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Analytical Methods

Fast screening of pesticide residues in fruit juice by solid-phase microextraction and gas chromatography–mass spectrometry

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Abstract

A new vanguard–rearguard analytical method for determining 54 pesticide residues in different fruit juices (natural and commercial orange, peach and pineapple juices were tested) is proposed. For that, in a first step, a fast screening (vanguard) method is applied for detecting those samples containing pesticides at concentrations above a pre-established cut-off value. In a second step, those samples are re-analyzed by a conventional pesticide residue (rearguard) method that confirms the presence of the pesticides and quantifies them. The sample process is very simple, fast and semiautomatic and therefore, it reduces significantly the average time required per sample, increases precision and minimizing human mistakes. Only 1 mL of juice sample is required for analysis. Pesticides are quickly extracted with ethyl acetate in a test tube, transferred to a mixture water:acetone 9:1 (v/v), and isolated by solid-phase microextraction (SPME). The SPME screening method only requires 10 min of SPME extraction. The SPME confirming/quantifying method requires 55 min of SPME extraction. The instrumental determination is carried out by gas chromatography–mass spectrometry (GC–MS) using a full scan acquisition mode for the screening method (less than 17 min of chromatographic run) and a tandem mass spectrometry (MS/MS) acquisition mode for the quantifying/confirming method (less than 70 min of chromatographic run). The use of full scan MS and tandem MS for the detection increase significantly the certainty of the results. Also, the combination of a solvent and SPME extractions and GC–MS/MS offers a significant selectivity and sensitivity with a proven reduction of false positive and negative cases. The use of a vanguard–rearguard strategy can reduce the 50% of the total time required for determining routinely juices in a laboratory by a traditional strategy (identification, confirmation and quantitation of the pesticides in the samples by a conventional analytical method).

Keywords: Screening; Vanguard–rearguard method; Pesticides; Juice; Solid-phase microextraction; Gas chromatography–mass spectrometry

1. Introduction

Pesticides are frequently used in pre- and post-harvest treatments for controlling diseases of fruits and vegetables. They may penetrate plant tissues and appear in processed products such as fruit juices, which are widely consumed, particularly by children that consume high amounts of juices and are more susceptible to chemicals (Albero, Sánchez-Brunete, & Tadeo, 2003; Albero, Sánchez-Brunete, & Tadeo, 2005; Goto et al., 2005; Topuz, Özhan, & Alpertunga, 2005).

Until now, European Union legislation has established maximum residue levels (MRLs) for raw fruit but not for processed products such as juice. However, it is also important to control pesticide residues in juice for assessing their significance to human exposition and therefore improving the protection of consumers' health.

Traditionally, conventional multiresidue methods have been developed for the analysis of pesticide residues in fruit juice (Chu, Hu, & Yao, 2005; Gomes et al., 2006; Sannino, Bolzoni, & Bandini, 2004; Schellin, Hauser, & Popp, 2004;

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Zhao, Han, Jiang, Wang, & Zhou, 2006). Unfortunately, multiresidue methods are frequently long and tedious and limit the laboratory throughput. Today, vanguard–rearguard strategies are beginning to be considered as an alternative to such traditional analytical methods establishing certain quality compromises (Baena & Valcárcel, 2003; Valcárcel & Cárdenas, 2002; Valcárcel & Cárdenas, 2005). Screening methods provide general answers based simply on binary yes/no responses rather than on detailed and discriminated chemical information (Simonet, Rios, & Valcárcel, 2004). Such binary responses are generated faster than results of conventional multiresidue methods and therefore, it is possible to take timely decisions. The screening method classifies samples between negative and potentially non-negative samples (those samples that contain any pesticide above a pre-established concentration called "cut-off value"). Later, a confirming/quantifying method (typically a conventional multiresidue method) is only applied to those samples previously classified as "potentially non-negative samples" in the previous fast screening method. The mentioned strategy is one of the solutions to the bottlenecks in analytical laboratories where, among others, preliminary operations and instrumental determinations are tremendously variable and complex and require close human attention.

Ideally, pesticide screening system should be based on rapid-response analytical tools and involving no sample treatment but it is often very difficult and frequently, it is necessary to apply at least a simple sample treatment. Operations such as sampling, storage, mass and volume measurement, dissolution, homogenization, purification, solvent changeover, reactions, non-chromatographic/chromatographic determination, and others prone to random and systematic errors, require qualified personnel, are expensive and reduce productivity of the laboratory (Valcárcel & Cárdenas, 2005). Automatization of such activities is highly recommended and the use of techniques such as solid-phase microextraction (SPME) can be considered as a selective and useful tool for automatic sampling and sample treatment (Farajzadeh & Hatami, 2004; Simplício & Vilas Boas, 1999; Zambonin, Quinto, De Vietro, & Palmisano, 2004).

In this paper, juice samples are screened by a rapid vanguard method that selects those samples that contain any pesticide above the cut-off value. In this way, the screening method acts as a sample filter selecting those juice samples that contain pesticides above the stated threshold limit in a fast and almost completely automated procedure. Due to the absence of legal threshold values, they have been considered as the MRL stated by the European Union for raw fruits. Later, those samples classified as "non-negative" samples are re-analyzed by a conventional quantitative multiresidue method. Both methods are based on the use of the selective combination of SPME together with gas chromatography–mass spectrometry (GC–MS). The mass analyzer is operated in full scan mode for the screening method and tandem MS (MS/MS) mode for the quantification/confirmation method. The full scan screening is fast and simple but selective enough for reducing considerably the risk of false positive or negative results at the typical concentrations of the pesticides in juices. The MS/MS mode increases selectivity and sensitivity being more adequate for quantitative purposes. It reduces drastically the negative influence of matrix interferences on quantitative data.

The proposed methodology has been applied to the analysis of 30 real juice samples of diverse types and compositions (natural and commercial orange, pineapple and peach juices). The proposed vanguard–rearguard methods reduced approximately a 50% of the total time required by a conventional GC–MS pesticide residue method.

2. Experimental

2.1. Reagents and materials

Individual pesticide standards (with purities always >95%) were obtained from Dr. Ehrenstorfer (Ausburg, Germany). Quintocene (1,2,3,4,5-pentachloro-6-nitro-benzene) was used as internal standard (IS). The solvents used, acetone, ethyl acetate, *n*-hexane, dichloromethane, and acetonitrile (with a quality for pesticide residue analysis) were used as provided by Scharlau (Barcelona, Spain). Stock standard solutions of individual compounds were prepared by exact weighing of the powder or liquid and dissolution in 100 mL of acetone (obtaining concentrations that ranged between 200 and 500 μg mL^{-1}), and stored in a freezer (−30 °C). A multicompound working standard solution was obtained by appropriate dilutions of stock solutions with acetone and stored under refrigeration (4 °C). Sodium chloride (extra pure quality) and standard solutions of HCl and NaOH were also obtained from Scharlau.

