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Determination of herbicide residues in juice by matrix solid-phase dispersion and gas chromatography–mass spectrometry☆

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Abstract

A rapid multiresidue method was developed for the determination of 15 herbicides in carrot, grape, and multivegetable juices. The analytical procedure was based on the matrix solid-phase dispersion of juice samples on Florisil, placed in glass columns, and subsequent extraction with ethyl acetate with assisted sonication. The recoveries through the method ranged from 82 to 115% with relative standard deviations equal or lower than 10% for all the herbicides studied. The analysis of samples was accomplished using gas chromatography–mass spectrometry with selected ion monitoring. Spiked blank samples were used as standards to counteract the matrix effect observed in the chromatographic determination. The detection limits ranged from 0.1 to 1.6 μg/l. The developed method was applied to the analysis of herbicide residues in commercial juice samples.

Keywords: Fruit juices; Matrix solid-phase dispersion; Extraction methods; Pesticides

1. Introduction

Herbicides are widely used in horticultural crops to control weeds that may produce important yield reductions. The introduction of these pesticides in the food chain via the environment can be considered a risk for human health due to the toxicity of most of these compounds.

Methods used to determine herbicide residues in fruits and vegetables are mainly based on liquid partitioning with organic solvents such as ethyl acetate, acetonitrile, and dichloromethane, usually followed by a solid-phase extraction cleanup step [1–6]. In the last years, new extraction procedures have been developed to overcome the drawbacks caused by using high amounts of glassware and toxic solvents in the classical liquid extraction methods. With this aim, several procedures based on solid-phase microextraction (SPME) [7], supercritical fluid extraction (SFE) [8–10], pressurized liquid extraction (PLE) [11], and microwave-assisted extraction (MAE) [12] have been used.

Matrix solid-phase dispersion (MSPD), based on the dispersion of the sample on an adsorbent, such as Florisil, C_{18}, alumina, or silica, allows the extraction and cleanup of analytes in one single step. The dispersion of solid samples is previously done in a mortar and then the mixture is transferred to the extraction columns [13]. In the case of liquid samples, the dispersion of the matrix in the adsorbent can be done directly in the extraction columns [14,15].

The determination of herbicides in fruits and vegetables can be performed by gas chromatography with electron-capture detection (ECD) [11,13] or nitrogen–phosphorus detection (NPD) [6,12], when herbicide molecules have functional groups giving a selective and sensitive response with those detectors. Gas chromatography coupled with mass spectrometry has also been employed in the determination of herbicides due to its high selectivity when used in the selected ion monitoring (SIM) mode [2,4,5,7,10]. Herbicides with a low volatility or thermally unstable are determined by liquid chromatography with ultraviolet [3,4] or fluorescence detection [5]. The application of enzyme-linked immunosorbent assays (ELISA) in the determination of herbicides has also been reported [16,17].

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The aim of this work was to develop a multiresidue method, based on matrix solid-phase dispersion, for the determination of 15 herbicides belonging to different chemical classes (triazines, dinitroanilines, thiocarbamates, and chloroacetanilides) in carrot, grape, and multivegetable juices. Residues were determined by GC–MS with selected ion monitoring and the developed method was applied to the determination of herbicide residues in commercial juices.

2. Experimental

2.1. Materials

2.1.1. Chemicals

Herbicide standards (99% purity) were purchased from Riedel-de Häen (Seelze, Germany). Ethyl acetate and methanol (pesticide grade) were obtained from Scharlau (Barcelona, Spain). Florisil 60–100 mesh, heated at 140 °C overnight before use, was purchased from Fluka (Buchs, Switzerland).

2.1.2. Herbicide solutions

Stock solutions (500 μg/ml) of each herbicide were prepared by dissolving 50 mg of the herbicide in 100 ml ethyl acetate and stored at 4 °C. Three stock solutions containing 0.2, 0.1, and 0.02 μg/ml of each pesticide in methanol were prepared and used to fortify the juice samples. Standard solutions in ethyl acetate were prepared to fortify blank juice samples used as chromatographic standards.

