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Multi-residue determination of pesticides in fruit-based soft drinks by fast liquid chromatography time-of-flight mass spectrometry

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ABSTRACT

This work reports a rapid and reliable method for the determination of 33 multi-class pesticides in fruit-based soft drinks. The proposed method consists of a sample treatment step based on solid-phase extraction using hydrophilic–lipophilic balanced polymer-based reverse-phase SPE cartridges, followed by identification and quantitation of the target pesticides by rapid resolution liquid chromatography using a short C_{18} column (4.6 mm × 50 mm) with 1.8 μm particle size and mass spectrometric detection using electrospray time-of-flight mass spectrometry (LC–TOFMS). The identification and confirmation of the compounds were based on retention time matching along with the accurate mass measurements of the protonated molecules ($[M+H]^+$) and their main fragment ions. Fruit-based soft drinks spiked at different fortification levels (10 and 50 μg L^{-1}) yielded average recoveries in the range 66–124% with RSD (%) below 14% ($n=6$). The obtained limits of quantitation varied in the range 0.02–2 μg L^{-1}. The proposed method was successfully applied to the analysis of 14 market-purchased fruit-based soft drinks samples collected in some European countries, showing its potential applicability and revealing the presence of some of the target species in the μg L^{-1} range.

1. Introduction

Pesticide residue research is important not only for trade purposes but also to preserve human health. Different regulations have been established for fruits and vegetables [1,2]. On the other hand, in the case of drinking water, the European Union Council Directive 98/83/EC of 3 November 1998 (80/778/EC) [3] on the "quality of water intended for human consumption" – regulated at the European level – establishes maximum admissible concentration for individual pesticides and related products in drinking water at 0.1 μg L^{-1} for each individual pesticide and at 0.5 μg L^{-1} for total amount of pesticides (i.e. the sum of all individual pesticides detected and quantified in the monitoring procedure). The EU regulations are the only ones that have agreed on a single value, which is low enough to ensure that no chemical is toxic to human. Therefore, there are regulations for pesticides in fruit, vegetables, baby food or drinking water.

In contrast, scarce attention has been paid to other derivate products, which may contain these commodities as an ingredient. Amongst these products, fruit-based soft drinks contain a percentage of fruit or juice extracts typically in the range of 3–10%. However unlike other kinds of commodities (fruit, vegetables, water) scarce or no attention has been paid to enforce the safety of these products in terms of chemicals.

In a recent work [4], over 100 fruit-based soft drink samples (purchased from 15 different countries from brands of companies distributed worldwide) were studied, revealing the presence of relatively large concentration levels of pesticides in fruit-based soft drinks. Some of the detected pesticides were those applied to crops at final stages of production (post-harvest treatment) and might have hazardous effects to infants, one of the main groups of consumers of soft drinks industry products. This first report indicated the importance of establishing comprehensive monitoring programs to control these kinds of derivate products [5–7]. For this reason, there is a need to develop rapid and reliable methods for the determination of pesticides in soft drinks. In this sense, the use of liquid chromatography coupled to mass spectrometry (LC–MS) has become a valuable technique for analyzing many residues and contaminants in complex matrices such as food and environmental samples as described extensively in the literature [8–13]. Recent reviews on pesticides in food and water have commented on the unique ability of accurate mass to identify both target compounds and non-targets by liquid chromatography time-of-flight mass spectrometry (LC–TOFMS) [14–16]. State-of-the-art

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LC–TOFMS instrumentation provide sensitive full-scan acquisition, becoming an excellent tool for the unambiguous target and non-target identification and confirmation of pesticides residues in vegetables and fruits [17–23].

This work reports on the development and validation of a fast multi-residue method for the determination of 33 representative multi-class pesticides in fruit-based soft drinks samples using LC–TOFMS. The analytical methodology used in this monitoring study consists of a sample treatment protocol based on solid-phase extraction using hydrophilic–lipophilic balanced polymer-based reverse-phase HLB Oasis cartridges followed with identification and quantitation of the targeted species by rapid resolution liquid chromatography–mass spectrometry using electrospray ionization in the positive ionization mode and a short C_{18} column (4.6 mm × 50 mm) with 1.8 μm particle size. The unambiguous confirmation of the compounds was based on retention time matching combined with accurate mass measurements of the protonated molecules ($[M+H]^+$) and their main fragment ions. The proposed method was successfully implemented to the analysis of 14 market-purchased fruit-based soft drinks samples collected in Europe, where selected pesticides were detected in the μg L^{-1} range.

2. Experimental

2.1. Chemicals and materials

Pesticide analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany) and from Riedel de Haën, Pestanal® quality (Seelze, Germany). Individual pesticide stock solutions of the studied compounds (*ca.* 500 μg mL^{-1} each) were prepared in methanol or acetonitrile and stored at −20 °C. HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used during the analyses. Oasis HLB™ SPE cartridges (200 mg, 6 mL) purchased from Waters (Milford, MA, USA) and a Supelco (Bellefonte, PA, USA) Visiprep™ SPE vacuum system were also used.

2.2. Selection of the targeted species

Analytes included in this study were selected on the basis of previous experience in pesticide testing and published literature [4]. They comprise a group of 33 agrochemicals belonging to different categories (acaricides, algicides, bird repellents, fungicides, herbicides, insecticides, nematicides, acaricides, etc.), corresponding to different chemical classes (benzimidazoles, carbamates, conazoles, nicotinoids, organophosphorous, phenylureas, pyrimidines, spiroketalamine group class, spynosin, strobilurin, etc.). Some of the selected agrochemicals belong to the Annex 1 of the European Union Directive (91/414/EEC). Some of them also belong to the list of the current European Union Proficiency Test (EUPT) for Fruits and Vegetables (www.crl-pesticides.eu) and others were selected according to www.pesticides-online.com database. Finally, imazalil and prochloraz metabolites were added because of its relevance and significant presence in citrus fruit samples.

2.3. Samples

Fourteen market-purchased citrus fruit-based soft drinks samples were studied. The samples were bottles and cans of different brands collected and purchased in Spain, France, Italy, Ireland, Czech Republic, Austria, Switzerland and the United Kingdom in 2009. Details of the samples and results are given in Section 3.4.

2.4. Sample treatment

The pesticides were extracted using solid-phase extraction with polymer-based hydrophilic–lipophilic balanced SPE cartridges (Oasis HLB). The cartridges were preconditioned with 5 mL of MeOH and 5 mL of mQ water at a flow rate of 2 mL min^{-1}. After the conditioning step, aliquots of 15 mL of sample (without pH adjustment) were loaded into the cartridge. Soft drink samples were passed through the cartridges at a flow rate of 3 mL min^{-1}. The retained analytes were eluted with 5 mL of MeOH at 1 mL min^{-1}. This eluate was then evaporated until near dryness by a gentle nitrogen stream and taken up with 500 μL of MeOH and 1000 μL of milli Q water (final preconcentration factor 1:10). Then this extract was filtered through a 0.45 μm PTFE filter (Millex FG, Millipore, Milford, MA, USA). For validation and quantitation purposes, matrix-matched standards were prepared by spiking the extracts with appropriate volume of working standard solutions of the studied analytes. For recovery studies, an orange-flavored soft drink sample was spiked before the SPE extraction procedure with the mixture of the studied fungicides at two concentration levels: 10 and 50 μg L^{-1}.

2.5. Chromatography

The separation of the species from the whole SPE soft drink extracts was carried out using an HPLC system (consisting of vacuum degasser, auto-sampler and a binary pump) (Agilent Series 1100, Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed phase C_{18} analytical column of 50 mm × 4.6 mm and 1.8 μm particle size (Zorbax Rapid Resolution Eclipse XDB-C18). 20 μL of soft drink extract was injected in each study. Mobile phases A and B were water with 0.1% formic acid and acetonitrile respectively. The chromatographic method held the initial mobile phase composition (10% B) constant for 1 min, followed by a linear gradient to 100% B at 11 min and held constant for 3 min at 100% B. The flow-rate used was 0.5 mL min^{-1}.

