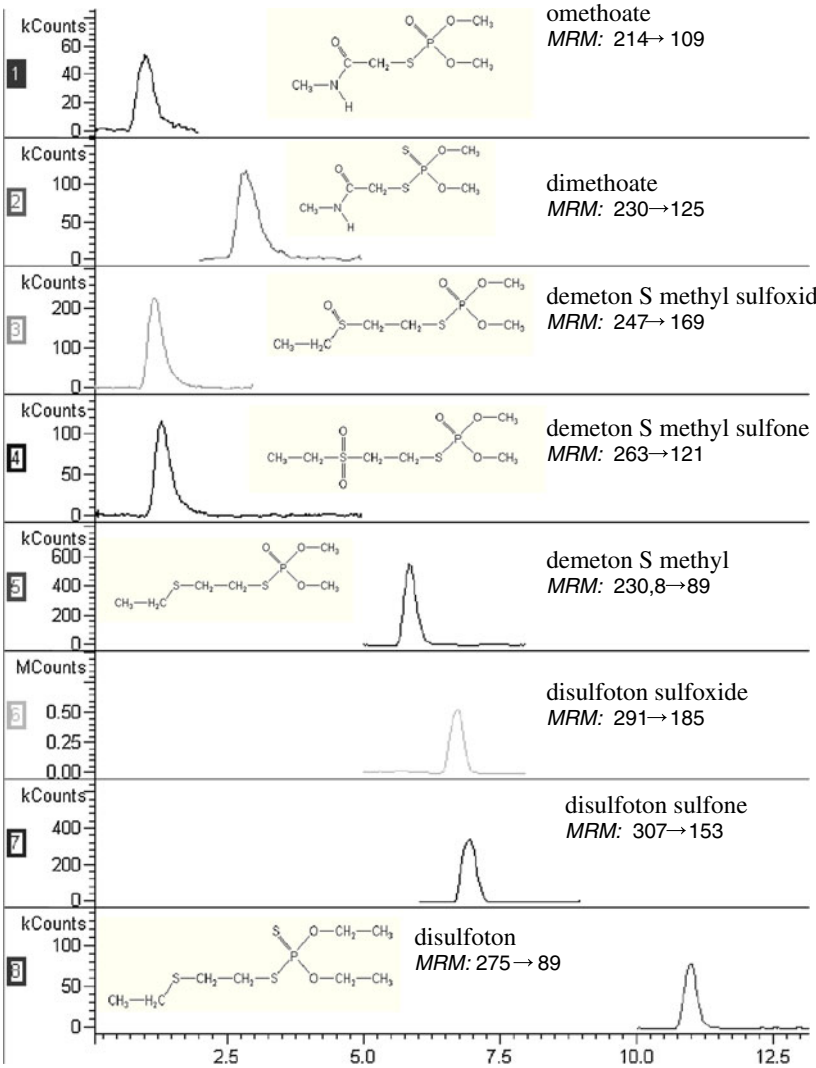