SPME fibers of polydimethylsiloxane (PDMS) with 100 μm of thickness and polydimethylsiloxane–divinylbenzene (PDMS–DVB) with 65 μm of thickness were purchased from Supelco (Bellefonte, PA, USA).

2.2. Apparatus

A Saturn 2000 GC–MS system from Varian (Walnut Creek, CA, USA) was used with a CP-8200 autosampler prepared to SPME analysis with an agitation device. The CP-3800 gas chromatograph was also fitted with a split/splitless programmed-temperature injector, a untreated fused silica capillary column 2 m × 0.25 mm i.d. from Supelco (Bellefonte, PA, USA) used as guard column, and a DB5-MS of 30 m × 0.25 mm i.d. × 0.25 μm film thickness (J&W Scientific, Folson, CA, USA) analytical column. The MS detector (an ion trap mass spectrometer) ionized the eluate using alternatively, chemical ionization (CI) and electron ionization (EI) modes in the same run. For screening purposes, the mass analyzer was operated in full scan mode. For confirming/quantifying purposes, it was programmed in MS/MS mode. The analyzer was composed of SilChrom coated electrodes to reduce chemical reactivity.

A National Institute of Standards and Technology (NIST) spectral library (version 2.0) and a MS/MS spectral library specially created for the target analytes under our experimental conditions were available. Helium (purity 99.9999%) was used as carrier and collision gas. A test tube shaker with a variable speed controller was purchased from Ika-Works, Inc. (Wilmington, NC, USA).

2.3. Extraction procedures

After sample homogenization, 1 mL of juice sample is mixed into a centrifuge tube with 1 mL of ethyl acetate using a test tube shaker for 2 min. The mixture is allowed to rest approximately 2 min until there is an effective separation among the phases. An aliquot of 0.5 mL of ethyl acetate extract is moved to a chromatography vial for its evaporation using a soft stream of nitrogen. One milliliter of a mixture water:acetone (9:1 v/v) containing the IS proposed (0.2 mg L^{-1}) is added to the vial. It is sealed and the dried residue is redissolved using the test tube shaker. The vial is placed into the carrousel of the GC autosampler to be automatically extracted by SPME. The SPME extraction to be applied is different for the screening and confirming/quantifying steps.

2.3.1. SPME extraction for screening pesticides

The PDMS–DVB fiber was introduced directly into the water:acetone extract for 10 min at ambient temperature and using the agitator device. After that, the analytes were desorbed introducing automatically the fiber into the injector port for 9 min. The injector temperature was programmed as follows: initial temperature set at 250 °C for 5 min, then it was increased to 300 °C at 100 °C min^{-1} (finally hold for 5 min). The split vent of the injector was kept closed during desorption time.

2.3.2. SPME extraction for confirming/quantifying the pesticides

The water:acetone (9:1 v/v) extracts of those samples considered in the screening step as non-negative samples were reanalyzed by SPME following the SPME extraction method described in Section 2.3.1 but modifying the absorption time from 10 to 55 min.

2.4. GC–MS analysis

2.4.1. Screening method

The carrier gas flow was kept constant at 1 mL min^{-1} and the column oven was programmed as follows: initial temperature set at 70 °C for 9 min, then it was increased to 300 °C at 50 °C min^{-1} (hold 4 min). The mass spectrometer was operated in full scan mode setting the ion trap, manifold and transfer line temperatures at 200, 50 and 280 °C, respectively. The multiplier voltage (1×10^5 gain) was 1600 V with a multiplier offset of +100 V. Automatic gain control (AGC) was turned on. For CI and EI operation, the AGC target value was always 20,000 counts; the emission current was 30 μA, the AGC pre-scan ionization time was 100 μs, the background mass and RF dump value were set at 85 and 450 Th, respectively. The mass analyzer was programmed for scanning between 85 and 450 Th. The compounds were identified monitoring their main characteristic full scan ions (highest sensitivity and selectivity). Such ions (Table 1) were also selected as parent ions for MS/MS processes in the confirming/quantifying method.

2.4.2. Confirming/quantifying method

The carrier gas flow was kept constant at 1 mL min^{-1} and the column oven was programmed as follows: initial temperature set at 70 °C for 9 min, then it was increased to 150 °C at 50 °C min^{-1}, then ramped at 5 °C min^{-1} to 180 °C (hold 6 min), then ramped to 195 °C at 1 °C min^{-1}, then to 243 °C at 50 °C min^{-1} and finally at 300 °C (hold 4 min) at 6 °C min^{-1}. For the mass spectrometer, the ion trap, manifold and transfer line temperatures were set at 200, 50 and 280 °C, respectively. The multiplier voltage (1×10^5 gain) was 1700 V with a multiplier offset of +200 V. Automatic gain control (AGC) was turned on. For CI and EI operation, the AGC target value was always 2000 counts; the emission current was 80 μA, the AGC pre-scan ionization time was 1500 μs, and the isolation window 3 Th. The rest of specific MS–MS conditions for the studied pesticides are listed in Table 1.

3. Results and discussion

3.1. Solvent extraction prior to the SPME process

A direct SPME extraction of juice samples may be compromised by the presence of interferences, specially caused by suspended matter as well as dissolved matter (in particular pectins) that are the main factors responsible for a reduced extraction efficiency by forming micelles, adsorbing the analytes and/or slowing down their diffusion towards the fiber (Simplício & Vilas Boas, 1999). A dilution of the juice sample with water was proposed as a solution to the interferences of pectins but if the juice is diluted with water before the extraction with SPME at the proposed ratios (between 1:50 and 1:100), sensitivity would decrease making difficult the determination of pesticides at low concentrations in juices. If the head space is analyzed by SPME, it is necessary to warm up significantly to get a partial volatilization of the pesticides (several pesticides present a moderate volatility). For that reason, we showed preference for evaluating a previous, simple and fast extraction with a small amount of organic solvent before SPME isolation. In that way, sensitivity is not drastically reduced and selectivity is clearly improved avoiding most matrix interferences.