2.6. LC/electrospray time-of-flight mass spectrometry

The HPLC system was connected to a time-of-flight mass spectrometer Agilent MSD TOF (Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray interface operating in positive ion mode, using the following operation parameters: capillary voltage: 4000 V; nebulizer pressure: 40 psig; drying gas: 9 L min^{-1}; gas temperature: 325 °C; skimmer voltage: 60 V; octapole DC 1: 37.5 V; octapole RF: 250 V; fragmentor voltage (in-source CID fragmentation): 190 V. LC/MS accurate mass spectra were recorded across the range 50–1000 *m/z*. Accurate mass measurements of each peak from the total ion chromatograms were obtained by means of an automated calibrant delivery system using a dual-nebulizer electrospray source that introduces the flow from the outlet of the chromatograph together with a low flow of a calibrating solution (calibrant solution A, Agilent Technologies), which contains the internal reference masses (purine ($C_5H_4N_4$ at *m/z* 121.050873 and HP-921 [hexakis-(1H,1H,3H-tetrafluoropentoxy)-phosphazene] ($C_{18}H_{18}O_6N_3P_3F_{24}$) at *m/z* 922.009798). Besides, a software package is auto-calibrating and recording continuously the results of the internal reference masses along with the raw data. The instrument worked providing a typical resolution of 9700 ± 500 (*m/z* 922). The full-scan data recorded were processed with Applied Biosystems/MDS-SCIEX Analyst QS software (Frankfurt, Germany) with accurate mass application-specific additions from Agilent MSD TOF software.

Table 1
Fragmentation study on the selected 33 multi-class pesticides: effect of the fragmentor voltage on CID fragmentation.

Compound	Activity[a] (chemical class)[b]	Ion	Theoretical *m/z*	Elemental composition	Relative abundance (%)			
					160 V	190 V	230 V	250 V
Carbendazim	F (III)	$[M+H]^+$	192.0767	$C_9H_{10}N_3O_2$	100	100	9	–
		Frg. 1	160.0505	$C_8H_6N_3O$	17	71	100	100
		Frg. 2	132.0556	$C_7H_6N_3$	–	–	–	10
Thiabendazole	F (III)	$[M+H]^+$	202.0433	$C_{10}H_8N_3S$	100	100	100	100
		Frg. 1	175.0326	$C_9H_7N_2S$	–	3	10	35
Monocrotophos	A,I (IX)	$[M+Na]^+$	246.0501	$C_7H_{14}NO_5PNa$	100	100	55	57
		$[M+H]^+$	224.0682	$C_7H_{15}NO_5P$	82	19	–	–
		Frg. 1	193.0260	$C_6H_{10}O_5P$	17	15.4	–	–
		Frg. 2	127.0154	$C_2H_8O_4P$	23	83.4	100	100
		Frg. 3	109.0049	$C_2H_6O_3P$	2	5	24	54
		Frg. 4	98.0600	C_5H_8NO	14	43	16	9
Thiamethoxam	I (VIII)	$[M+Na]^+$	314.0085	$C_8H_{10}N_5O_3SClNa$	100	100	100	100
		$[M+H]^+$	292.0266	$C_8H_{11}N_4O_3SCl$	79	20	2	–
		Frg. 1	211.0648	$C_8H_{11}N_4OS$	47	69	20	10
		Frg. 2	181.0537	$C_7H_9N_4S$	7	12	20	17
		Frg. 3	152.0275	$C_6H_6N_3S$	4	8	20	36
		Frg. 4	131.9669	$C_4H_3N_5Cl$	17	26	20	23
		Frg. 5	122.0712	$C_6H_8N_3$	2	3	9	16
Imazalil metabolite		$[M+H]^+$	257.0242	$C_{11}H_{11}N_2OCl_2$	100	100	100	100
Imidacloprid	I (VIII)	$[M+Na]^+$	278.0415	$C_9H_{10}N_5O_2ClNa$	31	91	5	–
		$[M+H]^+$	256.0596	$C_9H_{11}N_5O_2Cl$	100	100	78	49
		Frg. 1	210.0666	$[C_9H_{11}N_4Cl]^{+\bullet}$	9	45	14	10
		Frg. 2	209.0589	$C_9H_{11}N_5O_2Cl$	8	44	51	60
		Frg. 3	175.0978	$C_9H_{11}N_5O_2Cl$	12	73	100	100
		Frg. 4	126.0105	$C_9H_{11}N_5O_2Cl$	–	7	7	9
		Frg. 5	84.0556	$C_9H_{11}N_5O_2Cl$	–	4	7	6
Dimethoate	A,I,N (IX)	$[M+Na]^+$	251.9888	$C_5H_{12}NO_3PS_2Na$	100	10	–	–
		$[M+H]^+$	230.0075	$C_5H_{13}NO_3PS_2$	77	100	45	22.4
		Frg. 1	198.9647	$C_4H_8O_3PS_2$	41	24	–	–
		Frg. 2	170.9698	$C_3H_8O_2PS_2$	11	25	5	–
		Frg. 3	156.9541	$C_2H_6O_2PS$	3	8	7	–
		Frg. 4	142.9926	$C_2H_8O_3PS$	–	6	6	–
		Frg. 5	124.9821	$C_2H_6O_2PS$	28	66	100	100
		Frg. 6	93.0099	$C_2H_6O_2P$	–	–	8	32
		Frg. 7	88.0219	C_3H_6NS	10	12	3	–
		Frg. 8	78.9943	CH_4O_2S	–	–	15	46
Acetamiprid	I (VIII)	$[M+Na]^+$	245.0564	$C_{10}H_{11}N_4ClNa$	49	52	100	81
		$[M+H]^+$	223.0745	$C_{10}H_{12}N_4Cl$	100	100	21	6
		Frg. 1	126.0105	C_6H_5NCl	8	30	98	100
		Frg. 2	98.9996	C_5H_4Cl	–	–	16	37
		Frg. 3	72.9839	C_3H_2Cl	–	–	4	24
Butocarboxim	A,I (IV)	$[M+Na]^+$	213.0668	$C_7H_{14}N_2O_2SNa$	100	100	48	10
		Frg. 1	116.0528	$C_5H_{10}NS$	–	5	11	7
		Frg. 2	100.0215	C_4H_6NS	2	5	24	54
		Frg. 3	75.0262	C_3H_7S	–	–	5	8
Thiacloprid	I,M (VIII)	$[M+Na]^+$	275.0128	$C_{10}H_9N_4ClSNa$	–	44	79	75
		$[M+H]^+$	253.0309	$C_{10}H_{10}N_4ClS$	100	100	22	6
		Frg. 1	126.0105	C_6H_5NCl	3	9	100	100
		Frg. 2	98.9996	C_5H_4Cl	–	–	12	25
		Frg. 3	90.0338	C_6H_4N	–	–	7	15
		Frg. 4	72.9839	C_3H_2Cl	–	–	7	15
Prochloraz metabolite		$[M+H]^+$	282.0213	$C_{11}H_{15}NOCl_3$	100	100	100	100
Imazalil	F (V)	$[M+H]^+$	297.0555	$C_{14}H_{15}N_2OCl_2$	100	100	100	100
		Frg. 1	255.0086	$C_{11}H_9N_2OCl_2$	–	1	4	12
		Frg. 2	158.9763	$C_7H_5Cl_2$	–	1	9	37
		Frg. 3	109.0760	$C_6H_9N_2$	–	1	13	44
		Frg. 4	81.0447	$C_4H_5N_2$	–	–	–	5
		Frg. 5	69.0447	$C_3H_5N_2$	–	1	7	30
Pyrimethanil	F (XI)	$[M+H]^+$	200.1182	$C_{12}H_{14}N_3$	100	100	100	100
		Frg. 1	183.0916	$C_{11}H_{11}N_2$	–	–	5	13
Carbofuran	A,I,N (IV)	$[M+Na]^+$	244.0944	$C_{12}H_{15}NO_3Na$	60	68	69	41
		$[M+H]^+$	222.1125	$C_{12}H_{16}NO_3$	100	38	–	–
		Frg. 1	165.0910	$C_{10}H_{13}O_2$	33	100	42	9
		Frg. 2	123.0441	$C_7H_7O_2$	9	29	100	100
Spiroxamine	F (0)	$[M+H]^+$	298.2741	$C_{18}H_{36}NO_2$	100	100	100	86
		Frg. 1	144.1382	$C_8H_{18}NO$	3	8	56	100
		Frg. 2	100.1121	$C_6H_{14}N$	–	3	18	62
Metalaxyl	F (I)	$[M+Na]^+$	302.1362	$C_{15}H_{21}NO_4Na$	100	100	100	100
		$[M+H]^+$	280.1543	$C_{15}H_{22}NO_4$	50	63	6	–
		Frg. 1	248.1281	$C_{14}H_{18}NO_3$	8	4	2	–
		Frg. 2	220.1332	$C_{13}H_{18}NO_2$	18	6	19	11
		Frg. 3	192.1383	$C_{12}H_{18}NO$	10	3	21	15

Table 1 (*Continued*)

