Talanta 83 (2011) 1552–1561

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Pesticide residue analysis of fruit juices by LC–MS/MS direct injection. One year pilot survey

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ARTICLE INFO

Article history:
Received 5 August 2010
Received in revised form 3 November 2010
Accepted 22 November 2010
Available online 2 December 2010

Keywords:
Liquid chromatography coupled to tandem mass spectrometry
Fruit juice
Food analysis
Validation
Direct injection

ABSTRACT

For this work, thirteen types of fruit juices (orange, pineapple, peach, apple, multifruit, mango, strawberry, tomato, pear, mandarin, grape, banana and grapefruit) were selected to develop an analytical method for the analysis of 53 pesticides by direct injection in LC–MS/MS. The preparation of the samples was very simple: an aliquot of the juice was centrifuged and it was ten-times diluted prior to analysis, which allowed reducing considerably the time and cost of the analyses. Besides, dilution of the samples permits reducing the amount of matrix going into the system, and thus, decreasing the matrix effects, so common in this type of commodities, opening the possibility to perform quantification with solvent based standards. Validation of the method was carried out in accordance with EU guidelines. Calibration curves covering three orders of magnitude were performed, and they were linear over the concentration range studied for all the matrices (from 0.1 to 100 $\mu g\,L^{-1}$). Practical limits of quantification were in the low $\mu g\,L^{-1}$ range, far below the maximum residue levels (MRLs) of the EU regulations, which do not set specific MRLs for juices, and in this cases of processed food, MRLs of the raw product are applied. Repeatability of the instrumental method was studied in all matrices, obtaining good intra- and inter-day relative standard deviations (RSDs). The proposed method was applied to 106 real fruit juice samples purchased in different local markets during a one-year survey in order to validate the suitability for routine analysis. 43% of the analysed samples gave positive results (higher than the practical limits of quantification).

1. Introduction

Fruit juices are consumed daily in the European Union (EU) countries, due to their high nutritious values and to its benefits on the human health [1]. For this reason, monitoring of pesticide residue levels in this kind of commodity is essential, specially taking into account its high consumption by children. Nowadays, pesticide residues in food are an important issue in terms of food safety. On this account, legal authorities set maximum residue levels (MRLs) in order to reinforce convenient agricultural practices. In the EU, it is the European Commission who sets them to protect consumers from exposure to unacceptable levels of pesticides residues in food and feed [2]. This EU regulation does not set specific MRLs for juices, and so, in the cases of processed food, MRLs of the raw product are applied. Pesticide residue levels found in fruit juices depend on various factors such as type of pesticide, commodity, treatment applied and degradation processes involved [3]. However, the concentrations are often quite small, and therefore, appropriate methods of analysis are needed. According to the literature, traditional sample preparation methods for determining pesticides in juice are based on liquid–liquid extraction [4], but nowadays the analytical approach is based on solid–liquid extraction, due to the simplicity and robustness of these extraction procedures, together with the low requirement of organic solvents. Solid-phase extraction (SPE) [5–7] and solid-phase microextraction (SPME) [8] are widely used at present. Other employed techniques are headspace SPME [9], liquid-phase microextraction (LPME) [10], dispersive liquid–liquid microextraction (DLLME) [11,12] single-drop microextraction (SDME) [13,14] matrix solid phase dispersion (MSPD) [15–18] and dispersive SPE-QuEChERS [19,20].

Both gas and liquid chromatography techniques have been applied traditionally to pesticide analysis in juices, although in the past years liquid chromatography has experimented further development and has proved to be one of the most powerful techniques for the analysis of pesticides in a wide range of matrices, mostly when coupled to tandem mass spectrometry (LC–MS/MS). Gas chromatography has been used for juice analysis, coupled to nitrogen–phosphorus (NPD) [7,16], flame photometric (FPD) [13,14,21], electron capture (ECD) [22] or mass spectrometer (MS) detectors [6,9,11,15,18,20,23]. Liquid chromatography coupled to MS or MS/MS is the most common

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doi:10.1016/j.talanta.2010.11.061

approach in the last years, mainly working with triple quadrupole [19,24], time of flight (TOF) [5] or quadrupole-ion trap analyzers [8,17].

Matrix effect can be an important problem as it can severely compromise quantitative analysis of the compounds at trace levels as well as method reproducibility [25]. A possible approach in order to minimize it can be dilution of the extracts, obtaining this way the injection of less matrix load into the chromatographic system. The appearance of new generation analytical systems in the market makes this attempt possible, as highly sensitive instruments are available for these purposes. By this approach, matrix effect could be avoided to a great extent for many fruit and vegetable matrices, facilitating the quantification process. This was the objective of this work: the development and validation of a multiresidue method for the determination of pesticides in fruit juice without any sample extraction prior to the analysis. Only a dilution step, followed by LC–MS/MS analysis is performed.

2. Experimental

2.1. Chemicals and reagents

2.1.1. Standards

Pesticide analytical standards of high purity (>98%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and from Sigma–Aldrich (Steinheim, Germany). Individual pesticide stock solutions of all compounds (1000 mg L^{-1}) were prepared in acetonitrile or methanol and stored at −20 °C in the dark.

2.1.2. Solvents

HPLC-grade acetonitrile was supplied by J.T. Baker (Deventer, The Netherlands). 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.

2.1.3. Reagents

Formic acid was purchased from Fluka (Buchs, Switzerland).

2.2. Sample preparation

Three different types of juices were used for the development and validation of the method: orange juice, pineapple juice and peach juice. Commercial samples were acquired in a local supermarket and were stored at 4 °C until they were processed. A previous analysis of the samples was performed in order to ensure that they did not contain any of the studied compounds, and these samples were selected as blanks for spiking, calibration curves, and recovery purposes.

2.3. Real samples

In order to validate the method, one hundred and six samples of different types of juices (orange, pineapple, peach, apple, multifruit, mango, strawberry, tomato, pear, mandarin, grape, banana and grapefruit) were purchased in supermarkets from the South of Spain, in Almería, during a one-year period of time. All samples were stored in their original packaging under the recommended conditions until analysis.

2.4. Spiking procedure

Blank juice was placed in a 50 mL volumetric flask, along with 0.5 mL of the standard mixture of the desired concentration in acetonitrile. The flask was then shaken and taken to an ultrasonic bath for 5 min for correct homogenization of the sample prior to extraction. The final spiking concentration levels were of 0.02 and 0.2 mg L^{-1} in the juice samples, and 0.002 and 0.02 mg L^{-1} in the extract, as the samples were ten times diluted prior to analysis.

2.5. Extraction procedure

A representative 25 g aliquot of juice (previously homogenized) was weighed into a 50 mL disposable screw-capped polypropylene tube, and it was centrifuged at 3700 rpm for 4 min to sediment the solids. An aliquot (100 μL) of the supernatant solution of this centrifuged juice was then diluted with 900 μL of acetonitrile: high-purity water 1:9 (v,v), and it was filtered through a 0.45 μm polytetrafluoroethylene (PTFE) syringe filter (Millex FG, Millipore, Milford, MA), resulting in an extract containing 0.1 g sample per mL. This final solution was ready for injection into the LC–MS/MS.