2.1.3. Internal standard solutions

The internal standard solution was prepared by dissolving lindane in ethyl acetate to obtain a 500 μg/ml solution. A working internal standard solution of 0.05 μg/ml in ethyl acetate was prepared.

2.1.4. Columns

Glass columns (10 cm × 2 cm i.d.) were purchased from Pobel (Madrid, Spain) and Whatman No. 1 filter paper circles of 2 cm diameter, placed at the bottom end, were from Whatman (Maidstone, UK).

2.2. Apparatus

2.2.1. GC–MS

An Agilent 6890 gas chromatograph (Waldbronn, Germany) equipped with an automatic injector Model HP 7683 and a 5973 series mass selective detector was used. A fused silica capillary column (ZB-5MS), 5% phenyl polysiloxane as nonpolar stationary phase (30 m × 0.25 mm i.d.) and 0.25 μm film thickness, supplied by Phenomenex (Torrance, CA, USA), was employed. Operating conditions were as follows: injector port temperature 280 °C, injection volume 2 μl in pulsed splitless mode (pulsed pressure 45 psi for 1.5 min; 1 psi = 6894.76 Pa); helium as carrier gas at a flow-rate of 1.0 ml/min; oven temperature program: 70 °C (2 min), programmed at 25 °C/min to 150 °C, increased to 200 °C at 3 °C/min, followed by a final ramp to 280 °C at a rate of 8 °C, and held for 5 min. The total analysis time is 36.87 min and the equilibration time 2 min.

Mass spectrometric parameters: electron impact ionization mode with an ionizing energy of 70 eV scanning from *m*/*z* 60 to 500 at 4.45 scan/s, ion source temperature 230 °C, MS Quad temperature 150 °C, electron multiplier voltage maintained 1000 V above autotune; solvent delay, 5 min.

Analysis was performed with SIM based on the use of target and qualifier ions. Herbicides are identified according to the retention times, target ions and the qualifier-to-target ion ratios. The target and qualifier abundances were determined by injection of individual herbicide standards under the same chromatographic conditions in full-scan from *m*/*z* 60 to 500. Quantification was based on the peak area ratio of the target ion divided by the peak area of the internal standard. Standards were prepared spiking blank samples to counteract possible matrix effects. Table 1 lists the herbicides studied with their retention times, the target and qualifier ions together to the qualifier to target abundance ratios. The SIM program used to analyse herbicides in juice is summarised in Table 2.

Table 1
Retention time (t_R), molecular mass (M_r), target ion (*T*), qualifier ion (*Q*), and abundance ratio of qualifier ion/target ion (*Q*/*T*)[a] of the herbicides studied

	Pesticide	t_R(min)	M_r	*T*	*Q*	*Q*/*T* (%)
1	EPTC	7.94	189.3	128	189	23.7
2	Propachlor	12.33	211.7	120	176	36.6
3	Trifluralin	13.75	335.5	306	264	74.9
4	Simazine	15.24	201.7	201	186	60.2
5	Atrazine	15.50	215.7	200	215	61.1
6	Terbumeton	15.80	225.3	210	169	83.9
7	Lindane[b]	15.90	290.8	183	219	84.6
8	Terbuthylazine	16.22	229.7	214	229	29.6
9	Triallate	17.49	304.7	86	268	49.8
10	Metribuzin	18.88	214.3	198	199	39.7
11	Alachlor	19.68	269.8	160	188	89.8
12	Prometryn	19.99	241.4	241	184	78.4
13	Terbutryn	20.66	241. 4	226	241	48.7
14	Metolachlor	21.66	283.8	162	238	53.2
15	Cyanazine	22.06	240.7	212	213	38.4
16	Pendimethalin	23.55	281.3	252	281	10.7

[a] *Q*/*T* (%) are the results of abundance values of the qualifier ion (*Q*) divided by the abundance of the target ion (*T*) × 100.
[b] Internal standard.