The extraction efficiency was studied for various organic solvents and phase ratios. For that, *n*-hexane, dichloromethane, ethyl acetate and acetronitrile were evaluated for extracting 1 mL of orange juice spiked with the target pesticides at a single concentration of 0.1 mg L^{-1}. The phase ratios (juice:solvent) tested were 1:1, 1:2, 1.3 and

Table 1
Conditions MS/MS

Compound	Parent ion[a] (*m*/*z*)	Quantif. ion (*m*/*z*)	Excitation storage level (*m*/*z*)	Excitation amplitude (V)
Dichlorvos[b]	221	145 + 141	90	72
Propoxur[b]	168	111	70	40
Ethoprophos[b]	243	131 + 173	80	52
Diazinon[b]	304	179 + 162	110	85
Lindane	219	180:183	100	70
Pyrimethanil	198	102 + 129 + 155	75	81
Chlorthalonil	266	133	85	86
Etrimphos	292	181 + 263	70	45
Chlorpyriphos Met.	286	208	85	72
Parathion Met.	263	136 + 246	80	48
Metalaxyl	206	132 + 162	75	54
Pirimiphos Metil	290	151	85	64
Fenitrothion	260	125	71	59
Malation	173	99	75	51
Chlorpyriphos	314	258 + 286	170	100
Fenthion	278	135	112	92
Triadimephon	208	144 + 180	75	62
Tetraconazole	336	218	108	96
Dicofol	250	215	90	49
Pendimethalin	252	208 + 191 + 162	95	60
Penconazole	248	192 + 157	89	77
Isofenphos	213	185	93	52
Pyrifenox	262	189 + 227	100	90
Chlorfenvinphos	267	159	100	82
Procymidone	283	253:257	80	57
Triadimenol	168	70	48	38
Triflumizole	218	183	76	68
Chinomethionat	234	206	83	46
Endosulfan alfa	241	170:172	80	84
Hexaconazole	231	175 + 213	100	73
Fenamiphos	303	195	95	56
Buprofezin	249	191:195	80	50
Myclobutanil	179	125	80	65
Bupirimate	273	193	120	77
Chlorfenapyr	364	282 + 363 + 248	130	80
Endosulfan beta	241	170:172	80	84
Ethion	231	175 + 203	100	63
Benalaxyl	148	91	50	46
Carbofenothion	342	199 + 157	131	64
Endosulfan sulfate	272	235:238	80	64
Propiconazole	259	191 + 173	114	78
Nuarimol	235	139	75	56
Tebuconazole	250	125	75	63
Bromopropylate	341	181:187	70	45
Iprodione	314	245 + 271	125	88
Fenpropathrin	265	210	95	72
Tetradifon	229	197:203	100	97
Furatiocarb	325	194	140	77
Phosalon	182	111 + 138	80	77
Pirazophos	265	210	80	51
Pyridaben	309	147	126	81
Flucytrinate	157	107	79	69
Difenoconazole	323	265	122	87
Azoxystrobine	345	329	115	92

[a] Parent ions in MS/MS confirming/quantifying method and characteristic ions monitored in full scan mode for the screening method.
[b] Chemical ionization mode (methanol).

1:4. The best results were obtained when ethyl acetate was used at a phase ratio 1:1.

Prior to the SPME process, an aliquot of 0.5 mL of the extract was transferred to a chromatography vial of 2 mL and dried using a soft stream of nitrogen. The solution was not warmed up during the evaporation process. After that, the dried residue was re-dissolved in 1 mL of a mixture water:acetone (9:1 v/v) using a test tube shaker.

3.2. SPME extraction

Two different coating materials (PDMS and PDMS–DVB) were evaluated for the SPME extraction. For that, aliquots of water:acetone (9:1 v/v) spiked with the targets at the cut-off stated (their MRL in fruits) were extracted with the two tested SPME fibers using absorption times that ranged between 5 and 70 min. In general, the highest sensitivity was obtained for most pesticides absorbing them in the PDMS–DVB fiber for at least 55 min. This absorption time was set as optimum for the rearguard method, where a high sensitivity and precision is required for generating exact quantitative results. However, in order to simplify and reduce the total time of analysis of the vanguard (qualitative) method, its SPME absorption time was set at 10 min, the minimum time required for obtaining chromatographic signals with enough sensitivity and precision at the MRL of each pesticide (cut-off value).

Modifications of the ionic strength were also evaluated. For that, NaCl (5% w/v) was added ($n = 5$) to: (i) juice sample spiked with the target pesticides; or (ii) acetone:water (9:1 v/v) spiked with the target pesticides. The results showed that the modification of the ionic strength before the solvent extraction or the SPME extraction did not affect significantly to the final results obtained. It was observed that some compounds increased their sensitivity by a salting-out effect during the solvent extraction (lindane, malathion, iprodione) but others (bromopropylate, buprofezin, chlorpyriphos-methyl) reduced their instrumental response especially when the addition of salt was done just before the SPME extraction. It was attributed to a negative influence of the deposition of salt on the coating material. The influence of pH on the solvent and SPME extraction processes was also evaluated. Values between pH 1 and pH 9 were used, with diluted HCl and NaOH solutions being used to adjust the pH. Non-significant differences were observed in the extraction rates at the pH-values studied. So, it was not necessary to adjust the pH before the SPME extraction/clean-up.

After that, desorption process was optimized. The injector temperature was programmed to be increased from 70 to 300 °C in order to vaporize each pesticide at its optimum temperature avoiding possible thermal degradations. Long exposure periods of the PDMS–DVB fiber to 300 °C can degrade it, causing loss of active centers and therefore reduction of effectiveness, but at least 9 min of desorption time were necessary for completely desorbing some pesticides (synthetic pyrethroid insecticides like fenvalerate and deltamethrin). However, it was checked that the fibers can be used in the proposed experimental conditions for at least 75–100 analyses.

3.3. Gas chromatographic analysis

3.3.1. GC–MS (full scan mode) screening method

A fast GC–MS (full scan mode) analysis is proposed for screening the target pesticides in juice samples and therefore increasing the throughput of the laboratory. All the target pesticides were monitored in less than 17 min thanks to the fast GC temperature program set (a column temperature gradient of 50 °C min^{-1} was applied). It means a 4-fold gain in analysis time saved compared to conventional GC–MS methods (see Section 3.3.2). The gas chromatographic separation of the target analytes is not critical because it is not completely necessary to achieve an unequivocal identification of the targets by the screening method. Nevertheless, the high selectivity of the proposed extraction method (composed of two selective extraction steps) together with the selective MS experimental conditions set for the instrument (programmed for reducing the low mass MS noise caused by the matrix) helped to increase the identification capability of the proposed MS detection method. Fig. 1a shows the gas chromatograms obtained for the target compounds in the proposed full scan MS experimental conditions.