Compound	Activity[a] (chemical class)[b]	Ion	Theoretical *m/z*	Elemental composition	Relative abundance (%)			
					160 V	190 V	230 V	250 V
Diuron	AI,H (X)	$[M+Na]^+$	255.0062	$C_9H_{10}N_2OCl_2Na$	16	16	8	2
		$[M+H]^+$	233.0243	$C_9H_{11}N_2OCl_2$	100	100	36	9
		Frg. 1	72.0444	C_3H_6NO	4	20	100	100
Spinosad	I (II)	[c]$[M+H]^+$	732.4681	$C_{41}H_{66}NO_{10}$	100	100	100	100
[c]Spinosyn A		Frg. 1	544.3633	$C_{32}H_{50}NO_6$	4	4	3	3
[d]Spinosyn D		Frg. 2	142.1226	$C_8H_{16}NO$	–	–	2	4
		[d]$[M+H]^+$	746.4838	$C_{42}H_{68}NO_{10}$	100	100	100	100
		Frg. 1	558.3789	$C_{33}H_{52}NO_6$	5	5	4	3
		Frg. 2	142.1226	$C_8H_{16}NO$	–	–	3	5
Dimethomorph	F	$[M+Na]^+$	410.1129	$C_{21}H_{22}NO_4ClNa$	22	33	57	94
(Z + E isomers)	(VII)	$[M+H]^+$	388.1210	$C_{21}H_{23}NO_4Cl$	100	100	100	100
		Frg. 1	301.0626	$C_{17}H_{13}O_3Cl$	–	4	17	41
Prochloraz	F	$[M+H]^+$	376.0380	$C_{15}H_{17}N_3O_2Cl_3$	100	42	3	1
	(I)	Frg. 1	308.0006	$C_{12}H_{13}NO_2Cl_3$	25	100	100	100
		Frg. 2	265.9536	$C_9H_7NO_2Cl_3$	1	6	26	88
Cyproconazole	F	$[M+H]^+$	292.1211	$C_{15}H_{19}N_3OCl$	100	100	70	14
	(V)	Frg. 1	125.0153	C_7H_6Cl	–	3	28	31
		Frg. 2	70.0401	$C_2H_4N_3$	3	9	100	100
Methiocarb	A,B,I,M (IV)	$[M+Na]^+$	248.0715	$C_{11}H_{15}NO_2Na$	57	40	100	100
		$[M+H]^+$	226.0896	$C_{11}H_{16}NO_2$	96	8	–	–
		Frg. 1	169.0896	$C_9H_{13}OS$	100	100	61	63
Azoxystrobin	F (XII)	$[M+Na]^+$	426.1060	$C_{22}H_{17}N_3O_5Na$	48	78	83	100
		$[M+H]^+$	404.1241	$C_{22}H_{18}N_3O_5$	100	100	16	4
		Frg. 1	372.0979	$C_{21}H_{14}N_3O_4$	14	48	100	89
Triflumizole	F	$[M+H]^+$	346.0929	$C_{15}H_{16}N_3OF_3Cl$	100	27	23	9
	(V)	Frg. 1	278.0554	$C_{12}H_{12}NOF_3Cl$	40	100	100	30
		Frg. 2	69.0447	$C_3H_5N_2$	–	5	91	100
Malathion	A,I (IX)	$[M+Na]^+$	353.0252	$C_{10}H_{19}O_6PS_2Na$	100	100	100	100
		$[M+H]^+$	331.0433	$C_{10}H_{20}O_6PS_2$	60	17	–	–
		Frg. 1	285.0015	$C_8H_{14}O_5PS_2$	16	19	–	–
		Frg. 2	257.0066	$C_7H_{14}O_4PS_2$	3	9	3	–
		Frg. 3	127.0389	$C_6H_7O_3$	6	18	3	–
		Frg. 4	124.9821	$C_2H_6O_2PS_2$	9	11	20	23
		Frg. 6	99.0077	$C_4H_3O_3$	11	33	43	26.7
Chlorfenvinphos	A,I (IX)	$[M+Na]^+$	380.9587	$C_{12}H_{14}O_4PCl_3Na$	47	84	46	42
		$[M+H]^+$	358.9768	$C_{12}H_{15}O_4PCl_3$	100	100	4	–
		Frg. 1	330.9819	$C_{11}H_{15}O_3PCl_3$	3	6	–	–
		Frg. 2	204.9373	$C_8H_4Cl_3$	6	22	21	15
		Frg. 3	169.9684	$[C_8H_4Cl_2]^{+\bullet}$	2	8	13	21
		Frg. 4	155.0468	$C_4H_{12}O_4P$	20	54	17	4
		Frg. 5	127.0155	$C_2H_8O_4P$	5	23	19	6
		Frg. 6	98.9841	H_4O_4P	14	60	100	100
		Frg. 7	80.9736	H_2O_3P	4	17	17	28
Difenoconazole	F	$[M+H]^+$	406.0720	$C_9H_{18}N_3O_3Cl_2$	100	100	100	100
	(V)	Frg. 1	337.0392	$C_{17}H_{15}O_3Cl_2$	–	2	10	23
		Frg. 2	251.0024	$C_{13}H_9OCl_2$	1	4	20	68
Benalaxyl	F	$[M+Na]^+$	348.1570	$C_{20}H_{23}NO_3Na$	58	95	100	100
	(I)	$[M+H]^+$	326.1751	$C_{20}H_{24}NO_3$	100	100	11	–
		Frg. 1	294.1489	$C_{19}H_{20}NO_2$	5	15	7	–
		Frg. 2	208.1332	$C_{12}H_{18}NO_2$	7	26	42	16
		Frg. 3	148.1121	$C_{10}H_{14}N$	5	21	70	76
		Frg. 4	121.0886	$C_8H_{11}N$	–	7	23	35
Buprofezin	I	$[M+H]^+$	306.1634	$C_{16}H_{24}N_3OS$	100	100	48	4
	(VI)	Frg. 1	264.1165	$C_{13}H_{18}N_3OS$	–	–	5	5
		Frg. 2	250.1008	$C_{12}H_{16}N_3OS$	1	4	25	5
		Frg. 3	208.0539	$C_9H_{10}N_3OS$	–	1	21	13
		Frg. 4	201.1056	$C_9H_{17}N_2OS$	7	25	76	11
		Frg. 5	145.0430	$C_5H_9N_2OS$	1	2	27	8
		Frg. 6	116.0528	$C_5H_{10}NS$	–	1	7	1
		Frg. 7	106.0651	C_7H_8N	1	4	100	100
		Frg. 8	105.0447	$C_6H_5N_2$	–	1	11	13
		Frg. 9	86.0600	C_4H_8NO	–	1	12	6
		Frg. 10	59.9902	CH_2NS	–	–	5	7
		Frg. 11	57.0698	C_4H_9	–	–	1	6
Hexaflumuron	I	$[M+Na]^+$	482.9708	$C_{16}H_8N_2O_3F_6Cl_2Na$	36	49	25	13
	(VI)	$[M+H]^+$	460.9888	$C_{16}H_9N_2O_3F_6Cl_2$	100	100	19.5	4
		Frg. 1	158.0412	$C_7H_6NOF_2$	10	32.5	100	100
Diazinon	A,B,I (IX)	$[M+H]^+$	305.1083	$C_{12}H_{22}N_2O_3PS$	100	100	100	56
		Frg. 1	169.0794	$C_{12}H_{22}N_2O_3PS$	2	5	45	100
		Frg. 2	153.1021	$C_{12}H_{22}N_2O_3PS$	1	3	25	54
Pyriproxyfen	I (VI)	$[M+H]^+$	322.1438	$C_{20}H_{20}NO_3$	100	100	15	–
		Frg. 1	227.1067	$C_{15}H_{20}O_2$	3	17	52	17
		Frg. 2	185.0597	$C_{12}H_9O_2$	1	5	33	32

Table 1 (*Continued*)

Compound	Activity[a] (chemical class)[b]	Ion	Theoretical *m/z*	Elemental composition	Relative abundance (%)			
					160 V	190 V	230 V	250 V
		Frg. 3	134.0726	$C_9H_{10}O$	–	1	13	44
		Frg. 4	119.0491	C_8H_7O	–	2	12	11
		Frg. 5	96.0444	C_5H_6NO	6	39	100	100
		Frg. 6	78.0338	C_5H_4N	2	8	41	61
Spiromesifen	I (XIII)	$[M+Na]^+$	393.2036	$C_{23}H_{30}O_4Na$	37	40	47	63
		$[M+H]^+$	371.2271	$C_{23}H_{31}O_4$	9	5	–	1
		Frg. 1	295.1328	$C_{17}H_{21}O_3Na$	2	5	33	100
		Frg. 2	273.1485	$C_{17}H_{21}O_3$	100	100	100	97
		Frg. 3	227.1430	$C_{16}H_{19}O$	1	2	11	23
		Frg. 4	211.1481	$C_{16}H_{19}$	–	1	5	10
		Frg. 5	209.1324	$C_{16}H_{17}$	–	1	8	21
		Frg. 6	199.1481	$C_{15}H_{19}$	–	1	6	15
		Frg. 7	187.0753	$C_{12}H_{11}O_2$	1	2	8	14
		Frg. 8	131.0855	$C_{10}H_{11}$	–	1	5	17

[a] A: acaricide, Al: algicide, B: bird repellent, F: fungicide, H: herbicide, I: insecticide, M: molluscicide, N: nematicide.
[b] 0: spiroketalamine group, I: amide, II: antibiotic insecticide (subclass spynosin), III: benzimidazole, IV: carbamate, V: conazole, VI: chitin synthesis inhibitor/insect growth regulator, VII: morpholine, VIII: nicotinoid, IX: organophosphorous, X: phenylurea, XI: pyrimidine, XII: antibiotic fungicide (subclass strobilurin), XIII: tetronic acid.
[c] Spinosad isomer 1: Spinosyn A.
[d] Spinosad isomer 2: Spinosyn D.