2.6. LC–MS/MS analysis

Liquid chromatography–electrospray ionization-tandem mass spectrometry, in positive ion mode, was used to separate, identify, and quantify the target compounds. For the LC analysis, an Agilent 1200 HPLC system (Agilent Technologies, Wilmington, DE, USA) with a binary pump was used. The analytical column employed was a reversed-phase C8 of 150 mm × 4.6 mm and 5 μm particle size (Agilent Zorbax Eclipse XDB). The mobile phases, A and B, were acetonitrile and high-purity water with 0.1% formic acid, respectively. The gradient program started with 10% of A, constant for 0.5 min, followed by a linear gradient up to 100% A in 15 min, and finishing with 100% A constant for 10 min. After this 25 min run time, 10 min of post-time followed using the initial 10% of A. The flow rate was set constant at 0.6 mL min^{-1} during the whole process, and the injection volume was of 5 μL. For the mass spectrometric analysis, a 5500 QTRAP MS/MS system (AB Sciex Instruments, Foster City, CA) was used, equipped with a turboionspray source operating in positive ionization mode, set with the following parameters: Ion Spray (IS) voltage: 5500 V; curtain gas: 30 psi; nebulizer gas (GS1): 50 psi; auxiliary gas (GS2): 50 psi; source temperature: 550 °C. Nitrogen was used as the nebulizer and collision gas. Optimization of the compounds was performed by flow injection analysis (FIA), injecting individual standard solutions directly into the source. Table 1 shows the values of the instrumental settings optimized for each compound: declustering (DP) and entrance potential (EP) for precursor ions and collision energy (CE) for product ions. The best sensitivity in multiple reaction monitoring operation mode was achieved through the acquisition of selected reaction monitoring (SRM) transitions with "Scheduled MRM mode", with a time window of 90 s (the total number of SRM transitions was 106). For identification of the studied compounds two SRM transitions and a correct ratio between the abundances of the two optimized SRM transitions (SRM2/SRM1) were used, along with retention time matching. For quantitation, the most intense SRM transition was selected. AB SCIEX Analyst software 1.5 was used for data acquisition and processing.

2.7. Validation study

Method accuracy and precision were evaluated by recovery studies using blank matrices of the three studied juices (orange, pineapple and peach juice) spiked at two concentration levels, 0.2 mg L^{-1} and 0.02 mg L^{-1}. All experiments were tested with five replicates for each matrix, in accordance with EU guidelines [26]. Quantitation of the compounds in the spiked samples was carried out comparing the peak areas of the samples with those of matrix matched standard solutions. These, as well as the matrix-matched

Table 1
Values of the instrumental settings optimized for each compound: precursor ion, declustering potential (DP), entrance potential (EP), product ions and their collision energies (CE).

	Retention time (min)	Precursor ion (*m*/*z*)	DP (V)	EP (V)	Product ion 1 (*m*/*z*)	CE 1 (eV)	Product ion 2 (*m*/*z*)	CE 2 (eV)
Acephate	4.6	184.0	47.00	14.00	143.0	16.00	125.0	27.00
Acetamiprid	9.6	223.0	100.00	7.00	126.0	30.00	56.0	21.00
Albendazole	10.2	265.7	120.00	10.00	234.0	30.00	192.0	40.00
Azoxystrobin	14.0	404.2	100.00	5.00	372.0	21.00	343.9	33.00
Benalaxyl	15.7	326.2	110.00	8.00	208.1	21.00	294.2	16.00
Bupirimate	13.3	316.7	110.00	5.00	166.1	32.00	272.1	27.00
Cambendazole	8.3	303.0	144.00	7.00	261.1	26.00	217.0	40.00
Carbendazim	6.1	192.0	200.00	9.00	160.1	27.00	132.0	41.00
Carbofuran	12.1	222.0	91.00	5.00	165.1	18.00	123.1	31.00
Chloroxuron	13.8	291.0	110.00	7.00	72.1	54.00	164.1	24.00
Chromafenozide	14.8	395.2	62.00	9.00	175.1	29.00	132.9	34.00
Cyproconazole	13.6	292.1	170.00	9.00	70.1	58.00	125.0	44.00
Deet	12.5	192.0	160.00	11.00	119.1	26.00	91.0	41.00
Diazinon	16.3	304.6	100.00	8.00	169.0	30.00	153.1	27.00
Diethofencarb	13.9	268.0	76.00	4.00	226.1	14.00	180.1	23.00
Difenoconazole	15.4	405.9	100.00	5.00	250.9	36.00	337.0	24.00
Difenoxuron	12.4	287.0	150.00	12.00	72.0	23.00	123.3	25.00
Diuron	12.5	233.0	64.00	11.00	72.0	22.00	160.0	35.00
Ethiofencarb	12.7	226.1	60.00	5.00	107.0	28.00	164.1	11.00
Fenbendazole	11.6	300.1	150.00	8.00	268.0	27.00	159.0	47.00
Fenobucarb	14.0	208.1	94.00	4.00	94.9	25.00	152.1	12.00
Fenuron	12.4	165.1	40.00	10.00	72.0	26.00	120.0	24.00
Flazasulfuron	13.0	408.0	57.00	4.00	182.0	29.00	301.1	22.00
Fluacrypyrim	16.6	427.2	81.00	8.00	145.1	42.00	205.0	13.00
Fluometuron	12.3	233.1	73.00	13.00	72.0	34.00	160.1	39.00
Imazalil	9.9	297.8	125.00	5.00	158.9	34.00	255.9	24.00
Indoxacarb	16.3	528.0	120.00	13.00	249.2	24.00	292.9	18.00
Isoprocarb	13.1	194.0	60.00	6.00	94.9	18.00	152.1	11.00
Isoproturon	12.4	207.1	70.00	5.00	72.0	27.00	165.1	19.00
Metalaxyl	12.4	280.1	94.00	12.00	220.0	17.00	248.0	14.00
Metamitron	8.8	202.7	80.00	5.00	103.9	30.00	175.1	25.00
Methamidophos	4.0	142.0	73.00	5.00	94.0	21.00	125.2	19.00
Methoxyfenozide	14.6	369.3	65.00	12.00	149.0	23.00	133.1	35.00
Metolachlor	15.2	284.0	100.00	11.00	252.1	18.00	176.1	36.00
Monocrotophos	7.3	224.0	60.00	11.00	192.7	11.00	127.0	20.00
Monuron	11.1	199.1	120.00	10.00	72.0	23.00	126.0	37.00
Myclobutanil	14.2	289.1	38.00	6.00	70.0	23.00	125.1	46.00
Omethoate	5.5	214.0	66.00	8.00	125.0	29.00	155.0	23.00
Oxamyl	7.3	242.0	90.00	3.00	72.0	36.00	121.1	18.00
Pirimicarb	7.8	238.7	50.00	7.00	72.1	38.00	182.2	23.00
Pirimiphos methyl	16.4	306.1	110.00	11.00	108.0	42.00	164.1	28.00
Promecarb	14.2	208.1	80.00	9.00	151.1	14.00	109.0	23.00
Propamocarb	5.7	189.1	29.00	6.00	101.9	25.00	144.1	16.00
Propaphos	15.1	304.8	90.00	8.00	221.0	19.00	263.0	10.00
Propazine	13.6	230.0	100.00	3.00	146.0	30.00	188.0	24.00
Pyridaphenthion	14.4	341.0	100.00	7.00	189.1	36.00	205.0	34.00
Pyrimidifen	14.0	377.7	130.00	5.00	184.0	35.00	150.0	51.00
Pyriproxyfen	17.1	322.1	100.00	9.00	227.0	21.00	96.0	20.00
Tebuconazole	14.4	308.1	80.00	8.00	70.1	60.00	125.1	57.00
Tetraconazole	14.4	372.0	110.00	4.00	159.0	43.00	70.0	65.00
Thiacloprid	10.5	253.0	110.00	7.00	125.9	34.00	186.0	19.00
Triadimenol	13.5, 13.7	296.1	50.00	13.00	70.0	40.00	227.1	14.00
Triazophos	14.9	314.1	120.00	8.00	162.1	25.00	286.0	16.00