3.3.2. GC–MS/MS confirming/quantifying method

The target pesticides were GC separated using an oven temperature program that yielded their determination in approximately 70 min. The proposed separation avoids coelutions of more than 5–6 compounds and therefore, they could be determined simultaneously by MS/MS in the ion-trap analyzer without technical limitations (Garrido Frenich, González Rodríguez, Arrebola, & Martínez Vidal, 2005; Martínez Vidal, Arrebola, & Mateu-Sánchez, 2002). The specific MS/MS conditions applied increased significantly the selectivity of the detection reducing drastically the risk of false positives or negatives. Fig. 1b shows the gas chromatograms obtained for the target compounds in the proposed MS/MS experimental conditions.

3.4. Validation of the method

Validation of the method was carried out spiking blank commercial juices of orange, pineapple and peach with the target pesticides.

3.4.1. Validation of the screening method

The main aim of this screening method is to offer information about whether the concentration of a specific compound in a juice is under or over its considered MRL. Performance characteristics of any analytical method applied to monitor samples should be assessed but the lack of guidance dealing with screening method validation in the area of pesticide residues makes necessary selecting quality parameters based on the Eurachem Guide (CITAC/EURACHEM, 2002; Pulido, Ruisánchez, Bosque, & Rius, 2002) such as, selectivity, percent of false positives and negatives, cut-off, detection limit, and unreliability region.

The identification of the target pesticides in the juice samples was carried out by comparison of: (i) relative retention times (RRT) obtained when the main(s) characteristic ion(s) of the target compounds were monitored and (ii) the full

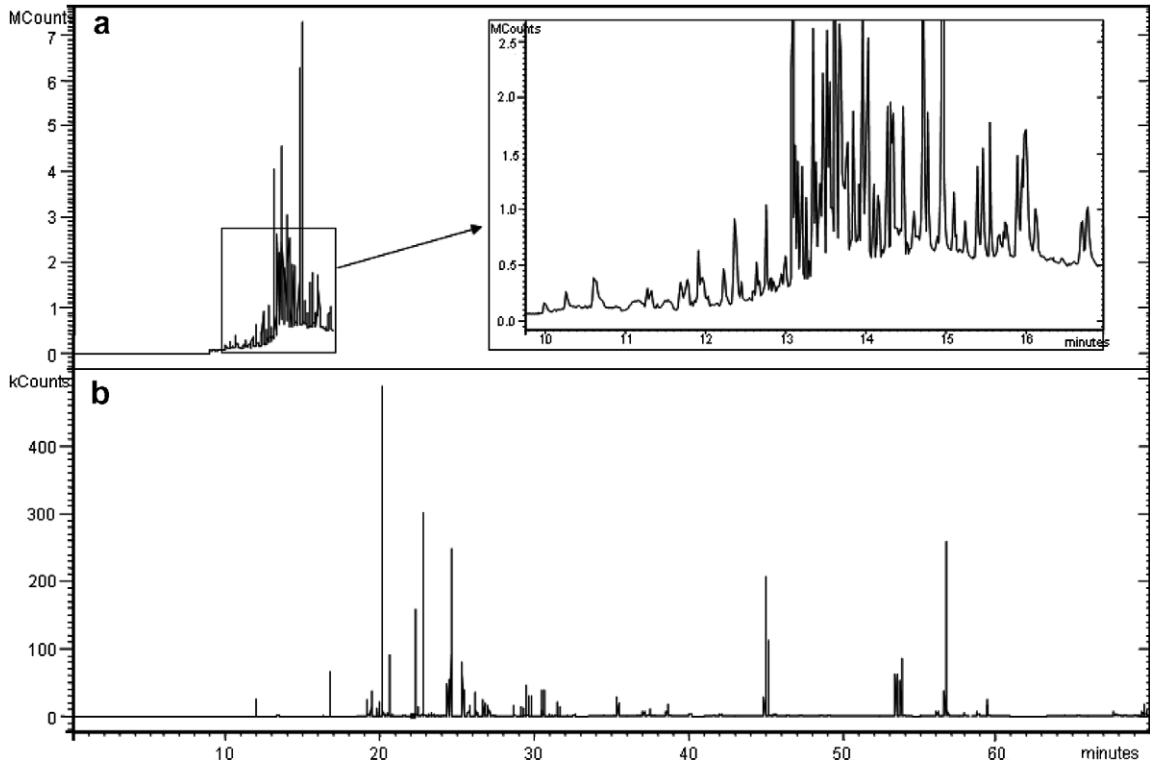

Fig. 1. Chromatograms of an orange juice spiked with the target pesticides at a concentration of 0.05 mg L^{-1} (a) obtained with the screening full scan method and (b) obtained with the confirming/quantifying MS/MS method.

scan MS spectrum obtained in the sample and the stored ones in the NIST spectral database. It is worth noting that the rearguard method is not applied to confirm negative samples and so, it is important to reduce the rate of false negatives in the screening method. Therefore, conservative identification criteria were stated for identification of the target pesticides by the screening method. So, a juice sample was considered as a potentially non-negative sample, when at least one chromatographic peak was detected into a relative retention time windows (RRTW) of the target compounds. They were defined as the average relative retention time (the ratio of the chromatographic retention time of the analyte to that of the IS) ±3 standard deviations of the relative retention times obtained when 6 blank juice samples spiked at the first calibration level of each compound were analyzed (Table 2 shows the RRTW stated for the chromatographic conditions of the vanguard method). Additionally, the mass spectra fit between the suspicious chromatographic peak and the NIST database should be higher than 500 (scaled to 1000). These criteria reduced drastically the risk of false negatives obtained with the screening method at the cut-off value. Such cut-off values were stated as the minimum MRL stated for raw fruits in the European Union legislation (see Table 2).

Limits of detection (LOD) were considered as the minimum concentration of analytes that generated a response three times greater than the noise level of the detection system ($n = 3$). They are shown in Table 2. They were all clearly below the cut-off stated.

A recovery and precision study was carried out at the cut-off concentration value for each target compound. For that, five blank samples were spiked with the studied pesticides and processed by the screening method. The recovery and precision (relative standard deviation, RSD%) data obtained are summarized in Table 2. All the recovery rates were between 73% and 96% and the RSD% was always lower than 25%. The unreliability region was considered as the analyte concentration range around the cut-off value in which false positive or false negative responses are produced with a statistical probability (a 5% error was fixed to obtain a false positive or a false negative). The concentration of the samples would be in two different regions: (i) region of reliability, if the concentration obtained was lower (or higher) than the lower (upper) limit, that means negative (or positive) sample, and (ii) region of unreliability, if the concentration obtained was lower (or higher) than the cut-off value corresponding to the region of false positive (or false negative) sample. All samples with concentrations of the target pesticides equal to or higher than the lower limit shall be confirmed by the rearguard method.