3. Results and discussion

3.1. Identification and confirmation of the targeted pesticides by LC–electrospray TOFMS: in-source CID fragmentation and accurate mass measurements

Standard electrospray ionization conditions were selected to achieve the best possible sensitivity and selectivity for the selected compounds. Standard values were set for drying and nitrogen flow rates, vaporizer and drying temperatures, and capillary voltage. Besides the typical electrospray parameters, the parameter associated with in-source CID fragmentation which had a strong influence on the sensitivity and relative abundance of protonated molecules as described elsewhere [24] were carefully studied. Table 1 shows the fragmentation of the studied pesticides and the relative abundances of the different species formed. The fragmentor voltage is the parameter that establishes the extent in which in-source CID fragmentation is carried out. There values are usually tested with the instrument used: 160 V (mild conditions), 190 V (medium fragmentation), 230 V (high fragmentation) and 250 V (extensive fragmentation). The extent of the fragmentation is primarily compound-dependent. For instance organophosphorus compounds such as monocrotophos, dimethoate, malathion or chlorfenvinphos yield several fragment ions even under mild conditions, while other compounds such as imazalil or pyrimethanil are difficult to cleave unless a high fragmentor voltage is applied. The highest fragmentor voltage value (250 V) gave extensive fragmentation of the protonated molecules in most cases. Only 7 out of 33 compounds still presented the protonated molecule as base peak under these circumstances. On the contrary, 160 V produced little or no fragmentation, so no additional structure information could be achieved for unambiguous confirmation of the target species. For this reason, the fragmentor voltage was set at 190 V, as a compromise value between sensitivity for quantitation and additional mass spectrum information for confirmation purposes.

Despite in-source CID is not as effective and specific selective as CID in a collision chamber, performing real MS/MS experiments, it is an interesting feature to add specific analyte information for unambiguous confirmation of the positive findings. Using the selected conditions, 25 out of 33 compounds (76%) gave useful fragmentation. It should be noted that the primary identification of the targeted species is performed by retention time matching and accurate mass measurements of the main characteristic ion with accuracy typically better than 3 ppm. In-source CID was characterized for complementary tool for confirmatory purposes. By using high resolution mass spectrometry data with high mass accuracies, as those shown in Table 2, unambiguous identification of the targeted species can be accomplished despite some of them might not have additional fragments ions.

Besides in-source CID fragmentation, a second feature that was explored as a tool for identification purposes was isotope profiles, i.e. the presence of chlorine or sulphur atoms in the targeted species. Considering this feature, 32 out of 33 compounds (except pyrimethanil) have either in-source CID or isotopic profile or both. This is a valuable indicator of the high degree of selectivity that can be obtained with LC–TOFMS instruments despite not real MS/MS experiments are done.

The identification of the targeted species was performed basically by retention time matching combined with accurate mass measurements of the targeted protonated molecules and, when available, their main fragment ions and or isotope signature (i.e. ^{37}Cl). In this sense, we notice that the combination of in-source CID and the comparison and evaluation of the theoretical and experimental isotope patterns (from the elemental composition of the species) are powerful tools for identification purposes in most of the targeted species. The accurate mass of characteristic isotopic signals, and the distance in the *m/z* axis between them can be combined by the software to provide a user-created weighted coefficient estimating how similar the experimental mass spectrum is when compared to that obtained with standards. Table 2 shows the results obtained for the accurate mass analysis of the selected pesticides in a fruit-based matrix-matched standard, spiked with 2 $\mu g\,L^{-1}$. From the data obtained, it can be concluded that the method offers a high degree of confirmation because of its very high mass accuracy, enabling accurate mass measurements of target ions within 2 ppm error in most cases.

For identification and quantitation purposes, we used extracted ion chromatograms (XICs) using a mass-window width of 20 mDa ($[M+H]^+ \pm 10$ mDa). The protonated molecule ($[M+H]^+$) was used for both confirmation and quantitation purposes for most of the species except when the relative intensity of a sodium adduct ($[M+Na]^+$) (e.g. thiametoxam) or characteristic common fragment ion (e.g. prochloraz) was higher than that of the protonated molecule in the selected conditions. As an example, Fig. 1 shows the total ion chromatogram obtained in the LC–TOFMS analysis of a soft drink extract spiked with 2 and 10 $\mu g\,L^{-1}$, together with

Table 2
LC–TOFMS accurate mass measurements of the protonated molecules and the main fragment ions of the pesticides studied in a fruit-based soft drinks matrix-matched standard (fragmentor voltage 190 V). Spiking level: 2 μg L^{-1}.