calibration curves, were prepared by spiking an aliquot of the blank extract with the desired amount of standard solution. The sensitivity of the method was calculated in terms of practical limit of quantitation, or reporting level, which was calculated as the minimum concentration of analyte that generated a signal to noise (S/N) ratio of 3, determined in the qualifier transition (SRM2). Linearity was evaluated both in solvent and matrix, using matrix-matched calibration curves prepared as described before, in a concentration range of 0.1–100 $\mu g\,L^{-1}$. The matrix effect was studied by comparison of the slopes of the calibration curves in solvent and in matrix. The repeatability of the instrumental method was estimated by determining the inter- and intra-day relative standard deviation (RSD, %) by the repeated analysis ($n = 5$) of a spiked matrix extract at 5 $\mu g\,L^{-1}$ level, from run-to-run over one day and five days, respectively.

3. Results and discussion

3.1. Optimization of LC–MS/MS parameters

The optimization of the compounds was made by flow injection analysis (FIA) of the individual standard solutions at a concentration of 0.1 $mg\,L^{-1}$ in methanol. In this process the precursor and the product ions were chosen, along with the optimum declustering and entrance potentials for the precursor ion and the collision energies for the product ions. The transitions of the most abundant product ions (SRM1) were used for quantitation and the second ones in abundance (SRM2), for identification.

The first step involved selecting the precursor ion for each compound. For the majority of them the protonated molecule $[M+H]^+$ was the most abundant, and so it was chosen as the precursor

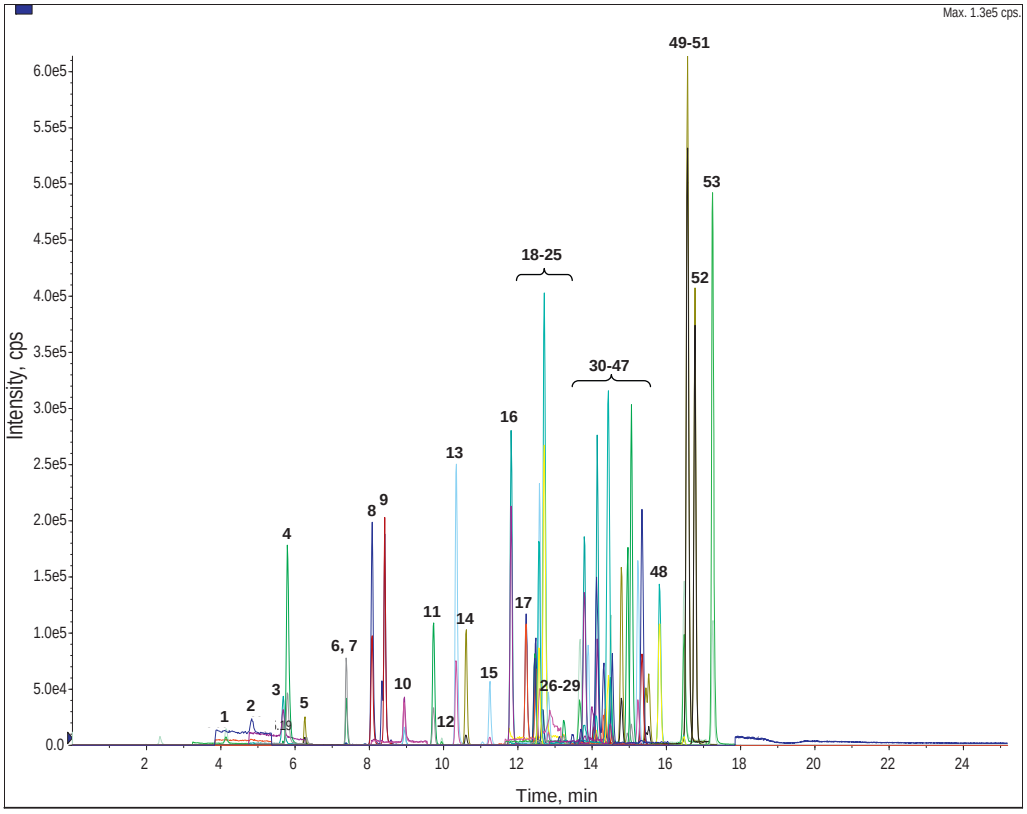

Fig. 1. Extracted ion chromatogram corresponding to the analysis of an orange juice extract spiked at 1 μg L^{-1} level with the studied compounds. (1, Methamidophos; 2, acephate; 3, omethoate; 4, propamocarb; 5, carbendazim; 6, monocrotophos; 7, oxamyl; 8, pirimicarb; 9, cambendazole; 10, metamitron; 11, acetamiprid; 12, imazalil; 13, albendazole; 14, thiacloprid; 15, monuron; 16, fenbendazole; 17, carbofuran; 18, fluometuron; 19, difenoxuron; 20, fenuron; 21, isoproturon; 22, metalaxyl; 23, deet; 24, diuron; 25, ethiofencarb; 26, flazasulfuron; 27, isoprocarb; 28, bupirimate; 29, triadimenol; 30, cyproconazole; 31, propazine; 32, chloroxuron; 33, diethofencarb; 34, azoxystrobin; 35, fenobucarb; 36, pyrimidifen; 37, myclobutanil; 38, promecarb; 39, pyridaphenthion; 40, tebuconazole; 41, tetraconazole; 42, methoxyfenozide; 43, chromafenozide; 44, triazophos; 45, propaphos; 46, metolachlor; 47, difenoconazole; 48, benalaxyl; 49, diazinon; 50, indoxacarb; 51, pirimiphos methyl; 52, fluacrypyrim; and 53, pyriproxyfen.)