3.4.2. Validation of the confirming/quantifying method

3.4.2.1. Identification and confirmation of target analytes. A first step in the identification of the pesticides was based on the MS screening method. In addition, the identification of the detected pesticides by the screening method was carried out by searching for them in the appropriate RRTWs of

Table 2
Relative retention time windows (RRTW), limits of detection (LOD), cut-off value, recovery (*R*%) and precision (RSD%) at the cut-off value concentration and unreliability interval for the vanguard method

Compound	RRTW (min)	LOD (μg L^{-1})	Cut-off conc. (mg L^{-1})	*R*%	RSD%	Unreliability interval (μg L^{-1})
Dichlorvos	0.86–0.91	12.1	0.05	84	8	0.042–0.056
Propoxur	0.93–0.98	2.5	0.01	75	15	0.005–0.014
Ethoprophos	0.97–1.02	1.8	0.01	86	5	0.006–0.013
Diazinon	0.99–1.05	1.7	0.02	75	6	0.016–0.024
Lindane	1.00–1.05	0.8	0.01	89	7	0.007–0.012
Pyrimethanil	1.00–1.05	2.3	0.02	81	6	0.015–0.023
Chlorthalonil	1.01–1.06	6.2	0.1	94	5	0.090–0.108
Etrimphos	1.00–1.05	2.3	0.01	86	6	0.005–0.014
Chlorpyriphos Met.	1.02–1.07	0.9	0.02	89	17	0.008–0.012
Parathion Met.	1.02–1.07	1.8	0.1	95	16	0.093–0.105
Metalaxyl	1.02–1.07	19.6	0.05	91	11	0.042–0.057
Pirimiphos Metil	1.02–1.07	1.5	0.01	90	7	0.004–0.016
Fenitrothion	1.03–1.08	2.6	0.1	74	14	0.092–0.108
Malation	1.03–1.08	2.1	0.1	78	18	0.091–0.108
Chlorpyriphos	1.03–1.08	0.8	0.02	83	18	0.015–0.023
Fenthion	1.03–1.08	3.1	0.02	89	13	0.014–0.024
Triadimephon	1.04–1.08	3.5	0.05	87	16	0.044–0.055
Tetraconazole	1.03–1.08	0.9	0.01	84	15	0.004–0.017
Dicofol	1.04–1.09	1.5	0.02	96	17	0.016–0.024
Pendimethalin	1.04–1.09	1.3	0.02	90	21	0.012–0.027
Penconazole	1.04–1.09	2.7	0.01	80	9	0.005–0.014
Isofenphos	1.04–1.09	3.1	0.01	84	6	0.006–0.013
Pyrifenox	1.05–1.10	1.7	0.05	87	25	0.041–0.056
Chlorfenvinphos	1.05–1.10	1.4	0.01	78	13	0.006–0.014
Procymidone	1.05–1.10	1.9	0.1	89	17	0.092–0.107
Triadimenol	1.05–1.10	9.1	0.05	73	16	0.044–0.055
Triflumizole	1.05–1.10	2.3	0.02	76	9	0.016–0.023
Chinomethionat	1.06–1.11	1.4	0.01	78	15	0.006–0.014
Endosulfan alfa	1.06–1.11	1.1	0.01	93	16	0.006–0.015
Hexaconazole	1.08–1.13	0.8	0.01	86	8	0.007–0.014
Fenamiphos	1.08–1.13	1.5	0.02	94	11	0.016–0.023
Buprofezin	1.08–1.13	2.7	0.01	76	14	0.005–0.015
Myclobutanil	1.09–1.14	2.1	0.01	78	18	0.005–0.014
Bupirimate	1.09–1.14	1.7	0.01	86	12	0.006–0.013
Chlorfenapyr	1.09–1.14	1.6	0.05	85	19	0.044–0.053
Endosulfan beta	1.09–1.14	1.3	0.02	89	11	0.016–0.023
Ethion	1.09–1.14	1.8	0.01	80	16	0.007–0.013
Benalaxyl	1.09–1.14	1.2	0.05	96	12	0.043–0.054
Carbofenothion	1.10–1.15	0.9	0.01	94	11	0.005–0.014
Endosulfan sulfate	1.10–1.15	1.4	0.02	92	15	0.014–0.023
Propiconazole	1.11–1.16	1.3	0.02	87	15	0.015–0.024
Nuarimol	1.11–1.16	0.6	0.01	84	10	0.006–0.013
Tebuconazole	1.11–1.16	1.2	0.02	73	9	0.015–0.024
Bromopropylate	1.12–1.17	0.4	0.05	90	14	0.043–0.054
Iprodione	1.11–1.16	1.3	0.1	78	8	0.091–0.107
Fenpropathrin	1.12–1.17	0.9	0.01	75	21	0.005–0.014
Tetradifon	1.14–1.19	0.7	0.01	86	20	0.005–0.013
Furatiocarb	1.14–1.19	1.1	0.01	89	14	0.006–0.013
Phosalon	1.14–1.19	1.5	0.1	80	6	0.095–0.104
Pirazophos	1.15–1.20	0.8	0.01	90	8	0.007–0.013
Pyridaben	1.19–1.24	1.3	0.01	91	18	0.006–0.014
Flucytrinate	1.23–1.28	0.8	0.01	78	19	0.005–0.014
Difenoconazole	1.28–1.33	1.1	0.01	76	6	0.005–0.013
Azoxystrobine	1.31–.1.35	7.1	0.05	79	17	0.041–0.058

the confirmation method (Table 3 shows such RRTW for the rearguard gas chromatographic conditions). Potentially positive samples were confirmed monitoring the product ion mass spectra (MS/MS spectra) obtained from the selected precursor ions after the collision-induced dissociation applied. The detected compounds were considered to be definitely confirmed if the abundance ratios for the main ions were within approximately 20% of those obtained on the same day from the calibration standard in the matrix at the concentration of the first calibration level.