Compound	RT (min)	Ion	Elemental compositions	Theoretical *m*/*z*	Experimental *m*/*z*	Error	
						mDa	ppm
Carbendazim	3.52	$[M+H]^+$	$C_9H_{10}N_3O_2$	192.0767	192.0763	−0.45	2.4
		Fragment 1	$C_8H_6N_3O$	160.0505	160.0504	−0.13	0.86
Thiabendazole	4.37	$[M+H]^+$	$C_{10}H_8N_3S$	202.0433	202.0427	0.25	1.3
		^{34}S ion	$C_{10}H_8N_3{}^{34}S$	204.0391	204.0397	−0.56	2.7
Monocrotophos	4.91	$[M+Na]^+$	$C_7H_{15}NO_5PNa$	246.0501	246.0499	−0.28	1.1
		Fragment 1	$C_2H_8O_4P$	127.0154	127.0156	0.13	1.0
Thiamethoxam	5.53	$[M+H]^+$	$C_8H_{11}N_5O_3SCl$	292.0265	292.0264	−0.16	0.56
		Fragment 1	$C_4H_3N_5Cl$	131.9669	131.9669	−0.025	0.19
Imazalil metabolite	5.87	$[M+H]^+$	$C_{11}H_{11}N_2OCl_2$	257.0242	257.0246	0.41	1.5
		^{37}Cl ion	$C_{11}H_{11}N_2OCl\ ^{37}Cl$	259.0213	259.0210	0.35	1.1
Imidacloprid	6.27	$[M+H]^+$	$C_9H_{11}N_5O_2Cl$	256.0596	256.0595	−0.08	0.31
		Fragment 1	$C_9H_{11}N_4$	175.0978	175.0983	0.37	2.1
Dimethoate	6.44	$[M+H]^+$	$C_5H_{13}NO_3PS_2$	230.0069	230.0072	0.30	1.3
		Fragment 1	$C_4H_8O_3PS_2$	198.9647	198.9652	0.50	2.5
		Fragment 2	$C_2H_6O_2PS$	124.9821	124.9819	−0.16	1.3
Acetamiprid	6.55	$[M+H]^+$	$C_{10}H_{12}N_4Cl$	223.0745	223.0746	0.1	0.44
		Fragment 1	C_6H_5NCl	126.0105	126.0102	−0.3	2.4
Butocarboxim	7.08	$[M+Na]^+$	$C_7H_{14}N_2O_2SNa$	213.0665	213.0666	0.059	0.27
		Fragment 1	C_3H_7S	75.0263	75.0266	0.30	4.0
Thiacloprid	7.18	$[M+H]^+$	$C_{10}H_{10}N_4ClS$	253.0309	253.0311	0.18	0.7
		Fragment 1	C_6H_5ClN	126.0105	126.0104	−0.10	0.8
Prochloraz	7.28	$[M+H]^+$	$C_{11}H_{15}NOCl_3$	282.0213	282.0209	−0.47	1.6
Metabolite		^{37}Cl ion	$C_{11}H_{15}NOCl_2\ ^{37}Cl$	284.0184	284.0181	−0.32	1.1
Imazalil	7.41	$[M+H]^+$	$C_{14}H_{15}N_2OCl_2$	297.0555	297.0562	0.61	2.0
		^{37}Cl ion	$C_{14}H_{15}N_2OCl\ ^{37}Cl$	299.0526	299.0530	0.35	1.2
Pyrimethanil	8.24	$[M+H]^+$	$C_{12}H_{14}N_3$	200.1182	200.1185	0.28	1.1
Spiroxamine	8.29	$[M+H]^+$	$C_{18}H_{36}NO_2$	298.2741	298.2744	0.34	1.2
		Fragment 1	$C_8H_{18}NO$	144.1382	144.1380	−0.3	2.0
Carbofuran	8.30	$[M+H]^+$	$C_{12}H_{16}NO_3$	222.1124	222.1126	0.13	0.58
		Fragment 1	$C_{10}H_{13}O_2$	165.0910	165.0912	0.19	1.2
		Fragment 2	$C_7H_7O_2$	123.0440	123.0441	0.044	0.35
Metalaxyl	8.74	$[M+H]^+$	$C_{15}H_{22}NO_4$	280.1543	280.1547	−0.32	1.1
		Fragment 1	$C_{13}H_{18}NO_2$	220.1332	220.1329	−0.30	1.4
Diuron	8.77	$[M+H]^+$	$C_{14}H_{15}N_2OCl_2$	233.0242	233.0246	0.30	1.3
		^{37}Cl ion	$C_{14}H_{15}N_2OCl\ ^{37}Cl$	235.0213	235.0216	0.25	1.1
Spinosad							
Spinosyn A	9.16	$[M+H]^+$	$C_{41}H_{66}NO_{10}$	732.4681	732.4686	0.48	0.67
		Fragment 1	$C_{32}H_{50}NO_6$	544.3632	544.3630	−0.26	0.48
Spinosyn D	9.53	$[M+H]^+$	$C_{42}H_{68}NO_{10}$	746.4837	746.4832	−0.57	0.77
		Fragment 1	$C_{33}H_{52}NO_6$	558.3789	558.3787	−0.21	0.38
Dimethomorph	9.24/9.42	$[M+H]^+$	$C_{21}H_{23}NO_4Cl$	388.1310	388.1313	0.27	0.69
		Fragment 1	$C_{17}H_{13}O_3Cl$	301.0626	301.0625	−0.09	0.32
Prochloraz	9.45	$[M+H]^+$	$C_{15}H_{19}N_3OCl$	376.0380	376.0383	0.38	1.3
		Fragment	$C_{12}H_{13}NO_2Cl_3$	308.0010	308.0012	0.13	0.4
Cyproconazole	9.66	$[M+H]^+$	$C_{15}H_{19}N_3OCl$	292.1211	292.1215	0.38	1.3
		Fragment	$C_2H_4N_3$	70.0401	70.0400	0.026	0.38
Methiocarb	9.70	$[M+Na]^+$	$C_{11}H_{15}NO_2SNa$	248.0715	248.0716	0.028	0.11
		$[M+H]^+$	$C_{11}H_{16}NO_2S$	226.0896	226.0894	−0.23	1.0
		Fragment 1	$C_9H_{13}OS$	169.0682	169.0681	−0.063	0.37
Azoxystrobin	10.00	$[M+H]^+$	$C_{22}H_{18}N_3O_5$	404.1241	404.1243	0.20	0.50
		Fragment 1	$C_{21}H_{14}N_3O_4$	372.0979	372.0978	−0.083	0.22
Triflumizol	10.52	$[M+H]^+$	$C_{15}H_{16}N_3OClF_3$	346.0928	346.0932	0.35	1.0
		Fragment 1	$C_{12}H_{12}NOClF_3$	278.0554	278.0560	0.60	2.1
Malathion	10.60	$[M+Na]^+$	$C_{10}H_{19}O_6PS_2Na$	353.0252	353.0256	0.31	0.87
		$[M+H]^+$	$C_{10}H_{20}O_6PS_2$	331.0433	331.0431	−0.25	0.75
		Fragment 1	$C_8H_{14}O_5PS_2$	285.0014	285.0021	0.61	2.1
		Fragment 2	$C_6H_7O_2$	127.0389	127.0391	0.13	1.0
Chlorfenvinphos	11.00	$[M+Na]^+$	$C_{12}H_{14}O_4{}^{35}Cl_3$ PNa	380.9587	380.9593	0.55	1.4
		$[M+H]^+$	$C_{12}H_{15}O_4{}^{35}Cl_3$ P	358.9768	358.9771	0.29	0.81
		$Cl_2{}^{37}Cl$ ion	$C_{12}H_{15}O_4{}^{35}Cl_2\ ^{37}Cl$ P	360.9738	360.9743	0.48	1.3
Difenoconazole	11.15	$[M+H]^+$	$C_{19}H_{18}N_3O_3Cl_2$	406.0719	406.0726	0.62	1.5
		$Cl^{37}Cl$ ion	$C_{19}H_{18}N_3O_3{}^{35}Cl\ ^{37}Cl$	408.0690	408.0698	0.77	1.9
Benalaxyl	11.23	$[M+Na]^+$	$C_{20}H_{23}NO_3Na$	348.1570	348.1576	0.58	1.7
		$[M+H]^+$	$C_{20}H_{24}NO_3$	326.1751	326.1756	0.53	1.6
		Fragment 1	$C_{12}H_{18}NO_2$	208.1331	208.1332	−0.10	0.50
Buprofezin	11.44	$[M+H]^+$	$C_{16}H_{24}N_3OS$	306.1634	306.1639	0.44	1.4
		Fragment 1	$C_9H_{17}N_2OS$	201.1056	201.1058	0.19	0.94
Hexaflumuron	11.51	$[M+Na]^+$	$C_{16}H_8N_2O_3F_6Cl_2Na$	482.9708	482.9702	−0.63	1.3
		$[M+H]^+$	$C_{16}H_9N_2O_3F_6Cl_2$	460.9888	460.9896	0.70	1.5
Diazinon	11.64	$[M+H]^+$	$C_{12}H_{22}N_2O_3PS$	305.1083	305.1083	0.47	1.5
		Fragment 1	$C_8H_{13}N_2S$	169.0793	169.0799	0.50	3.0
		Fragment 2	$C_8H_{13}N_2O$	153.1022	153.1023	0.06	0.4

Table 2 (*Continued*)

Compound	RT (min)	Ion	Elemental compositions	Theoretical *m/z*	Experimental *m/z*	Error	
						mDa	ppm
Pyriproxyfen	12.61	$[M+H]^+$	$C_{20}H_{20}NO_3$	322.1437	322.1440	0.23	0.71
		Fragment 1	$C_{15}H_{12}O_2$	227.1066	227.1071	0.44	1.9
		Fragment 2	C_5H_6NO	96.0443	96.0445	0.11	1.1
Spiromesifen	13.36	$[M+Na]^+$	$C_{23}H_{30}O_{10}Na$	393.2038	393.2036	0.17	0.43
		$[M+H]^+$	$C_{23}H_{31}O_{10}$	371.2217	371.2216	−0.086	0.23
		Fragment 1	$C_{17}H_{21}O_3$	273.1485	273.1483	0.38	1.4

the extracted ion chromatograms (XICs) for some of the studied species.

The separation of the targeted species was achieved in less than 14 min, obtaining satisfactory resolution with average peak widths of 10 s, which compares well against the typical analytical columns usually 20–40 s average of peak width at baseline). Compared to the previously reported method [4], the use of small particle size column provides several advantages: (1) the total run time is reduced over 2-fold (from 47 min to less than 20 min including cleaning and re-equilibration of the column); (2) the use of organic solvent (acetonitrile) is minimized *ca.* 60%, being therefore more environmentally friendly; (3) the average base-line peak width is reduced 2-fold, which involves an increase in analyte S/N ratio at low concentrations, thus improving the limits of detection of the method. Finally, we should note that the use of this type of columns is fully compatible with non-high pressure systems. Therefore, these advantages can be exploited using a conventional HPLC instrumentation, because the operating pressure of the columns is typically less than 100 bars.

3.2. Sample treatment and recovery studies

From previous experience on the same matrix, and problems related with the matrix effects and instrument cleaning, the preconcentration factor was set at 10:1 [4], with 15 mL of sample loaded in the SPE cartridge. Preconcentration factors of 25:1 or higher involved complex extracts that yielded signal/sensitivity losses and soiled the MS inlet, being necessary a dedicated and daily cleaning and maintenance of the source. In addition, when using large sample volumes and preconcentration factors, matrix effects were remarkable (over 40% suppression in most of the studied analytes). In contrast, the use of preconcentration factors of 10:1 (or lower) did not affect strongly the sensitivity and signal stability of the MS source, over large periods of operation. The matrix effects (see next section) were also minimized using this approach.