2.2.2. Laboratory equipment

An ultrasonic water bath (Raypa, Barcelona, Spain) was used in the extraction procedure. The generator of this apparatus has an output of 150 W and a frequency of 33 kHz. A 12-port vacuum manifold Visiprep (Supelco, Madrid, Spain) was employed.

Table 2
SIM program used to determine herbicides in juice

Group	Time (min)	Pesticide	*m/z*	Dwell time (ms)	Scan rate (cycles/s)
1	5.00	EPTC	128, 189	100	4.26
2	11.70	Propachlor, trifluralin	120, 176, 264, 306	100	2.15
3	14.00	Simazine, atrazine	186, 200, 201, 215	100	2.15
4	15.70	Terbumeton, lindane (IS)[a]	169, 183, 210, 219	100	2.15
5	16.10	Terbuthylazine	214, 229	100	4.26
6	17.00	Triallate	86, 268, 270	100	2.86
7	18.00	Metribuzin	198, 199	100	4.26
8	19.30	Alachlor, prometryn	160, 188, 241,184	100	2.15
9	20.40	Terbutryn	226, 241	100	4.26
10	21.55	Metolachlor, cyanazine	162, 238, 212, 213	100	2.15
11	23.00	Pendimethalin	252, 281	100	4.26

[a] IS: internal standard.

2.3. Juice samples

Various commercial brands of carrot, grape, and multivegetable (made mainly from tomato, carrot, pepper, and onion) juices were purchased from supermarkets in Madrid. A total of eight different juices, four carrot, two grape, and two multivegetable, were analysed.

2.4. Procedure

The sample preparation is based on a previously published method for the determination of insecticide and fungicide residues in fruit juices [14,15]. Glass columns, with Whatman No.1 filters placed at the bottom end, were filled with 2 g of Florisil. A 1 ml volume of juice was applied to the column, fortified when required with 0.5 ml of herbicide standard mixture in methanol. A 0.5 ml volume of methanol was added instead to unfortified samples. Methanol was used to achieve a better distribution of the sample throughout the column. The columns were placed in a tube rack and closed with one-way stopcocks. Juice samples were extracted twice with 5 ml ethyl acetate for 15 min in an ultrasonic bath at room temperature. Water level in the bath was adjusted to solvent level inside the columns. After extraction, solvent was filtered by vacuum in a manifold equipment. The extracts were collected in 10 ml graduated glass tubes and concentrated with a gentle stream of air to a volume of 4 ml for the highest fortification level, 2 ml for the intermediate level, and 1 ml for the lowest spiking level. A 0.5 ml of internal standard was added before the chromatographic analysis. For each fortification level, blank sample extracts were obtained and spiked with herbicides to be used as chromatographic standards.

3. Results and discussion

3.1. Determination of herbicide residues

Herbicide residue levels were determined by (GC–MS-SIM) to achieve the sensitivity necessary for trace quantitative analysis. The use of qualifier and target ions, in addition to the retention time, allowed to confirm positive identification of the herbicides studied. Moreover, the use of the internal standard minimized the possible variations in retention time and peak areas improving the reliability of the method.

The possible matrix effect on the chromatographic response was studied. When standards were prepared by spiking blank juice samples with known amounts of herbicides, higher peak areas were accomplished for the same herbicide concentration. Fig. 1 shows the different response obtained with standard mixtures prepared in ethyl acetate or with a blank juice sample. There is an evident matrix effect that enhances the chromatographic response of these herbicides. This effect has been previously described for other pesticides in food matrices [18,19]. Therefore, the quantification of herbicides was performed with fortified blank samples.