Table 3
Relative retention time windows (RRTW), limits of detection (LOD) and quantitation (LOQ), calibration range, recovery (*R*%) and precision (RSD%) for the rearguard method

Compound	RRTW (min)	LOD (μg L^{-1})	LOQ (μg L^{-1})	Range (mg L^{-1})	*R*% (RSD%)		
					Peach	Pineapple	Orange
Dichlorvos	0.57–0.67	8.3	25	0.05–0.5	91 (4)	90 (4)	98 (3)
Propoxur	0.80–0.90	1.7	5	0.01–0.1	91 (5)	90 (5)	102 (11)
Ethoprophos	0.82–0.92	0.3	1	0.01–0.1	92 (4)	90 (8)	93 (3)
Diazinon	0.98–1.08	0.08	0.25	0.02–0.2	87 (3)	88 (2)	93 (3)
Lindane	0.96–1.06	0.08	0.25	0.01–0.1	89 (5)	95 (4)	90 (6)
Pyrimethanil	1.00–1.10	0.08	0.25	0.02–0.2	90 (2)	95 (4)	91 (3)
Chlorthalonil	1.00–1.11	0.11	0.33	0.1–1	97 (2)	91 (7)	98 (2)
Etrimphos	1.02–1.12	0.07	0.2	0.01–0.1	92 (4)	96 (2)	100 (5)
Chlorpyriphos Met.	1.11–1.21	0.08	0.25	0.02–0.2	88 (3)	95 (5)	88 (9)
Parathion Met.	1.14–1.24	0.33	1	0.1–1	98 (4)	94 (4)	102 (8)
Metalaxyl	1.17–1.27	16.7	50	0.05–0.5	99 (9)	94 (5)	102 (5)
Pirimiphos Metil	1.22–1.32	0.05	0.16	0.01–0.1	92 (4)	88 (3)	93 (3)
Fenitrothion	1.23–1.33	1.33	4	0.1–1	93 (5)	96 (3)	101 (7)
Malation	1.27–1.37	1.67	5	0.1–1	96 (4)	94 (2)	106 (14)
Chlorpyriphos	1.29–1.39	0.05	0.15	0.02–0.2	86 (6)	89 (2)	71 (9)
Fenthion	1.32–1.42	0.17	0.5	0.02–0.2	94 (5)	90 (2)	90 (6)
Triadimephon	1.34–1.44	0.17	0.5	0.05–0.5	94 (7)	88 (2)	100 (10)
Tetraconazole	1.35–1.45	0.33	1	0.01–0.1	94 (6)	93 (2)	98 (8)
Dicofol	1.36–1.46	0.13	0.4	0.02–0.2	86 (6)	84 (2)	86 (9)
Pendimethalin	1.45–1.55	0.04	0.12	0.02–0.2	86 (2)	91 (2)	75 (12)
Penconazole	1.48–1.58	0.33	1	0.01–0.1	88 (3)	89 (6)	95 (5)
Isofenphos	1.49–1.59	0.07	0.2	0.01–0.1	92 (2)	89 (4)	95 (2)
Pyrifenox	1.50–1.60	0.33	1	0.05–0.5	90 (5)	92 (6)	108 (15)
Chlorfenvinphos	1.50–1.60	0.17	0.5	0.01–0.1	91 (4)	90 (4)	95 (5)
Procymidone	1.55–1.65	1.67	5	0.1–1	95 (3)	88 (5)	107 (13)
Triadimenol	1.59–1.69	8.3	25	0.05–0.5	95 (4)	89 (6)	104 (6)
Triflumizole	1.57–1.67	0.33	1	0.02–0.2	94 (4)	95 (4)	93 (3)
Chinomethionat	1.60–1.69	0.08	0.25	0.01–0.1	88 (3)	91 (6)	87 (12)
Endosulfan alfa	1.65–1.75	0.33	1	0.01–0.10	89 (3)	91 (7)	85 (12)
Hexaconazole	1.75–1.85	1.67	5	0.01–0.1	95 (2)	88 (5)	98 (3)
Fenamiphos	1.75–1.85	0.67	2	0.02–0.2	91 (2)	86 (4)	88 (5)
Buprofezin	1.89–1.98	0.17	0.5	0.01–0.1	90 (3)	90 (8)	87 (7)
Myclobutanil	1.87–1.97	0.17	0.5	0.01–0.1	90 (2)	93 (2)	85 (10)
Bupirimate	1.91–2.01	0.17	0.5	0.01–0.1	91 (2)	89 (4)	92 (3)
Chlorfenapyr	1.97–2.07	0.33	1	0.05–0.5	81 (5)	84 (8)	96 (14)
Endosulfan beta	2.04–2.14	0.67	2	0.02–0.2	91 (3)	89 (5)	90 (10)
Ethion	2.15–2.25	0.07	0.2	0.01–0.1	79 (11)	86 (2)	71 (7)
Benalaxyl	2.30–2.40	0.33	1	0.05–0.5	97 (6)	91 (5)	97 (6)
Carbofenothion	2.32–2.42	0.67	2	0.01–0.1	95 (5)	90 (5)	97 (6)
Endosulfan sulfate	2.33–2.43	1.33	4	0.02–0.2	87 (2)	91 (4)	88 (8)
Propiconazole	2.42–2.52	0.67	2	0.02–0.2	90 (2)	89 (2)	93 (7)
Nuarimol	2.48–2.58	1.67	5	0.01–0.1	92 (7)	95 (4)	93 (3)
Tebuconazole	2.51–2.61	1.33	4	0.02–0.2	91 (5)	90 (5)	92 (2)
Bromopropylate	2.76–2.86	0.03	0.1	0.05–05	80 (3)	85 (2)	74 (10)
Iprodione	2.74–2.84	0.33	1	0.1–1	89 (3)	89 (2)	91 (3)
Fenpropathrin	2.83–2.93	0.33	1	0.01–0.1	90 (4)	93 (3)	97 (16)
Tetradifon	2.88–2.99	0.07	0.2	0.01–0.1	77 (6)	84 (3)	78 (13)
Furatiocarb	2.90–3.01	0.17	0.5	0.01–0.1	90 (6)	84 (5)	101 (11)
Phosalon	2.91–3.02	0.01	0.03	0.1–1	85 (6)	89 (2)	89 (4)
Pirazophos	3.05–3.16	0.03	0.1	0.01–0.1	89 (7)	93 (5)	95 (2)
Pyridaben	3.17–3.27	0.33	1	0.01–0.1	84 (7)	90 (8)	72 (14)
Flucytrinate	3.34–3.44	0.07	0.2	0.01–0.1	86 (6)	84 (8)	79 (17)
Difenoconazole	3.51–3.62	0.07	0.2	0.01–0.1	88 (5)	88 (2)	92 (3)
Azoxystrobine	3.58–3.68	1.67	5	0.05–0.5	91 (4)	94 (5)	95 (8)

3.4.2.2. Limits of detection (LOD) and quantitation (LOQ). Limits of detection (LOD) of the rearguard method were calculated as previously described in Section 3.4.1 for the vanguard method. Limits of quantitation (LOQ) were stated as the minimum concentration of the analytes that were quantified with a precision of 15% (expressed as relative standard deviation, RSD). The LOD values ranged between 0.01 and 16.7 μg L^{-1} and

the LOQ values ranged between 0.1 and 50 μg L^{-1} (Table 3). In all cases, LOD and LOQ were lower than the cut-off values stated and always lower than those obtained in the full scan (vanguard) method.