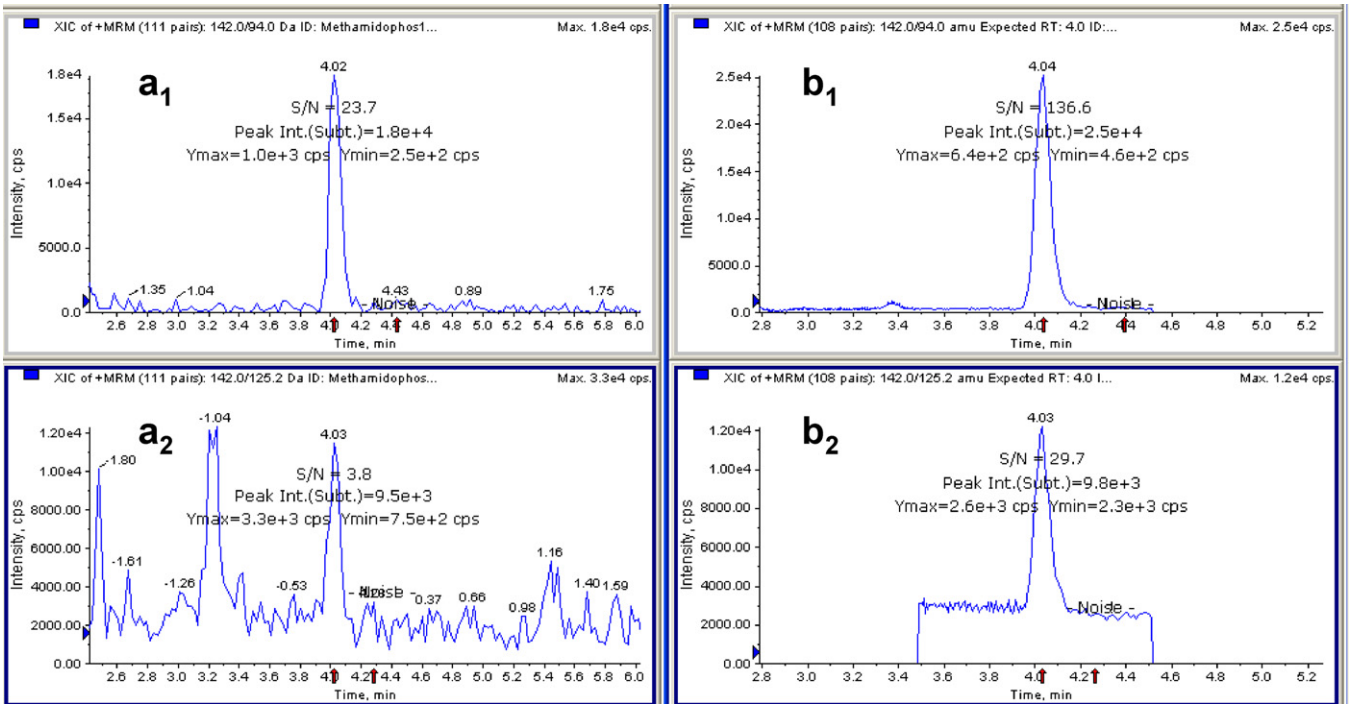

Fig. 2. Comparison of the "MRM" mode (a_1 for quantifier and a_2 for qualifier) and the "Scheduled MRM" mode (b_1 for quantifier and b_2 for qualifier) for metamidophos in pineapple juice extract spiked with a standard solution mix at 1 μg L^{-1}.

Table 2
Recovery values and relative standard deviations (RSDs) of the target compounds in different juice matrices. (Spiking levels: 0.02 and 0.2 mg L^{-1}.).