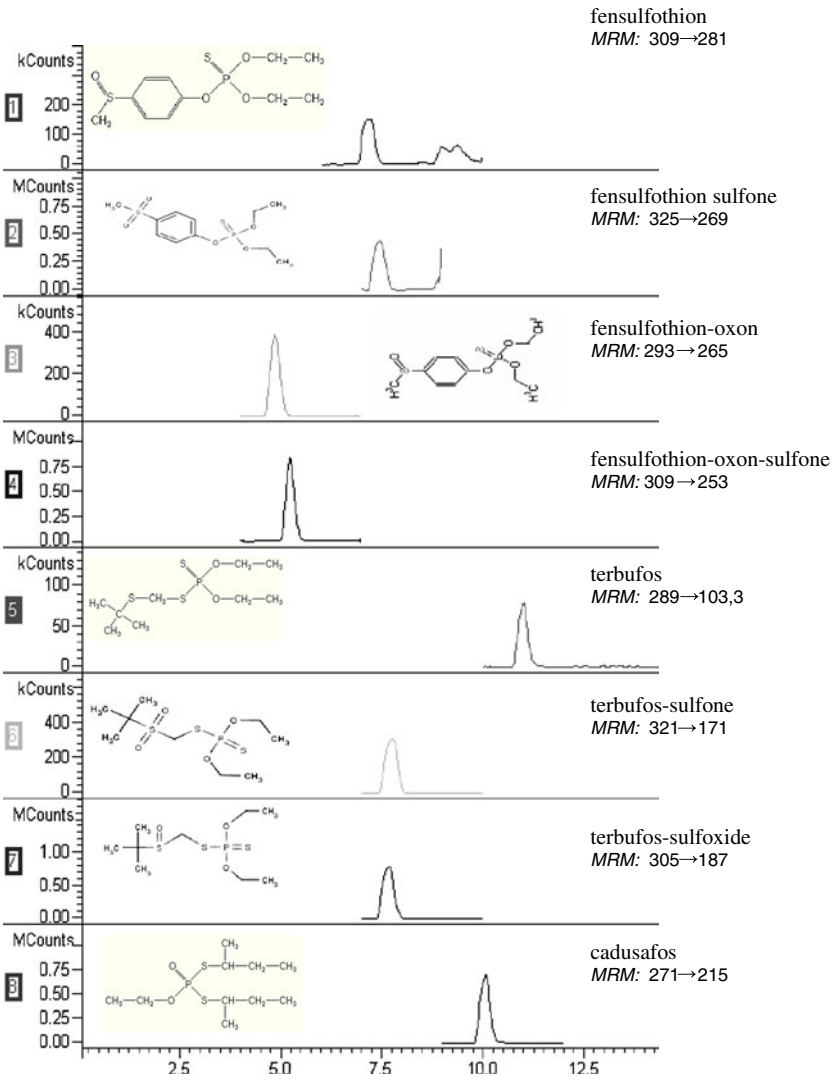