3.4.2.3. Quantitation of target analytes. Samples were quantified analyzing aliquots of blank sample spiked with the pesticides at three different concentration levels to perform the calibration curves. The concentrations of the calibration levels depended on the cut-off values set and are shown in Table 3. The first calibration level was always equal to, or lower than, the MRL established for raw fruits. Linear or polynomial calibration graphs were constructed considering relative areas (analyte/IS) of the calibration standards. Good fit was found in the concentration range studied, with determination coefficients always higher than 0.99.

3.4.2.4. Trueness and precision. Recovery efficiency data were obtained by analyzing uncontaminated juices ($n = 6$) spiked at the first and second calibration level. Values between 71% and 108% were obtained for all compounds. These values indicated acceptable recovery for the assay procedure. The intra-assay (repeatability) precision was assessed, at the two concentration levels of the recovery studies, by extraction and analysis on the same day of six fortified vegetable samples for each level (Table 3). Precision

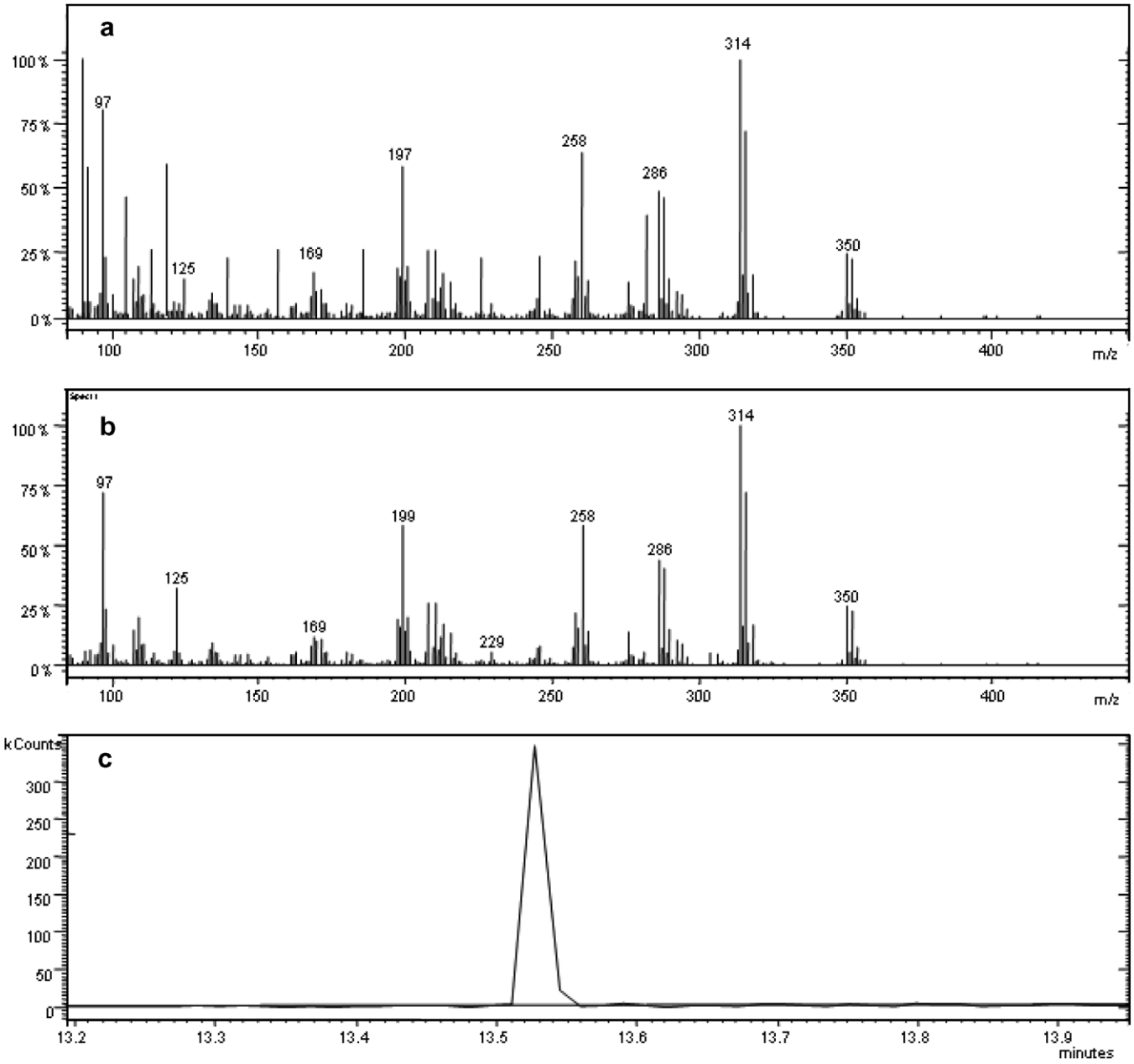

Fig. 2. (a) Full scan mass spectrum obtained screening a real orange juice sample that contained chlorpyriphos above the cut-off value (0.02 mg L^{-1}); (b) mass spectrum obtained from the library; (c) gas chromatogram (monitoring of m/z 314) obtained with the vanguard method.

values, expressed as relative standard deviation (RSD), were lower than 16% for all pesticides.

3.5. Analysis of real samples

Various real juice samples were analyzed to test the feasibility of the proposed analytical methodology for the analysis of pesticide residues in juice: 15 juice samples commercially available (orange, pineapple and peach) and 15 natural juice samples obtained in the laboratory from fresh fruits (also orange, pineapple and peach). For that, various internal quality criteria have been established to assure that the measurement chemical processes (vanguard and rearguard methods) are under statistical control. The set of samples analyzed each day was always processed together with: (i) a blank sample extract analyzed with the vanguard and rearguard methods that eliminates a false positive caused by contamination in the extraction process, instrument or chemicals used; (ii) a blank extract spiked at a concentration of the first calibration level (cut-off value) in order to identify potentially positive samples by the screening method; (iii) a calibration curve for quantifying the results in the rearguard method and (iv) a blank extract spiked at a concentration of second calibration level in order to assess the extraction efficiency for the vanguard and rearguard methods. Recovery rates between 60 and 120% are accepted if (a) the majority of recoveries are within the 70–110% range and, (b) samples which contain residues in a batch are reanalyzed and the results reported are within the 70–110% range.