	Orange juice		Pineapple juice		Peach juice	
	R, % (RSD, %)[a]		*R*, % (RSD, %)[a]		*R*, % (RSD, %)[a]	
	0.02 mg L^{-1}	0.2 mg L^{-1}	0.02 mg L^{-1}	0.2 mg L^{-1}	0.02 mg L^{-1}	0.2 mg L^{-1}
Acephate	102.5 (5.4)	120.2 (3.7)	118.0 (8.3)	104.8 (9.8)	104.9 (10.8)	124.1 (9.7)
Acetamiprid	110.2 (2.1)	104.0 (3.3)	108.3 (19.1)	104.3 (9.3)	120.6 (11.5)	96.1 (9.0)
Albendazole	78.5 (0.6)	116.7 (5.2)	124.3 (13.6)	110.9 (3.9)	112.1 (16.9)	82.5 (11.8)
Azoxystrobin	119.0 (8.5)	91.9 (6.2)	105.0 (2.0)	82.9 (1.0)	102.3 (4.0)	107.8 (3.7)
Benalaxyl	122.2 (4.1)	78.3 (3.6)	100.0 (13.0)	88.8 (5.9)	76.5 (5.3)	62.4 (5.8)
Bupirimate	114.0 (7.9)	104.4 (10.6)	125.8 (4.5)	106.7 (12.4)	107.6 (20.0)	95.5 (2.9)
Cambendazole	86.6 (2.5)	113.1 (3.8)	116.2 (3.8)	117.6 (14.8)	113.4 (15.7)	112.5 (15.4)
Carbendazim	88.6 (1.1)	114.5 (13.8)	112.3 (12.7)	116.7 (6.5)	123.5 (6.9)	98.4 (30.4)
Carbofuran	106.8 (1.5)	124.3 (1.5)	120.7 (5.0)	117.0 (7.6)	125.9 (11.5)	119.0 (7.1)
Chloroxuron	106.2 (13.5)	91.2 (1.6)	93.1 (12.7)	104.0 (6.7)	68.0 (10.7)	72.9 (7.7)
Chromafenozide	110.0 (9.7)	95.5 (3.4)	119.0 (2.0)	79.6 (21.4)	86.8 (20.5)	76.8 (6.4)
Cyproconazole	68.8 (3.3)	105.1 (11.4)	126.0 (18.4)	105.3 (3.6)	89.1 (24.2)	84.3 (3.1)
Deet	99.9 (3.1)	108.6 (8.1)	125.7 (9.5)	127.0 (3.9)	116.3 (7.7)	115.1 (9.9)
Diazinon	73.5 (7.5)	73.3 (4.9)	75.7 (8.8)	80.1 (12.8)	81.4 (16.7)	72.6 (15.9)
Diethofencarb	75.7 (2.6)	103.5 (11.4)	127.4 (14.1)	122.6 (7.2)	118.9 (19.1)	88.1 (10.1)
Difenoconazole	72.1 (7.8)	25.3 (12.6)	79.5 (2.3)	76.0 (19.3)	75.6 (17.5)	72.5 (19.5)
Difenoxuron	63.2 (4.3)	120.8 (7.2)	119.6 (5.9)	118.5 (6.2)	98.2 (11.9)	112.8 (14.4)
Diuron	84.6 (2.3)	115.3 (6.8)	107.1 (14.2)	124.5 (11.1)	112.1 (7.6)	97.1 (9.9)
Ethiofencarb	99.6 (6.6)	115.1 (6.9)	115.6 (13.2)	108.7 (12.2)	110.9 (8.5)	105.5 (12.8)
Fenbendazole	120.4 (3.2)	89.5 (8.4)	116.2 (6.4)	111.3 (3.3)	82.3 (2.2)	71.8 (3.7)
Fenobucarb	86.1 (1.0)	115.7 (2.2)	100.5 (16.6)	120.0 (8.6)	106.7 (6.6)	111.2 (12.7)
Fenuron	92.7 (4.9)	117.4 (8.4)	118.8 (9.2)	113.2 (1.5)	118.6 (7.9)	121.7 (7.8)
Flazasulfuron	116.0 (1.9)	74.4 (9.3)	77.5 (17.3)	78.0 (15.5)	74.4 (19.7)	79.4 (18.9)
Fluacrypyrim	77.4 (14.2)	74.9 (24.6)	78.3 (7.5)	84.9 (15.8)	96.2 (18.4)	76.9 (19.9)
Fluometuron	106.6 (3.7)	107.9 (7.2)	119.9 (12.9)	112.5 (6.3)	106.6 (11.0)	122.1 (3.9)
Imazalil	79.1 (3.3)	115.6 (8.1)	125.8 (20.0)	115.2 (18.6)	124.2 (14.5)	119.9 (17.6)
Indoxacarb	98.7 (8.9)	114.1 (16.4)	114.0 (16.6)	121.7 (20.0)	80.6 (5.8)	119.0 (16.2)
Isoprocarb	89.3 (3.2)	114.7 (14.3)	119.8 (7.0)	112.9 (9.7)	120.7 (3.8)	120.5 (7.4)
Isoproturon	93.8 (3.4)	114.0 (3.8)	112.9 (7.5)	115.4 (3.6)	115.0 (3.2)	115.0 (3.0)
Metalaxyl	99.9 (2.1)	120.2 (4.7)	116.3 (5.7)	116.5 (4.5)	118.8 (2.2)	119.5 (5.8)
Metamitron	104.3 (2.9)	111.6 (14.7)	114.9 (18.8)	120.4 (5.2)	124.6 (9.0)	125.0 (14.5)
Methamidophos	103.8 (1.0)	113.8 (7.3)	93.4 (12.7)	117.3 (9.6)	100.4 (14.7)	110.3 (1.9)
Methoxyfenozide	114.6 (19.0)	94.7 (5.1)	109.6 (1.3)	100.4 (7.5)	102.5 (8.0)	87.2 (15.3)
Metolachlor	85.6 (3.6)	115.5 (2.6)	122.7 (4.8)	98.3 (4.8)	114.9 (3.5)	99.5 (5.3)
Monocrotophos	99.8 (2.1)	105.4 (11.2)	119.5 (7.8)	112.2 (12.0)	101.4 (17.3)	123.2 (11.4)
Monuron	105.2 (2.8)	121.7 (4.0)	112.1 (1.1)	116.8 (11.5)	114.6 (10.1)	112.7 (1.9)
Myclobutanil	120.6 (4.6)	111.1 (9.7)	110.0 (6.5)	88.5 (9.1)	82.9 (9.9)	75.8 (8.6)
Omethoate	108.9 (2.0)	122.6 (10.2)	102.8 (16.4)	121.4 (7.8)	109.3 (13.2)	113.8 (18.2)
Oxamyl	98.3 (8.1)	105.8 (2.3)	80.9 (9.9)	97.5 (10.1)	110.6 (6.8)	104.0 (8.7)
Pirimicarb	90.2 (3.5)	112.9 (4.1)	113.8 (2.5)	107.8 (11.8)	105.0 (11.7)	125.1 (8.3)
Pirimiphos-methyl	111.7 (1.6)	96.4 (1.9)	113.6 (8.3)	104.3 (4.2)	79.3 (2.0)	78.2 (4.8)
Promecarb	71.0 (2.1)	118.1 (8.3)	113.9 (0.2)	125.4 (4.1)	102.0 (6.0)	110.8 (12.7)
Propamocarb	105.0 (1.5)	105.2 (5.8)	114.5 (2.4)	96.7 (3.2)	103.5 (9.6)	118.4 (8.5)
Propaphos	118.9 (14.7)	71.0 (8.2)	81.8 (15.6)	77.1 (18.0)	75.5 (10.7)	78.9 (9.1)
Propazine	72.1 (1.8)	122.7 (1.7)	118.9 (3.9)	117.0 (1.8)	109.4 (12.8)	105.7 (4.4)
Pyridaphenthion	118.4 (7.5)	83.3 (4.8)	104.2 (22.0)	105.5 (7.0)	99.8 (16.0)	72.9 (3.7)
Pyrimidifen	119.1 (6.6)	96.5 (3.6)	111.2 (14.8)	91.8 (8.2)	83.1 (5.9)	68.2 (0.5)
Pyriproxyfen	76.0 (14.3)	76.1 (14.9)	89.2 (3.4)	85.1 (13.2)	82.9 (15.5)	90.2 (20.1)
Tebuconazole	118.2 (4.2)	82.7 (8.1)	96.0 (4.6)	78.8 (9.4)	79.7 (19.3)	75.7 (14.0)
Tetraconazole	104.1 (13.3)	92.1 (6.0)	84.6 (18.6)	83.7 (13.0)	81.4 (14.5)	88.7 (10.6)
Thiacloprid	97.3 (3.6)	111.7 (8.4)	105.5 (17.2)	115.5 (9.4)	110.5 (12.3)	102.0 (2.6)
Triadimenol	76.2 (1.4)	107.4 (8.0)	118.6 (3.4)	107.5 (6.8)	105.4 (2.0)	94.8 (6.3)
Triazophos	107.2 (11.4)	91.0 (3.5)	108.0 (4.9)	85.2 (6.8)	76.0 (4.2)	72.2 (10.8)

[a] Mean recovery and relative standard deviation from analysis of spiked samples ($n = 5$).

ion. For oxamyl the most considerable ion was the sodium adduct $[M+Na]^+$. Then, the optimum declustering and entrance potentials were chosen for the precursor ions. The selected conditions are shown in Table 1.

Afterwards, in the *Product Ion* mode, two product ions for each compound were selected, along with their corresponding optimum collision energies. Acetamiprid (*m*/*z* 56.0), chloroxuron (*m*/*z* 72.1), cyproconazole (*m*/*z* 70.1), difenoxuron (*m*/*z* 72.0), diuron (*m*/*z* 72.0), fluometuron (*m*/*z* 72.0), isoproturon (*m*/*z* 72.0), monuron (*m*/*z* 72.0), myclobutanil (*m*/*z* 70.0), oxamyl (*m*/*z* 72.0), pirimicarb (*m*/*z* 72.0), tebuconazole (*m*/*z* 70.1), and triadimenol (*m*/*z* 70.0) yielded low mass ions. Obtaining such low masses represents a disadvantage as it entails a decrease in specificity. Nevertheless these ions were chosen for product ions as no other higher mass ions were sensitive enough. Fig. 1 shows an extracted ion chromatogram (XIC) corresponding to all the SRM transitions obtained at a concentration of 1 μg L^{-1} in orange juice extract.

The use of "Scheduled MRM" mode implies a great advance in terms of sensibility. Working this way, no dwell time is needed, and instead of that, each transition is only scanned at a certain retention time window. However, the retention time of the pesticides is needed before acquisition with this mode. An example of the improvement in terms of signal to noise ratio is illustrated in Fig. 2, which shows both SRM transitions for methamidophos in pineapple juice extract spiked with a standard solution mix at 1 μg L^{-1}, working in normal SRM mode (a) and with "Scheduled MRM mode" (b).

3.2. Method validation

Validation was performed in accordance with EU guidelines [26] of method validation procedures for pesticide residue analysis in food and feed. Performance characteristics studied were accuracy and precision of the extraction method, linearity, matrix effects, practical limits of quantitation, instrumental precision and specificity.