Fig. 4 Reconstructed ion chromatogram by LC–MS–MS for cereal-based infant food extract spiked at 3 μg/kg using the MRM transitions for omethoate, dimethoate, demeton-*S*-methyl sulfoxide, demeton-*S*-methyl sulfone, demeton-*S*-methyl, disulfoton-sulfoxide, disulfoton-sulfone, disulfoton, fensulfothion, fensulfothion sulfone, fensulfothion-oxon, fensulfothion-oxon-sulfone, terbufos, terbufos-sulfone, terbufos-sulfoxide, cadusafos, fipronil, and fipronil-desulfinyl

Fig. 4 (continued)



where $S_{y/C} = \sqrt{\frac{\sum_i (y_i - \bar{y})^2}{n-2}}$, $(y_i - \bar{y})$ is the residual for the *i*th point and *b* the slope of the regression line and *n* is the number of the data points in the calibration.

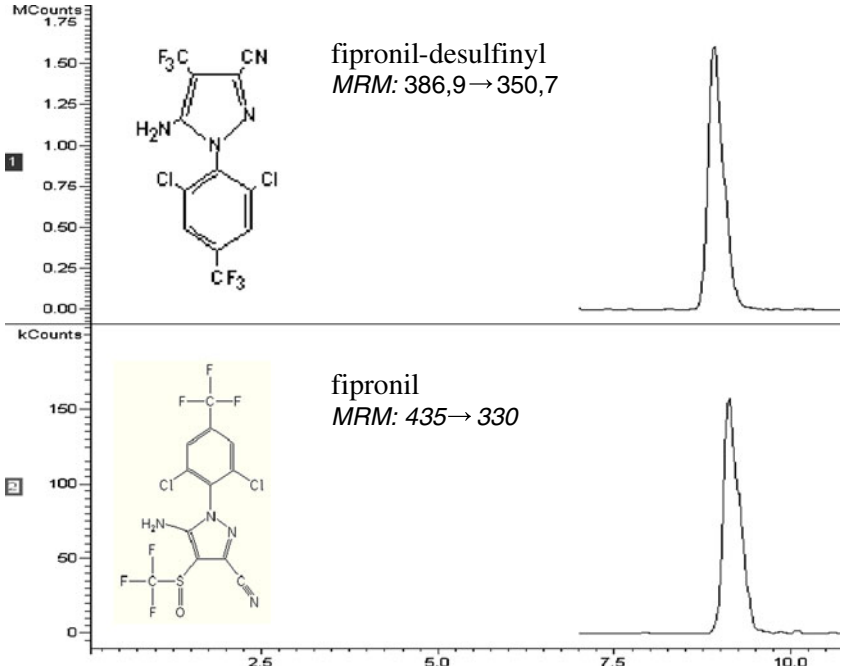
Good linearity was achieved in all cases with correlation coefficients better than 0.990 in most cases, 0.98 in the cases of diethofencarb, difenoconazole, ethoprophos, fenhexamid, fentin, haloxyfop-ethoxyethylester, haloxyfop-methoxyethylester, methiocarb sulfone, profenofos, quinoxyfen, terbufos, tetraconazole, and 0.944–0.955 in the cases of hexaconazole, tebuconazole, tolylfluanid.

Trueness, Precision

Recoveries and repeatability of the method were established in order to evaluate the methods' trueness and precision, respectively. Mean recoveries of 70–120% with relative standard deviations (SD_r)≤20% are acceptable, while in certain cases, typically with multiresidue methods, recoveries outside this range may be acceptable. In routine analysis, the acceptable recoveries are in the range of the mean recovery ±2 SD_r (European Commission 2009). The mean recoveries were determined from spiked cereal-based infant food samples, at two concentration levels. The lowest fortification level was 3 μg/kg for the analytes of Appendix IV of the Commission Directives 2006/141/EC and 2006/125/EC and 10 μg/kg for the rest of the analytes. The second fortification level was ten times higher (30 or 100 μg/kg). The recoveries were calculated using matrix-matched, calibration standards.

As shown in Table 3, the recoveries at the lowest level for 114 of the 120 analytes ranged from 60.5% to 125.4% (91 of the 114 compounds gave recoveries within 70–120%) with SD_r less than 29.7% and 56.2–123.1% (108 of the 114 compounds gave recoveries within 70–120%) with SD_r less than 26.1% for the highest. The majority of the analytes (91 analytes) gave recoveries and SD_r values within the accepted

Fig. 4 (continued)



values. In the cases of 23 analytes, as shown in Table 3, the recovery values at the lowest concentration level was below 70% (16 analytes) or over 120% (seven analytes), but consistent (low SD_r values) and are considered acceptable (European Commission 2009). Therefore, the method still is able to serve as a semiquantitative method to detect and confirm their presence in samples.

The analytes acephate, aldicarb sulfoxide, metoxuron, phosmet, tetraconazole, tolyfluanid, and omethoate gave recovery and SD_r values outside the acceptable ranges, so for these analytes, further investigation is required.