Alternatively to the dilution of the extracts, a clean-up step could have been added to the method, but we considered that the sensitivity and detection limits of the method were enough for the application tested. Moreover, by avoiding the implementation of clean-up steps, the additional use of reagents (sorbents, solvents, etc.) is avoided being the protocol straightforward and environmentally friendly. This extraction method could be easily automated using a SPE–LC–MS assembly, thus increasing the throughput and automation degree of the procedure.

To evaluate the effectiveness of the extraction method, different recovery studies were carried out using an orange flavored soft drink sample, in which the presence of pesticides was examined to make sure that the matrix does not contain the studied analytes. Several soft drink aliquots were spiked at two different concentration levels (10 and 50 $\mu g\,L^{-1}$) with the working standard solution. These concentration levels were selected taking into consideration the concentration levels of the pesticides found in this kind of samples (up to 40 $\mu g\,L^{-1}$) [4] and the minimum MRLs values established for food. The spiked samples were centrifuged and extracted with the SPE method described. The obtained extracts were analyzed with the developed LC–MS method, obtaining recoveries between 64 and 123% for the 33 studied pesticides with RSD (%) below 10% in most cases, as can be seen in Table 3. These results show the feasibility of the studied extraction method for multi-residue pesticide analysis in fruit-based soft drinks.

3.3. Analytical performance

To evaluate the analytical features of the proposed method, calibration curves of the targeted 33 compounds were constructed at different concentrations, in the range 1–500 $\mu g\,L^{-1}$ using fruit-based soft drink extracts to prepare matrix-matched standards at 6–7 concentration levels (0.1–50 $\mu g\,L^{-1}$ in soft drink sample considering the SPE (10:1 preconcentration factor)). The results obtained are shown in Table 4 where the calibration curves are summarized together with the limits of quantitation, matrix effects and RSD (%). The linearity of the analytical response across the studied range is excellent, taking into account that the calibration curves of the analyzed fungicides showed correlation coefficients higher than 0.995 in most cases. The relative standard deviation (RSD) ($n=6$) values for run-to-run study were in the range 1.4–5.9%. Inter-day RSD ($n=5$) weekly values are typically in the range 5–15%. These results demonstrate the precision of the developed method and the potential of the proposed approach for quantitative purposes. The limits of quantitation (LOQs) were estimated as the minimum concentration of analyte corresponding to a signal-to-noise ratio (S/N) = 10:1. This was experimentally calculated from the injection of matrix-matched standard solutions at low concentration levels, using the more abundant ion for each compound based on the signal from high-resolution extracted ion chromatograms with narrow mass windows. The results obtained for each fungicide are also shown in Table 4. The LOQs obtained are as low as 0.02 $\mu g\,L^{-1}$ for diazinon and pyriproxyfen and below 0.5 $\mu g\,L^{-1}$ for most of the studied agrochemicals. Compared to the concentration levels that were found so far in the studied soft drinks samples (up to 30 $\mu g\,L^{-1}$ for an individual compound, and typically in the range 2–30 $\mu g\,L^{-1}$ [4]), the LOQs reported here are very satisfactory for the targeted application. We should keep in mind that there is no regulation available related to the maximum residue levels (MRL) authorized of pesticides in this kind of derivate products. The more stringent regulation available in the EU establishes a MRL of 10 $\mu g\,kg^{-1}$ for pesticide residues in baby food and for non-authorized compounds. Using this standard, the proposed method would fulfil the requirements.

Matrix effects which might have an important impact on the quality of the quantitative data generated by the method were also addressed. Matrix components can both reduce or enhance the signal given by the analytes when they achieve the detector. The problem is originated in the interface (source) when the matrix constituents influence the ionization of a coeluted analyte, causing ion suppression. The sample treatment protocol was designed aiming at minimizing the potential matrix effects, using a reduced preconcentration factor. To evaluate the impact of the

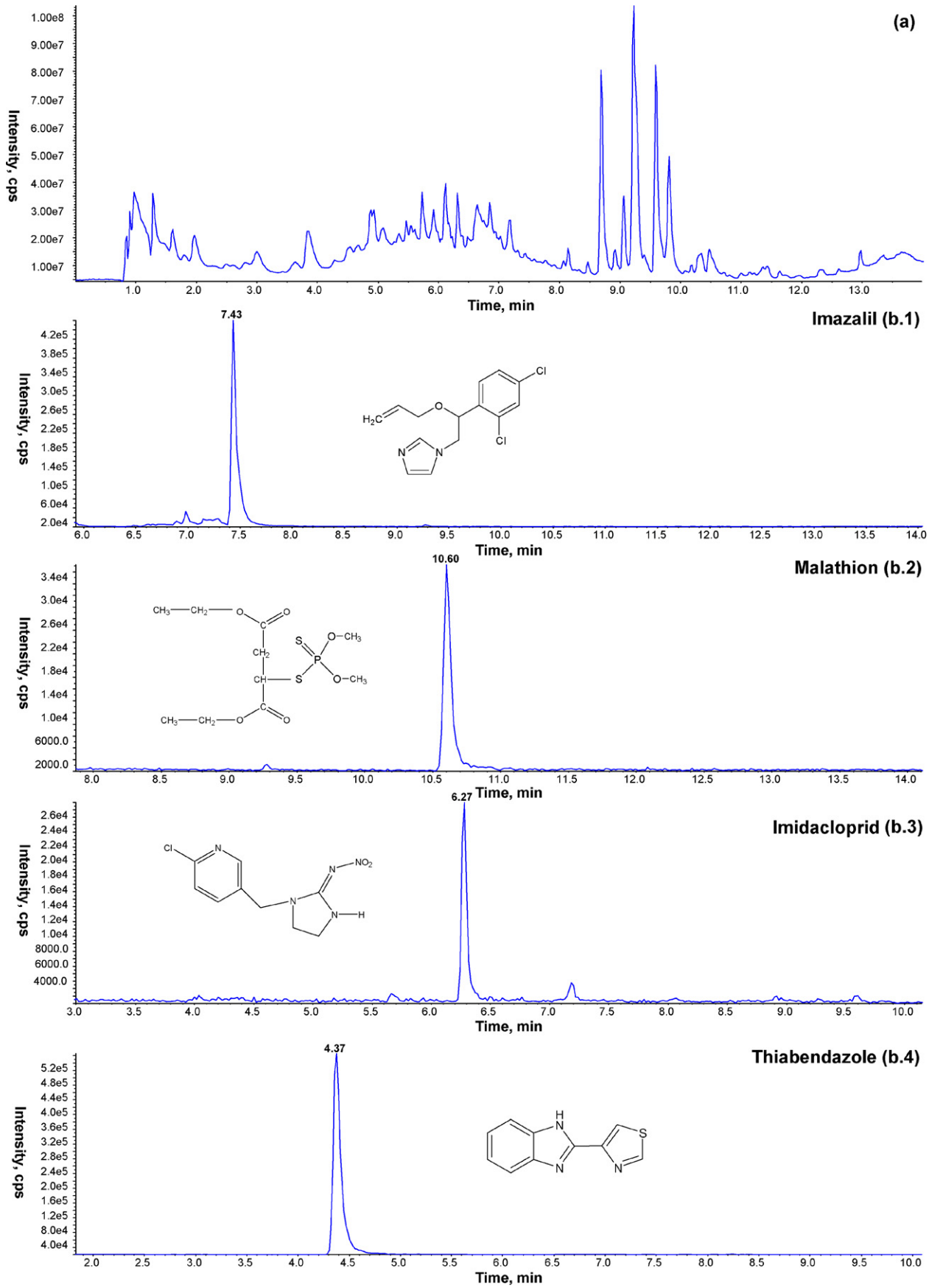

Fig. 1. (a) Total ion chromatogram of a fruit-based soft drink extract spiked with selected pesticides. (b) Extracted ion chromatograms. (b.1) Imazalil, 2 μg L^{-1}; 297.055 ± 0.01; (b.2) malathion, 2 μg L^{-1}; 353.025 ± 0.01; (b.3) imidacloprid, 10 μg L^{-1}; 256.060 ± 0.01; (b.4) thiabendazole, 10 μg L^{-1}; 202.045 ± 0.01.

Table 3
Recovery studies on fruit-based beverages extracts fortified with the pesticide mixture at two concentration levels: 10 and 50 μg L^{-1}.