3.2.1. Recoveries

The accuracy of the method was verified by measuring recoveries from spiked blank samples of the different matrices investigated at two concentrations levels, 0.020 and 0.200 mg L^{-1}. These fortification levels were selected according to the expected concentration in real samples. These two levels also represent values at the lower and at the higher part of the linear range, as after the tenfold dilution, those concentrations would imply 0.002 and 0.020 mg L^{-1} in the extract. All experiments were performed by quintuplicate for each matrix. Mean recovery data and relative standard deviations (RSDs) obtained, expressing the precision of the extraction method, are given in Table 2.

3.2.2. Linearity

Linearity was evaluated using solvent and matrix-matched calibration curves at five concentration levels covering three orders of magnitude: from 0.1 to 100 μg L^{-1}, based on linear regression and squared correlation coefficient, R^2. The linearity of the analytical response for all the studied compounds within the studied range of three orders of magnitude was very good, with correlation coefficients higher than 0.995 in all cases.

3.2.3. Matrix effect

Matrix effect was also evaluated during the validation of the method, as signal suppression or enhancement as a result of matrix effect can severely compromise quantitative analysis of the compounds at trace levels, as well as it can greatly affect the method reproducibility and accuracy [25]. The matrix effect was studied by comparison of the slopes of the calibration curves in solvent and in matrix. Signal enhancement occurs if the percentage of the difference between these slopes is positive. If its negative, its indicative of signal suppression. Depending on the value of this percentage, different matrix effects could be observed. A percentage between −20% and 20% was considered as no matrix effect, because this variation is close to the repeatability values. A medium matrix effect occurred when the values were between −50% and −20% or 20% and 50%, and a strong matrix effect would be below −50% or above +50%. Table 3 shows the percentage of signal suppression or enhancement for the three juice types evaluated. As illustrated in Fig. 3, most of the compounds did not present relevant matrix effect in the juices investigated. The only one that showed strong signal suppression was peach juice, but only for diazinon, flazasulfuron and indoxacarb. Orange juice presented the lowest matrix effect, as 94% of the compounds did not show this kind of effect. This fact enhances the advantage of working with a high sensitivity equipment, which permits dilution of the samples. This entails the possibility of performing quantitation with solvent based calibration curves, avoiding the use of matrix-matched calibration curves without an important increase of the uncertainty, and hence, simplifying the number of matrix matched standard controls.

Table 3
Matrix effect of the different juice matrices.

Pesticide	Matrix effect (%)[a]		
	Orange juice	Pineapple juice	Peach juice
Acephate	18.9	20.7	22.2
Acetamiprid	3.5	6.0	−6.7
Albendazole	−0.8	6.4	7.4
Azoxystrobin	0.9	6.6	10.1
Benalaxyl	15.2	25.7	18.6
Bupirimato	1.0	10.0	11.0
Cambendazole	9.1	27.8	0.2
Carbendazim	−9.4	17.8	13.5
Carbofuran	−3.9	6.9	6.7
Chloroxuron	2.6	10.3	14.9
Chromafenozide	7.3	18.2	20.4
Cyproconazole	1.7	21.1	19.8
Deet	11.6	18.8	19.4
Diazinon	−8.0	4.4	−65.3
Diethofencarb	20.7	13.6	17.3
Difenoconazole	−10.4	10.1	7.2
Difenoxuron	1.8	12.1	15.7
Diuron	−5.6	5.1	−2.7
Ethiofencarb	−6.0	15.1	14.9
Fenbendazole	−3.5	0.2	15.8
Fenobucarb	−2.0	10.0	3.6
Fenuron	−3.8	5.2	6.0
Flazasulfuron	−39.8	−38.5	−72.9
Fluacrypyrim	9.8	10.6	−10.5
Fluometuron	−0.9	18.2	7.6
Imazalil	4.7	20.0	−20.1
Indoxacarb	0.0	−2.8	−57.0
Isoprocarb	9.2	11.7	22.7
Isoproturon	−10.1	0.9	4.8
Metalaxyl	9.5	11.1	8.7
Metamitron	−10.6	−25.6	−4.9
Methamidophos	2.6	8.8	20.6
Methoxyfenozide	0.3	10.1	9.7
Metolachlor	3.7	10.1	12.0
Monocrotophos	−14.9	−20.7	−7.0
Monuron	−0.4	9.0	−0.4
Myclobutanil	−4.1	13.5	5.6
Omethoate	1.7	−6.6	−3.6
Oxamyl	−10.8	−5.9	6.6
Pirimicarb	−3.4	2.3	13.1
Pirimiphos-methyl	−3.7	0.5	−8.2
Promecarb	3.9	19.0	20.9
Propamocarb	5.8	13.0	−2.3
Propaphos	8.7	15.0	12.6
Propazine	4.1	26.4	12.3
Pyridaphenthion	−2.4	5.5	1.1
Pyrimidifen	1.0	3.5	12.7
Pyriproxyfen	−23.0	−7.7	−1.9
Tebuconazole	−3.7	9.6	8.6
Tetraconazole	−3.6	6.9	−4.0
Thiacloprid	−7.1	14.1	13.0
Triadimenol	1.5	14.8	11.1
Triazophos	−5.7	5.7	6.4

[a] Expressed as percentage of the difference between the slopes of the corresponding calibration curves in solvent and in matrix. Negative values stand for signal suppression and positive values for signal enhancement.

3.2.4. Precision

In order to evaluate the repeatability of the instrumental method, the intra- and inter-day RSD were studied. Method repeatability was determined at a concentration level of 5 μg L^{-1}, by analysis of five spiked matrix extracts ($n = 5$) for each matrix tested. Table 4 shows the RSD for all matrices. RSDs for within analysis ranged between 0.4% and 14.0%, although in most of the cases it was below 5%. Inter-day RSD was calculated during five days, and it varied from 0.8% to 24.9%, being 10% the average value for all matrices. This demonstrates the repeatability of the method and therefore its effectiveness for quantitative purposes.

3.2.5. Practical limits of quantitation

The sensitivity of the method was calculated in terms of practical limit of quantitation or reporting level, which was estimated as the minimum concentration of analyte that generated a S/N of 3, determined in the qualifier transition (SRM2), and taking into account the 10-fold dilution that takes place during sample preparation

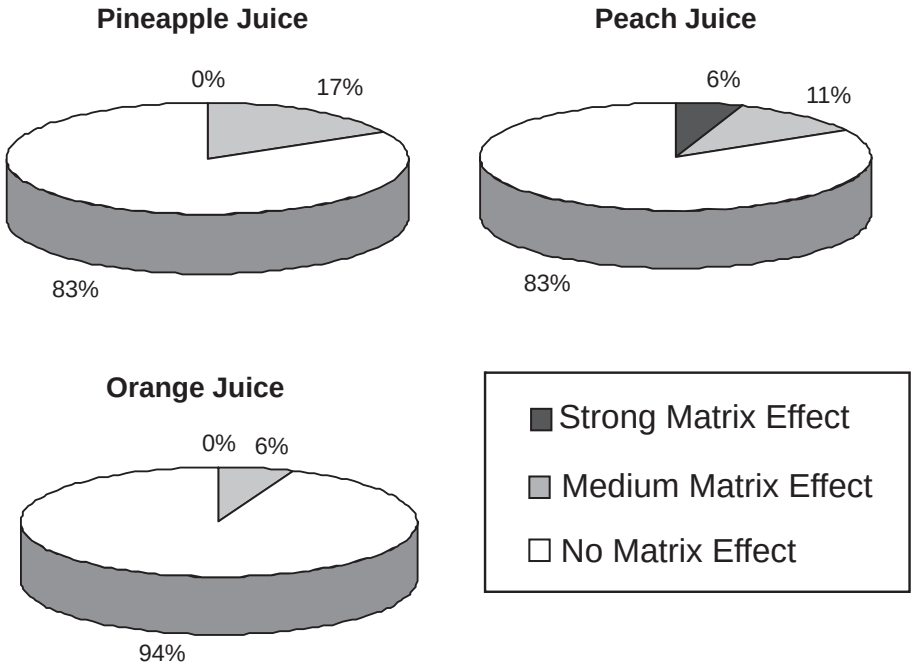

Fig. 3. Illustration of matrix effects in the different commodities used in this study. Matrix effect is categorized into negligible (white), medium (grey) and strong (black).