Limit of Quantification

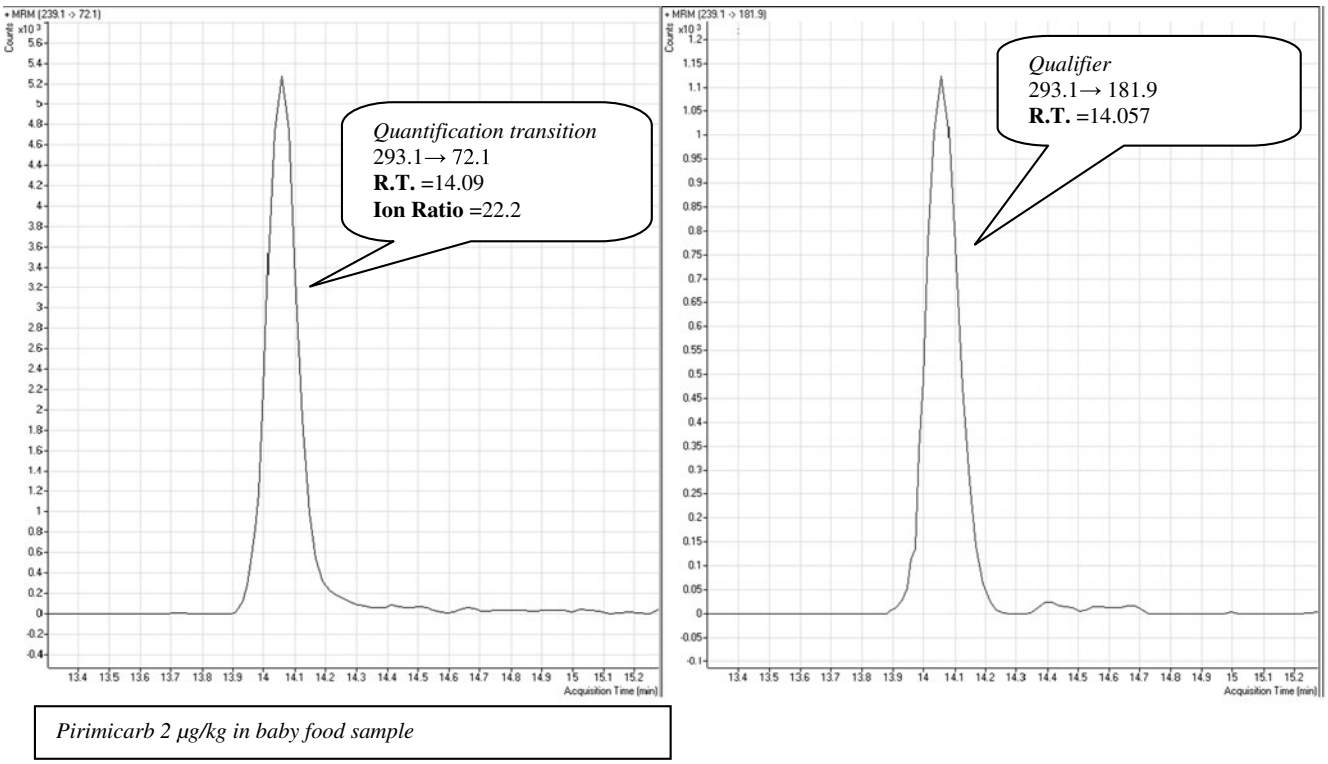
The LOQ was established as the lowest concentration tested for which recovery and SD_r values were satisfactory in accordance with the criteria established for analysis of pesticide residues in foods (European Commission 2009) and with S/N ratio higher than 10. Therefore, as LOQ, the lowest validated level with acceptable accuracy and precision results was selected. In Table 3, the S/N ratio at the LOQ is presented. As shown in Fig. 4, the analytes with concentration at the LOQ gave good peak shape with S/N >10.

Analysis of Real Samples

The proposed methodology was applied for the analysis of real baby food samples. Sixteen cereal-based infant food samples were analyzed (rice cereal, farine lactée, biscuit purée, cereal purée, and baby food desserts). In one sample, traces of the pesticide pirimicarb were found at concentrations below the LOQ of the method (2 μg/kg). The quantification of pirimicarb was conducted by single-level calibration. Single-level calibration may provide more accurate results than multi-level calibration if the detector response is variable with time. For the comparison, the sample response should be within ±50% of the calibration standard response. Matrix-matched analytical standards in blank extract from cereal-based baby food, previously analyzed for the absence of peaks interfering with the peaks of the analytes, were used. Figure 5 shows the chromatogram of the baby food sample that contained pirimicarb at a concentration of 2 μg/kg.