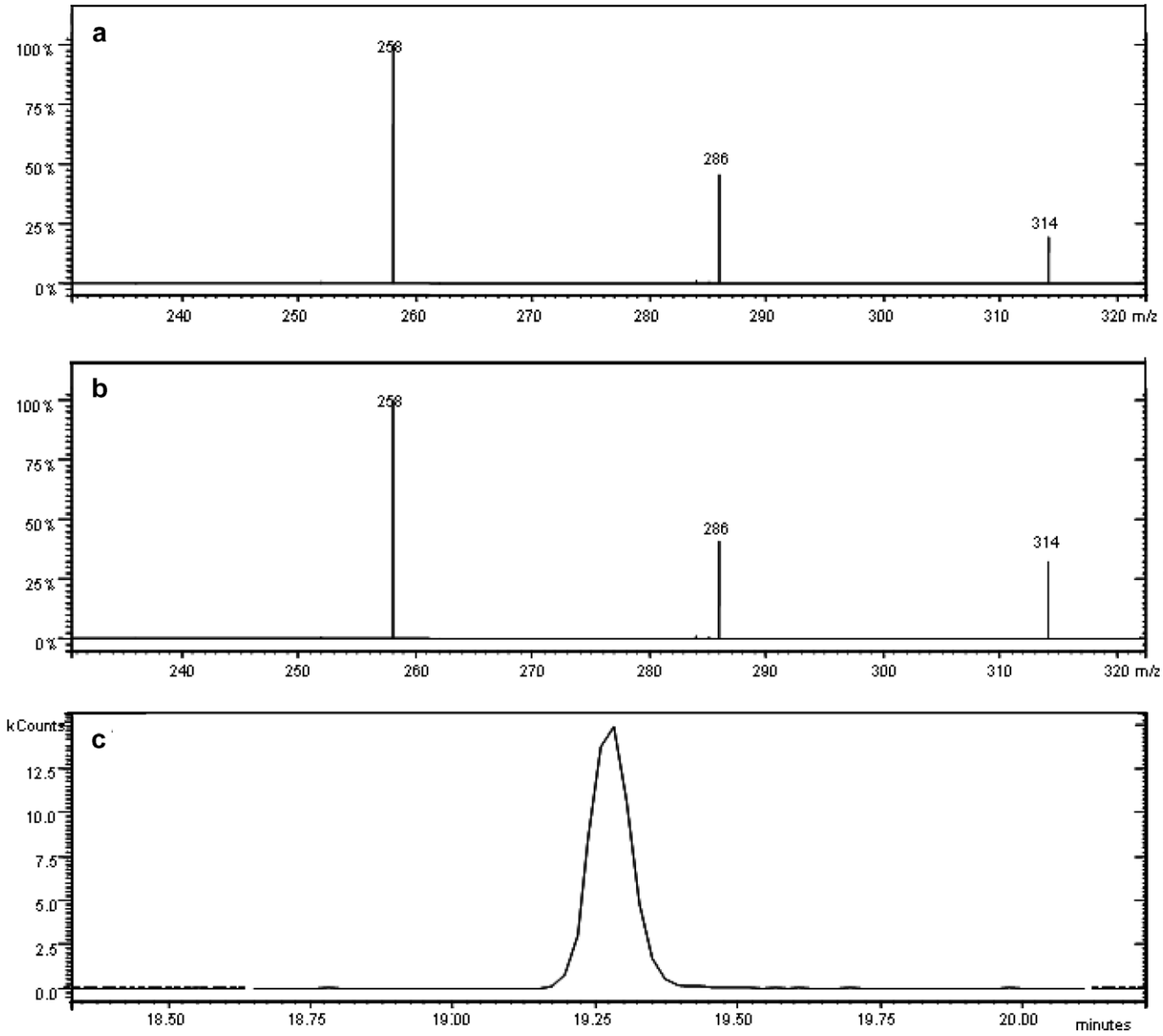

Fig. 3. (a) MS/MS mass spectrum obtained confirming/quantifying the real orange juice sample that contained chlorpyriphos (0.048 mg L^{-1}); (b) MS/MS mass spectrum stored in the library; (c) gas chromatogram obtained with the rearguard method.

None of the analyzed commercial samples presented pesticides at concentrations higher than the cut-off value but six natural juices contained trace amounts of some pesticides (pyrethroids) below the cut-off value and one natural orange juice contained chlorpyriphos above the cut-off value (0.02 mg L^{-1}) of the screening method. Such result was confirmed and quantified by the rearguard method determining 48 μg L^{-1} of the mentioned organophosphorus insecticide. Figs. 2 and 3 shows the chromatographic signals and MS and MS/MS spectra (experimental spectra compared with the spectra stored in the libraries) obtained for this sample using the screening and confirming/quantifying methods.

It is important to mention that the analysis of the 30 juice samples by a conventional multiresidue method would have required approximately 62.5 h (i.e. the proposed for confirming/quantifying that needs 55 min of sample treatment plus 70 min of GC determination). However, the screening of all the samples needed only 12.5 h and the confirmation/quantitation of the 7 non-negative samples (approximately a 23% of the total analyzed samples) consumed approximately 15 h more (the whole process required a total of 27.5 h). Therefore, the use of a vanguard/rearguard strategy implies a reduction of more than 50% of the time required using a conventional strategy.

4. Conclusions

The proposed methodology is an application of the vanguard–rearguard analytical strategy to the control of pesticide residues in juice fruits (orange, pineapple and peach). The process can be summarized as follow: (i) fast and miniaturized extraction of the juice samples with 1 mL of ethyl acetate; (ii) fast screening of all the samples by SPME–GC–MS (full scan mode) for labeling the samples as negatives or potentially non-negatives; (iii) analysis of the potential non-negative samples by SPME–GC–MS–MS for confirming and quantifying the pesticide residues of the samples. The extraction process is simple and selective due to a double extraction/purification step: a fast organic solvent extraction and SPME isolation before GC–MS analyses that minimize matrix interferences and achieve enough sensitivity at the MRL stated by the EU for raw fruits. The screening and confirming/quantifying methods were validated following the CITAC/EURACHEM recommendations and applied to the analysis of 30 real juice samples (natural and commercial juices) demonstrating the feasibility of the proposed methodology for its application in routine laboratories where the sample throughput can be significantly improved (more than a 50%).

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