The effect of sonication in the extraction step was assayed by comparing a set of juice samples fortified at 0.05 μg/ml and extracted with assisted sonication with another set extracted without sonication. Recoveries obtained without sonication were in the range of widely accepted values (70–120%). Nevertheless, a small increase in recovery with sonication was observed for many compounds from carrot and multivegetable juices. The enhancement on recovery was clearly observed in grape juice (Table 3) where differences in the range 4–13% were obtained for most compounds. These results are in agreement with those found in a previous work on organophosphorus pesticide determination in fruit juices, where pesticide recoveries were improved by sonication particularly in thicker juices [15]. Therefore, extraction assisted with sonication was applied throughout the work.

3.2. Validation of the analytical method

3.2.1. Linearity

The linearity of all the herbicides was determined using blank carrot juice samples fortified in the range from 0.01 to 0.05 μg/ml and with the internal standard at 0.025 μg/ml. The detector response was linear in the range

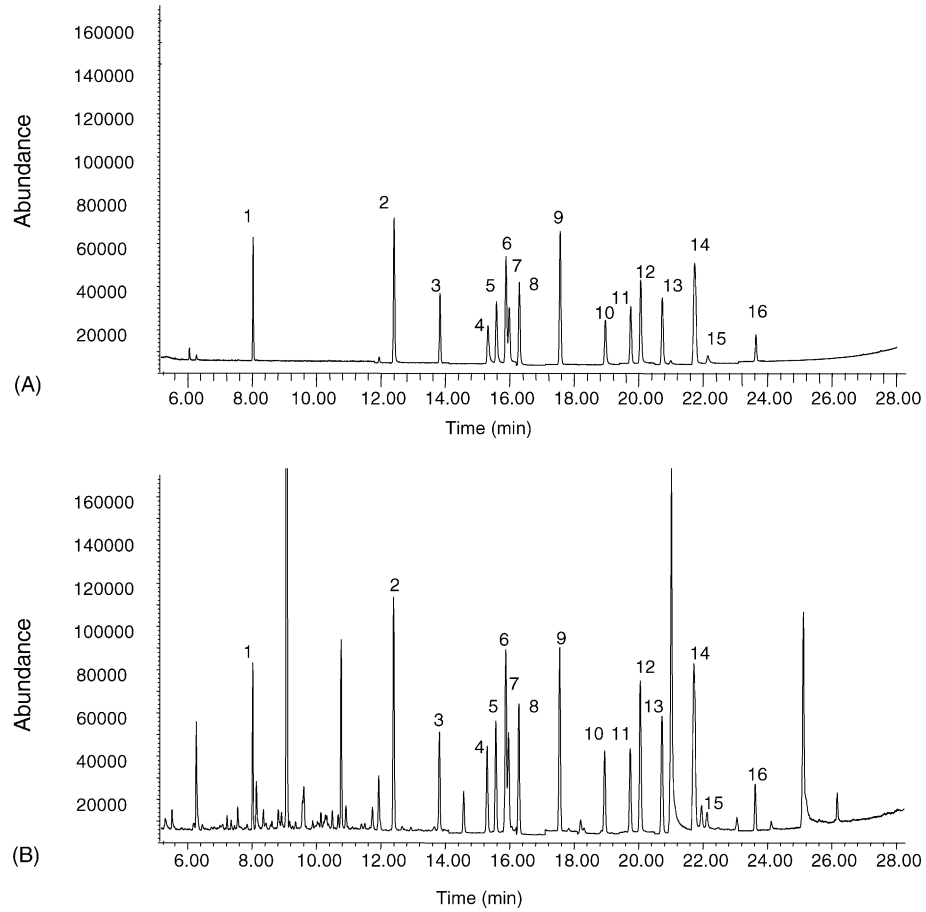

Fig. 1. GC–MS-SIM chromatograms of a standard mixture solution at 0.025 μg/ml (A) and a blank carrot juice sample fortified at 0.025 μg/ml (B). See Table 1 for peak identification.

of concentrations studied. When other blank juices, grape or multivegetable, were used similar responses were obtained. Table 4 summarises the calibration data of the studied herbicides. The slope and intercept values together with their standard deviations were determined applying regression analyses. Good determination coefficients were obtained for all the compounds ranging from 0.9963 to 0.9999.