prior to analysis. The reporting levels of the studied compounds in the different matrices range from 0.1 to 5 $\mu g\,L^{-1}$ for more than 90% of the analytes in the three matrices studied, and have a maximum value of 10 $\mu g\,L^{-1}$, which is the case of acephate in pineapple juice and oxamyl in peach juice, which is enough to verify compliance of products with legal tolerances and to monitor the occurrence of undesirable substances in this type of food matrices, and what is more important, enough to guarantee children's safety, as the reporting levels are far below the maximum levels for pesticide residues in baby food [27].

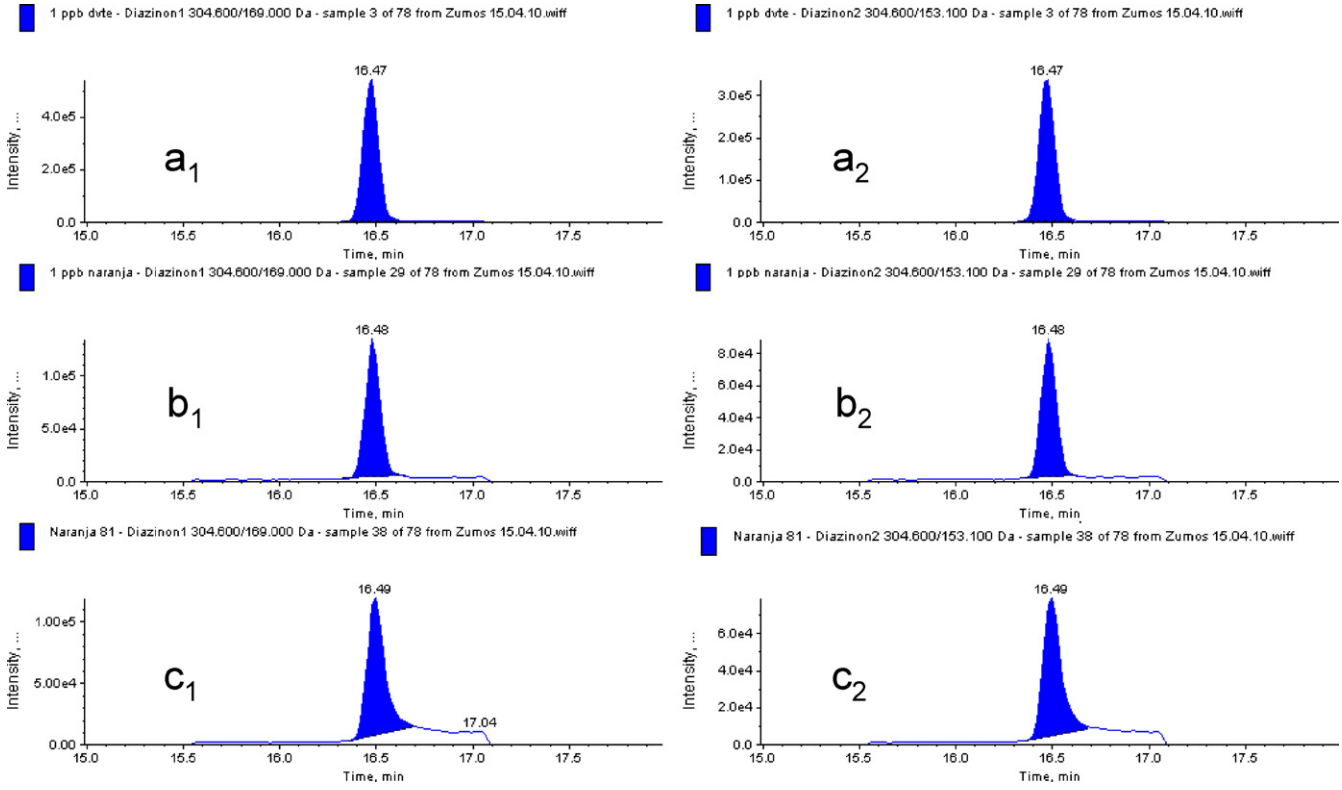

Fig. 4. Extracted ion chromatogram of the two transitions corresponding to diazinon: (a) in a 1 $\mu g\,L^{-1}$ calibration standard in solvent (a_1 for quantifier and a_2 for qualifier), (b) in a 1 $\mu g\,L^{-1}$ calibration standard in orange juice (b_1 for quantifier and b_2 for qualifier) and (c) in a diluted real sample (orange juice containing 10 $\mu g\,L^{-1}$ of diazinon) (c_1 for quantifier and c_2 for qualifier).

Table 4
Method repeatability expressed as inter- and intra-day relative standard deviation (RSD) calculated at a concentration level of 5 μg L^{-1}.