Conclusions

In conclusion, the QuEChERS method for the extraction procedure combined with the LC–ESI–MS–MS was found to be a sensitive method for the determination of 120 pesticides and metabolites according to the Commission Directives 2006/141/EC and 2006/125/EC in cereal-based infant food. Slightly modifications either on the extraction procedure or the LC conditions made possible the confirmation and quantification of pesticides that require special conditions like fentin, haloxyfop, and its esters. Although for some pesticides and metabolites the recovery values were low, still confirmation is feasible and quantification of the analyte can be achieved. The



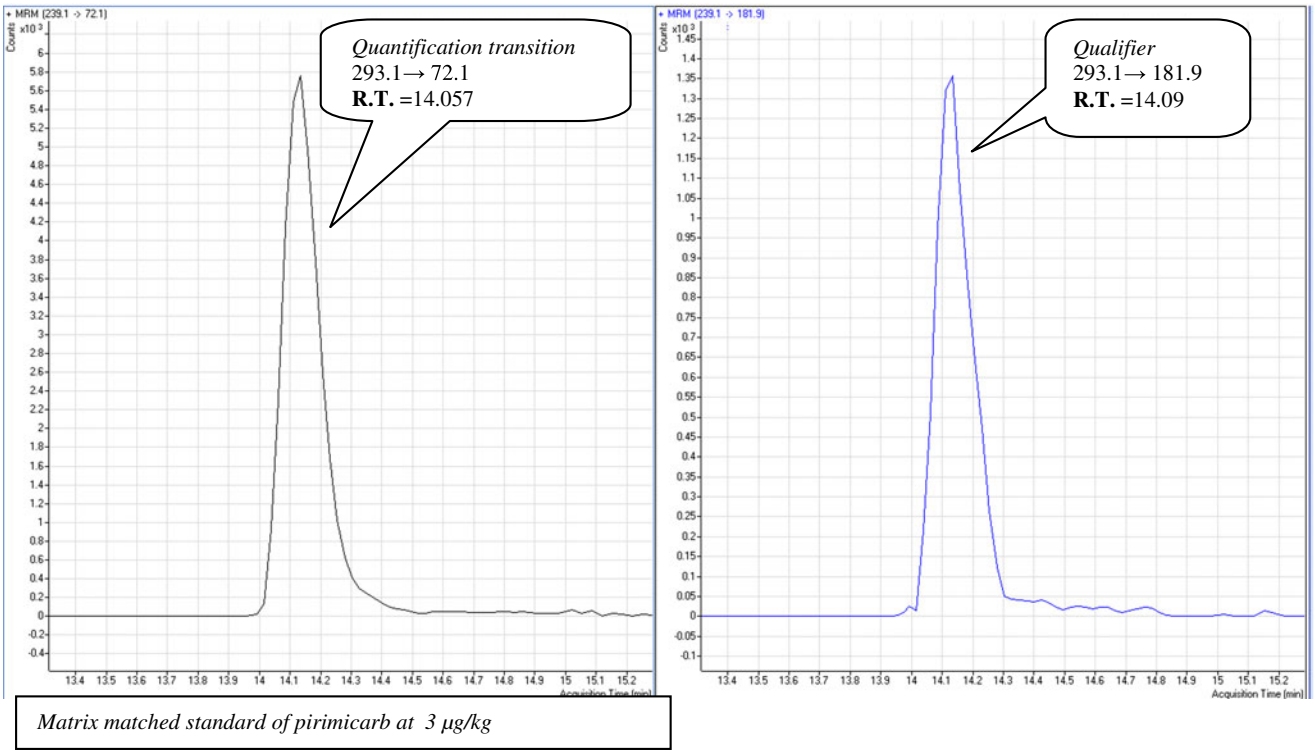


Fig. 5 LC–MS–MS chromatogram of a real baby food sample that contained pirimicarb at 2 µg/kg and a matrix-matched standard of pirimicarb 3 µg/kg at baby food matrix extract

method is simple, fast, and suitable for routine analysis for the determination of pesticides in cereal-based infant food and other products with high protein or high starch content meeting the EU guidelines method performance criteria.

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