Table 3
Influence of sonication on herbicide recovery in the extraction procedure

	Recovery (%) ± R.D.S. (%, $n = 4$)	
	Without sonication	With sonication
EPTC	82 ± 5	90 ± 7
Propachlor	82 ± 5	92 ± 5
Trifluralin	86 ± 9	94 ± 10
Simazine	88 ± 6	96 ± 7
Atrazine	89 ± 7	94 ± 7
Terbumeton	86 ± 7	90 ± 6
Terbuthylazine	89 ± 6	97 ± 7
Triallate	80 ± 5	93 ± 8
Metribuzin	86 ± 9	93 ± 8
Alachlor	88 ± 8	94 ± 7
Prometryn	88 ± 7	95 ± 9
Terbutryn	90 ± 7	94 ± 8
Metolachlor	86 ± 7	91 ± 7
Cyanazine	102 ± 5	98 ± 5
Pendimethalin	86 ± 7	90 ± 7

Grape juice samples were fortified at 0.05 μg/ml.

3.2.2. Repeatability

The repeatability of the chromatographic method was determined by performing the analysis of a standard solution at 0.025 μg/ml. The solution was injected 10 times with an automatic injector and the relative standard deviations (R.S.D.s) obtained for retention times ranged from 0.03 to 0.08%, whereas for relative peak areas the values were lower than 5.1% (Table 4). Therefore, the repeatability achieved with the chromatographic method in the conditions assayed was very good.

The repeatability of the whole analytical procedure was also determined by replicate analysis of a fortified sample during different days. The repeatability of the method, expressed as R.S.D., was lower than 11% for all the studied herbicides.

3.2.3. Specificity

The specificity of the proposed MSPD procedure was assessed by analysing control blank juice samples. The absence of background peaks, above the signal to noise ratio

Table 4
Calibration data and repeatability[a] of the studied herbicides

Herbicide	Calibration data			Repeatability (R.S.D. %)[b]	
	Slope (mean ± S.D.)	Intercept (mean ± S.D.)	Determination coefficient	t_R	Peak area
EPTC	1.54 ± 0.02	−0.03 ± 0.03	0.9997	0.08	3.5
Propachlor	2.49 ± 0.04	0.11 ± 0.05	0.9995	0.05	4.1
Trifluralin	0.93 ± 0.02	−0.008 ± 0.024	0.9992	0.07	3.9
Simazine	1.01 ± 0.02	0.01 ± 0.02	0.9994	0.06	3.6
Atrazine	1.35 ± 0.03	0.07 ± 0.05	0.9987	0.06	4.7
Terbumeton	2.25 ± 0.13	0.20 ± 0.20	0.9966	0.06	3.6
Terbuthylazine	2.14 ± 0.03	0.07 ± 0.04	0.9998	0.06	3.7
Triallate	1.75 ± 0.01	0.07 ± 0.02	0.9999	0.05	3.3
Metribuzin	1.42 ± 0.06	−0.01 ± 0.08	0.9963	0.05	4.9
Alachlor	0.98 ± 0.03	0.05 ± 0.04	0.9977	0.05	3.4
Prometryn	1.88 ± 0.04	0.15 ± 0.06	0.9994	0.05	4.1
Terbutryn	1.49 ± 0.03	0.08 ± 0.04	0.9996	0.05	3.8
Metolachlor	3.25 ± 0.04	0.37 ± 0.06	0.9998	0.04	4.4
Cyanazine	0.37 ± 0.01	−0.04 ± 0.01	0.9985	0.04	5.1
Pendimethalin	0.74 ± 0.02	0.06 ± 0.02	0.9994	0.03	4.4

[a] Repeatability of the chromatographic method.
[b] Relative standard deviations of retention times and peak areas relative to the internal standard ($n = 10$).

of 3, at the retention times of the herbicides, showed that no interferences occurred.