Pesticide	Spiking level (μg L^{-1})	Recovery (%)	RSD (%)[a]
Carbendazim	10	101.5	8.5
	50	102.0	4.5
Thiabendazole	10	88.6	8.0
	50	104.7	3.4
Monocrotophos	10	102.8	7.5
	50	101.6	3.5
Thiametoxam	10	103.4	5.5
	50	93.8	4.6
Imazalil metabolite	10	78.6	7.5
	50	82.2	5.2
Imidacloprid	10	122.6	5.5
	50	84.2	6.2
Dimethoate	10	115.7	4.1
	50	96.2	4.1
Acetamiprid	10	66.5	3.9
	50	79.0	2.5
Butocarboxim	10	107.2	7.9
	50	100.8	4.1
Thiacloprid	10	101.2	4.8
	50	105.9	1.5
Prochloraz metabolite	10	72.8	5.7
	50	78.9	4.1
Imazalil	10	75.8	10.0
	50	96.9	3.6
Pyrimethanil	10	109.9	5.7
	50	101.2	6.5
Carbofuran	10	90.5	6.7
	50	100.5	2.9
Spiroxamine	10	101.7	9.1
	50	100.2	3.4
Metalaxyl	10	96.6	5.4
	50	110.2	3.3
Diuron	10	98.4	3.9
	50	95.0	4.8
Spinosad	10	83.9	8.5
(Spinosyn A + D)	50	92.1	5.6
Dimethomorph	10	100.0	10.9
(Z + E isomers)	50	122.9	2.6
Prochloraz	10	85.1	7.1
	50	93.2	4.7
Cyproconazole	10	94.2	4.6
	50	98.8	2.6
Methiocarb	10	96.5	8.9
	50	96.5	2.1
Azoxystrobin	10	83.9	7.3
	50	110.6	2.3
Triflumizol	10	71.2	10.7
	50	73.3	12.2
Malathion	10	88.1	4.9
	50	100.2	2.3
Chlorfenvinphos	10	84.9	5.0
	50	90.4	2.5
Difenoconazole	10	75.3	13.7
	50	79.9	4.2
Benalaxyl	10	97.0	7.7
	50	93.9	2.2
Buprofezin	10	101.7	5.9
	50	94.8	2.1
Hexaflumuron	10	64.3	11.5
	50	65.8	7.7
Diazinon	10	79.8	9.9
	50	65.0	2.2
Pyriproxyfen	10	72.8	5.7
	50	82.1	4.8
Spiromesifen	10	72.3	4.4
	50	68.1	5.6

[a] $n = 6$.

matrix on the ionization suppression/enhancement on the analytes (compared to neat standards), the slopes obtained in the calibration with matrix-matched standards were compared with those obtained with solvent-based standards, calculating slope ratios matrix/solvent for each of the 33 studied agrochemicals. The results are summarized in Table 4. Signal suppression equal or major than 25% only occurred in 4 out of the 33 compounds studied (12%), while over 60% of the compounds showed minimal matrix effects, lower than 15%. These values are low enough to provide accurate quantitative data if matrix-matched standard calibration curves

Table 4
Analytical parameters for the analysis of pesticides in fruit-based soft drink samples by LC–TOFMS method.

Compound	Conc. range tested ($\mu g\,L^{-1}$)	Regression equation	Matrix effect[a] (Δ %)	Linearity (r)	LOQ ($\mu g\,L^{-1}$)	RSD (%)[b] ($n=6$)
Carbendazim	1–50	$y=1.49\times10^3C-5.45\times10^2$	0.89 (−11)	0.99880	0.1	5.8
Thiabendazole	0.1–50	$y=5.07\times10^4C+5.24\times10^5$	0.87 (−13)	0.99870	0.05	4.6
Monocrotophos	2–50	$y=2.16\times10^3C+3.76\times10^4$	0.69 (−31)	0.99830	1.2	5.3
Thiametoxam	2–50	$y=1.09\times10^3C-6.33\times10^3$	0.77 (−23)	0.99985	1.6	5.9
Imazalil metabolite	0.1–50	$y=3.61\times10^4C+2.27\times10^5$	0.82 (−18)	0.99980	0.06	4.2
Imidacloprid	0.1–50	$y=2.99\times10^3C-6.24\times10^4$	0.61 (−39)	0.99760	0.8	3.4
Dimethoate	0.1–50	$y=1.09\times10^3C-3.13\times10^4$	0.83 (−17)	0.99690	1.2	6.7
Acetamiprid	0.1–50	$y=1.06\times10^4C-7.33\times10^4$	0.84 (−16)	0.99985	0.2	5.1
Butocarboxim	2–50	$y=4.52\times10^3C-6.81\times10^4$	0.68 (−32)	0.99980	2	4.7
Thiacloprid	0.1–50	$y=1.02\times10^4C-1.79\times10^4$	1.07 (+7)	0.99995	0.3	2.8
Prochloraz metabolite	0.1–50	$y=3.03\times10^4C-1.28\times10^4$	0.97 (−3)	0.99999	0.04	3.3
Imazalil	0.1–50	$y=5.29\times10^4C+1.00\times10^6$	0.84 (−16)	0.99514	0.03	2.1
Pyrimethanil	0.1–50	$y=1.26\times10^5C+7.86\times10^4$	0.88 (−12)	0.99549	0.05	1.9
Spiroxamine	0.1–50	$y=1.26\times10^5C+2.00\times10^6$	0.78 (−22)	0.99212	0.03	1.4
Carbofuran	0.1–50	$y=7.20\times10^3C+1.18\times10^5$	0.98 (−2)	0.99725	0.2	4.1
Metalaxyl	0.1–50	$y=2.70\times10^4C+3.75\times10^5$	0.93 (−7)	0.99855	0.05	2.1
Diuron	0.1–50	$y=1.07\times10^4C+5.29\times10^3$	0.90 (−10)	1.00000	0.2	3.2
Spinosad	0.1–50	$y=4.77\times10^4C-2.54\times10^5$	0.84 (−16)	0.99980	0.07	1.9
Dimethomorph	0.1–50	$y=1.69\times10^4C+3.36\times10^5$	0.78 (−22)	0.99975	0.5	2.6
Prochloraz	0.1–50	$y=2.99\times10^4C-3.21\times10^5$	1.01 (+1)	0.99941	0.03	2.9
Cyproconazole	0.1–50	$y=5.67\times10^4C-4.26\times10^4$	0.90 (−10)	0.99960	0.07	3.1
Methiocarb	0.1–50	$y=1.05\times10^3C+1.27\times10^4$	1.01 (+1)	0.99965	0.1	3.2
Azoxystrobin	0.1–50	$y=2.61\times10^4C+4.64\times10^5$	0.96 (−4)	0.99710	0.08	2.0
Triflumizol	0.1–50	$y=3.62\times10^3C+5.94\times10^3$	0.61 (−39)	0.99995	0.1	2.7
Malathion	0.1–50	$y=3.23\times10^3C+5.01\times10^4$	1.05 (+5)	0.99815	0.05	3.1
Chlorfenvinphos	1–50	$y=9.90\times10^3C+2.80\times10^4$	0.93 (−7)	0.99970	0.2	4.6
Difenoconazole	0.1–50	$y=3.93\times10^4C+4.06\times10^5$	0.99 (−1)	0.99649	0.05	2.9
Benalaxyl	0.1–50	$y=5.43\times10^4C+2.45\times10^5$	0.96 (−4)	0.99875	0.1	2.8
Buprofezin	0.1–50	$y=1.62\times10^5C+1.00\times10^6$	1.00 (0)	0.99358	0.05	3.0
Hexaflumuron	2–50	$y=1.23\times10^3C+5.10\times10^3$	0.96 (−4)	0.99915	1.5	5.1
Diazinon	0.1–50	$y=2.14\times10^5C+3.00\times10^6$	0.79 (−21)	0.98919	0.02	2.6
Pyriproxyfen	0.1–50	$y=2.49\times10^5C+5.91\times10^4$	1.04 (+4)	0.99965	0.02	1.7
Spiromesifen	0.1–50	$y=3.92\times10^4C+5.41\times10^4$	1.17 (+17)	0.99880	0.1	3.7

[a] Ratio: matrix-matched calibration slope/solvent calibration slope.
[b] Concentration level: 20 $\mu g\,L^{-1}$.

were used throughout the study to minimize errors due to matrix effects. Further dilution of the extracts would further minimize the matrix effects although the method detection limits would be affected by the dilution factor applied, approximately.