	Orange juice		Pineapple Juice		Peach juice	
	Intra-day RSD (%)[a]	Inter-day RSD (%)[a]	Intra-day RSD (%)[a]	Inter-day RSD (%)[a]	Intra-day RSD (%)[a]	Inter-day RSD (%)[a]
Acephate	14.0	21.3	3.7	14.9	8.5	20.3
Acetamiprid	1.8	7.5	6.2	6.2	2.8	4.3
Albendazole	3.2	12.9	1.9	5.5	1.0	10.0
Azoxystrobin	0.9	8.4	5.2	3.2	2.7	4.9
Benalaxyl	1.5	10.2	2.1	21.5	1.4	2.8
Bupirimato	1.6	4.3	1.6	6.9	3.4	9.7
Cambendazole	2.0	4.9	2.6	2.2	8.5	2.5
Carbendazim	8.4	24.9	10.9	12.7	7.2	18.9
Carbofuran	5.8	8.2	3.8	4.6	3.8	6.1
Chloroxuron	5.9	7.4	2.9	2.2	7.0	15.7
Chromafenozide	1.7	17.7	5.8	13.9	4.6	3.2
Cyproconazole	6.4	8.0	2.7	9.1	2.8	6.1
Deet	2.5	12.7	1.5	6.4	2.9	23.5
Diazinon	1.9	12.7	2.1	25.5	6.9	21.1
Diethofencarb	7.0	11.8	3.0	6.0	5.8	8.0
Difenoconazole	2.5	15.1	1.8	24.9	2.2	4.3
Difenoxuron	2.0	6.0	2.7	5.5	8.9	7.9
Diuron	2.9	1.4	7.8	2.7	3.5	6.5
Ethiofencarb	2.0	7.2	4.5	7.5	1.1	5.4
Fenbendazole	7.4	14.7	5.5	12.8	0.6	18.6
Fenobucarb	2.5	7.2	2.3	10.9	1.9	8.5
Fenuron	4.0	7.1	4.9	5.2	1.8	11.9
Flazasulfuron	4.4	10.5	8.6	16.5	6.6	15.2
Fluacrypyrim	3.9	18.2	3.0	13.3	6.6	4.5
Fluometuron	4.4	9.8	7.7	9.0	4.6	9.6
Imazalil	2.5	9.5	7.2	7.2	7.4	12.5
Indoxacarb	1.5	10.6	3.8	8.2	2.0	3.2
Isoprocarb	2.5	11.7	4.7	0.8	2.4	1.2
Isoproturon	2.1	11.4	6.1	2.7	2.2	1.6
Metalaxyl	3.8	9.4	3.3	2.2	2.4	7.6
Metamitron	6.8	14.1	5.4	8.9	2.4	15.3
Methamidophos	6.6	22.2	9.1	11.9	1.4	15.8
Methoxyfenozide	3.0	17.6	7.4	15.9	13.9	1.6
Metolachlor	3.8	7.8	0.8	21.3	2.4	5.4
Monocrotophos	7.4	14.2	6.9	4.8	1.6	6.5
Monuron	5.7	9.8	3.4	5.1	3.0	13.9
Myclobutanil	5.9	7.0	2.5	12.0	4.2	9.8
Omethoate	7.3	21.0	8.5	7.8	0.4	3.3
Oxamyl	8.4	15.6	7.8	12.3	3.6	9.2
Pirimicarb	1.6	8.6	5.2	6.7	8.1	7.2
Pirimiphos-methyl	0.8	11.5	0.6	8.9	2.0	6.1
Promecarb	3.9	7.9	3.0	3.3	1.4	5.3
Propamocarb	4.5	8.9	4.9	13.9	0.5	14.0
Propaphos	2.1	6.1	2.2	26.1	0.9	9.4
Propazine	3.1	13.2	4.5	4.3	10.9	11.8
Pyridaphenthion	4.2	7.0	2.5	14.9	4.6	9.9
Pyrimidifen	2.4	10.6	2.5	7.7	1.3	9.1
Pyriproxyfen	1.3	17.4	0.9	13.0	2.9	15.7
Tebuconazole	2.5	9.3	3.6	23.1	3.9	11.1
Tetraconazole	0.9	6.6	6.5	14.2	1.0	6.9
Thiacloprid	9.9	12.0	2.6	5.2	5.7	3.0
Triadimenol	0.8	9.4	2.7	5.6	4.9	2.5
Triazophos	3.4	8.4	2.2	10.6	8.5	6.3

[a] Mean value (n = 5).

3.2.6. Specificity

The specificity of the method was tested by analyzing blank samples. For this purpose, a further identification step via the SRM ratio was used for the unambiguous identification of the present compounds. This ratio was calculated as the quotient between the qualifier and the quantifier transitions. The recommended maximum permitted tolerances for relative ion intensities are given in the SANCO analytical quality control procedures for pesticide residue analysis [26]. The tolerance range indicated in these guidelines for LC–MS techniques is from 20% to 50%. In order to validate this SRM ratio in matrix we calculated them for the standard solutions in solvent and in each matrix. Then we obtained the average value for a range of concentration from 1 to 100 μg L^{-1}. The identification criteria set for each compound was very stable all throughout the defined linearity range, with values of RSD <20%.

3.3. Survey of the studied pesticides in real samples

In order to prove the effectiveness of the validated method and its suitability for routine analysis, it was applied to real samples. One hundred and six juice samples were purchased in different local markets in Almería, in the South of Spain, during a one-year period of time. Orange, pineapple, peach, apple, multifruit, mango, strawberry, tomato, pear, mandarin, grape, banana and grapefruit juices were analysed. The results, displayed in Table 5, show that 57% of the juices were blank in our scope, or contained pesticides at levels lower than the practical limits of quantification, while 43% of them contained one or more of the pesticides studied. A total number of nine compounds were found, among which one – diazinon – is not included in Directive 91/414/EEC, and another one, deet, is not authorized in the EU, and therefore any of them should

Table 5
Occurrence of pesticides in fruit juices purchased from the local stores during a one-year survey.

Food commodity	Pesticides	No. samples	Range of concentrations ($\mu g\,L^{-1}$)	No. of non-compliance samples
Orange juice (34 samples)	Carbendazim	15	5–12	0
	Diazion	1	10	1
	Imazalil	6	8–60	0
	Blanks	15	–	0
Pineapple juice (22 samples)	Carbendazim	2	14–43	0
	Triadimenol	2	7–10	0
	Blanks	18	–	0
Peach juice (14 samples)	Carbendazim	5	6–33	0
	Deet	1	16	1
	Metalaxyl	1	20	0
	Methoxyfenozide	1	19	0
	Tebuconazole	6	6–17	0
	Triadimenol	1	8	0
	Imazalil	2	6	0
	Blanks	4	–	0
Apple juice (8 samples)	Carbendazim	1	5	0
	Omethoate	1	7	0
	Imazalil	3	5–127	0
	Blanks	5	–	0
Multifruit juice (7 samples)	Carbendazim	1	16	0
	Imazalil	3	6–13	0
	Blanks	3	–	0
Mango juice (5 samples)	Imazalil	1	12	0
	Blanks	4	–	0
Strawberry juice (4 samples)	Triadimenol	1	7	0
	Imazalil	1	6	0
	Blanks	2	–	0
Tomato juice (4 samples)	Blanks	4	–	0
Pear juice (3 samples)	Imazalil	1	279	0
	Blanks	2	–	0
Mandarin juice (2 samples)	Imazalil	2	29–54	0
	Blanks	0	–	0
Grape juice (1 samples)	Blanks	1	–	0
Banana juice (1 samples)	Blanks	1	–	0
Grapefruit juice (1 samples)	Blanks	1	–	0

be used in the EU. Carbendazim and imazalil were the pesticides mostly found in the samples. As an example, Fig. 4 shows the specific SRM transitions of diazinon in a real sample (freshly squeezed orange juice), confirming the suitability of the developed method for monitoring of juices.

With the aim of verifying how the matrix components could affect quantitation, all the samples were re-analysed quantifying with solvent based calibration curves. The results obtained for all the pesticides found in the samples were similar to those calculated with matrix-matched calibration curves, with variations within the range of 20%, which reveal the possibility of performing quantitation with solvent based calibration curves, avoiding the use of matrix-matched calibration curves without an important increase in the uncertainty of the results.

4. Conclusions

The developed method allows qualitative and quantitative analysis of 53 pesticides in fruit juice by direct injection in LC–MS/MS, without any sample extraction, which results in a quick and simple analysis. Dilution of the sample permits reducing the amount of matrix going into the system, and thus, to decrease the matrix effects, so common in this type of commodities, opening the possibility to perform quantification with solvent based standards in the majority of the cases. Compound identification has been performed using SRM ratio calculations. The method has been validated for routine analysis, and has been applied to real samples as part of a one year survey of pesticides in fruit juices. As a result of these analyses, two non-authorized pesticides in the EU, diazinon and deet, were found in fruit juices.

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