3.4. Determination of pesticides in market-purchased fruit-based soft drink samples

The proposed method was applied to the analysis of 14 market-purchased fruit-based soft drinks samples collected from different European countries. As an example, Fig. 2 shows the analysis of orange-flavored fruit-based soft drink sample, which contained both thiabendazole (0.60 $\mu g\,L^{-1}$) and imazalil (3.2 $\mu g\,L^{-1}$). The positive findings of the detected fungicides were confirmed by LC–TOFMS accurate mass analysis (obtaining mass accuracy <3 ppm error), thus showing the usefulness of LC–TOFMS for the multi-residue analysis of agrochemicals in fruit-based soft drink samples. The results obtained in the 14 studied samples are shown in Table 5. 13 out of 14 samples contained at least one pesticide residue, with total pesticide concentration levels in the range from 2.3 to 21.5 $\mu g\,L^{-1}$ of the studied pesticides. The analytes detected were carbendazim, thiabendazole, imazalil and its metabolite and prochloraz with its metabolite. The results show the ability of the proposed method for pesticide testing and quantitation in

Table 5
Application of the proposed fast liquid chromatography electrospray time-of-flight mass spectrometry method for the monitoring of selected citrus-flavored soft drinks samples collected in the European-market during 2009. Concentrations are expressed $\mu g\,L^{-1}$.

S. No.	Carbendazim	Thiabendazole	Imazalil	Imazalil metab	Prochloraz	Prochloraz metab	Rest of analytes	Total
1	2.3	ND	ND	ND	ND	ND	ND	2.3
2	0.48	0.60	3.2	0.06	ND	ND	ND	4.2
3	0.60	1.6	5.3	0.15	ND	ND	ND	7.6
4	1.8	2.1	16.1	0.30	1.1	0.40	ND	21.5
5	1.1	ND	10.4	0.20	1.0	0.59	ND	13.3
6	ND	ND	4.7	0.09	ND	ND	ND	4.8
7	ND	ND	ND	ND	ND	ND	ND	ND
8	1.40	0.47	6.8	0.12	0.58	0.49	ND	9.9
9	ND	ND	4.1	ND	1.8	0.28	ND	6.2
10	ND	ND	1.3	ND	0.2	ND	ND	1.5
11	0.85	0.5	2.5	ND	0.4	0.16	ND	4.4
12	ND	0.56	6.3	ND	1.9	ND	ND	8.8
13	ND	5.5	11.2	<LOQ	LOD	0.10	ND	16.8
14	ND	<LOQ	2.0	ND	ND	ND	ND	2.0

Samples tested: (1) Lemon Flavored (LF), Czech Republic; (2) Orange Flavored (OF) Switzerland; (3) OF, Austria; (4) LF, Spain (6% juice); (5) LF, France (5% juice); (6) LF, Italy (12% juice); (7) Pineapple, Spain (10% juice); (8) LF, United Kingdom (6% juice); (9) OF, Spain (6% juice); (10) LF, Spain (1% juice); (11) OF, Spain (8% juice); (12) LF, Ireland (6% juice); (13) OF, France (14% juice); (14) OF, France (12% juice).

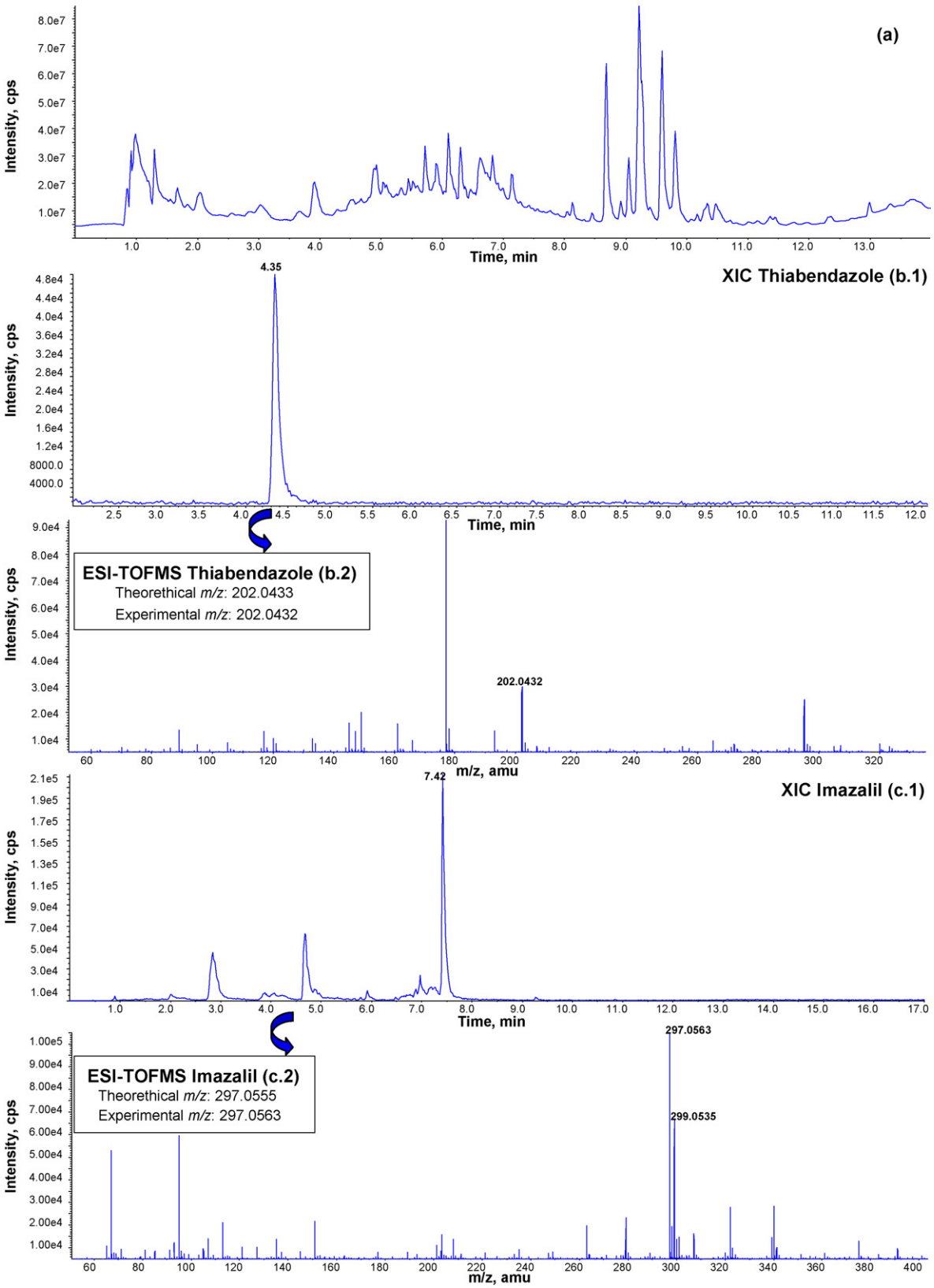

Fig. 2. Example of a positive fruit-based soft drink sample containing both imazalil (3.2 $\mu g\,L^{-1}$) and thiabendazole (0.60 $\mu g\,L^{-1}$). (a) Total ion chromatogram of a fruit-based soft drink; (b.1) extracted ion chromatogram of thiabendazole (m/z range 202.045 ± 0.010); (b.2) accurate mass spectrum at retention time 4.35 min in which thiabendazole (theoretical mass: 202.0433; experimental mass: 202.0432) was identified. (c.1) Extracted ion chromatogram of imazalil (m/z range 297.055 ± 0.010); (c.2) accurate mass spectrum at retention time 7.42 min in which imazalil (theoretical mass: 297.0555; experimental mass: 297.0563) was identified.

fruit-based soft drink samples at low concentration levels. A comprehensive survey is currently being performed in our laboratory using the proposed method described here with samples collected in Spain and other European countries and will be reported in due course.

4. Conclusions

To our knowledge, this work described the development and validation of the first multi-residue method for the determination of multi-class pesticides in fruit-based soft drinks, an emerging matrix in pesticide residue analysis field. The method uses SPE with polymer-based cartridges and LC–TOFMS using a high-resolution small-particle size column, which fosters the throughput of the method. Satisfactory recoveries were obtained for the different classes of pesticides assayed, which presages the application of the proposed method to large-scale multi-residue methods (i.e. covering >100 pesticides). The results shown that the sensitivity obtained with the proposed method is appropriate for the multi-residue analysis of pesticides in the tested samples. The high sensitivity attained by rapid resolution LC–TOFMS (i.e. LOQs as low as 0.02 $\mu g\,L^{-1}$ for diazinon or pyriproxyfen) is in compliance with the requirements of the application assayed, and with the typical concentration levels found in the tested samples so far. The potential of the proposed method was demonstrated by analyzing 14 market-purchased samples with excellent selectivity and sensitivity. The proposed LC–TOFMS method also offers the possibility of performing *a posteriori* (non-target) analysis of the samples. All the data are saved and can be re-examined to check for compounds that previously were not expected or were not subjected to control. This is an additional attractive feature that highlights the potential application of this method based on LC–TOFMS in pesticide residue laboratories worldwide.

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