3.2.4. Recovery

Table 5 shows the herbicide recovery results obtained. Juice samples, previously analysed to verify the lack of the compounds studied, were fortified at 0.10, 0.05 and 0.01 μg/ml before extraction and analysed by GC–MS. The recoveries obtained for all compounds ranged from 82 to 115% with relative standard deviations equal or lower than 10%. These results show that good recoveries from juice samples were obtained throughout the proposed method. Representative chromatograms of a fortified juice sample and a blank sample are depicted in Fig. 2.

3.2.5. Detection and quantification limits

The limits of detection (LODs) of the proposed method were determined by considering a value three times the background noise obtained for blank samples, whereas the limits of quantification (LOQs) were determined considering a value 10 times the background noise. Table 6 summarises the detection and quantification limits obtained for each herbicide.

3.3. Real samples

The developed MSPD procedure was applied to the determination of herbicides in commercial juices. Carrot, grape, and multivegetable juices of different brands were purchased

Table 5
Herbicide recoveries obtained from juice samples[a]

	Carrot, fortification levels (μg/ml)			Grape, fortification levels (μg/ml)			Multivegetable, fortification levels (μg/ml)		
	0.1	0.05	0.01	0.1	0.05	0.01	0.1	0.05	0.01
EPTC	88 ± 2	90 ± 7	82 ± 8	84 ± 8	90 ± 7	90 ± 9	97 ± 7	93 ± 8	102 ± 9
Propachlor	92 ± 2	93 ± 6	91 ± 5	89 ± 7	92 ± 5	94 ± 10	92 ± 7	89 ± 6	95 ± 10
Trifluralin	91 ± 5	90 ± 6	95 ± 7	89 ± 8	94 ± 10	91 ± 7	101 ± 5	86 ± 6	109 ± 8
Simazine	93 ± 3	92 ± 6	93 ± 7	89 ± 7	96 ± 7	102 ± 6	96 ± 6	90 ± 9	101 ± 5
Atrazine	93 ± 2	93 ± 5	97 ± 5	88 ± 7	94 ± 7	101 ± 3	102 ± 6	95 ± 7	109 ± 10
Terbumeton	91 ± 4	85 ± 9	93 ± 10	85 ± 6	90 ± 6	103 ± 6	95 ± 4	90 ± 5	101 ± 8
Terbuthylazine	93 ± 3	94 ± 4	96 ± 5	88 ± 7	97 ± 7	103 ± 5	97 ± 6	89 ± 9	104 ± 8
Triallate	88 ± 3	91 ± 5	91 ± 9	85 ± 8	93 ± 8	105 ± 8	98 ± 6	86 ± 8	110 ± 8
Metribuzin	97 ± 6	91 ± 9	96 ± 5	88 ± 6	93 ± 8	103 ± 5	104 ± 7	90 ± 9	115 ± 4
Alachlor	91 ± 3	91 ± 5	91 ± 7	86 ± 7	94 ± 7	105 ± 9	95 ± 5	89 ± 8	101 ± 9
Prometryn	90 ± 3	94 ± 5	99 ± 6	91 ± 7	95 ± 9	103 ± 4	103 ± 6	90 ± 10	108 ± 8
Terbutryn	91 ± 2	99 ± 5	97 ± 9	90 ± 7	94 ± 8	104 ± 5	98 ± 6	87 ± 9	109 ± 8
Metolachlor	90 ± 2	94 ± 4	96 ± 3	84 ± 7	91 ± 7	107 ± 5	103 ± 6	92 ± 6	112 ± 7
Cyanazine	90 ± 8	89 ± 8	95 ± 6	84 ± 9	94 ± 8	108 ± 3	98 ± 8	95 ± 4	102 ± 6
Pendimethalin	85 ± 4	90 ± 5	99 ± 8	82 ± 7	90 ± 7	104 ± 6	100 ± 4	84 ± 8	109 ± 9

[a] Recovery % ± R.S.D. % ($n = 4$ at each fortification level for each juice sample).

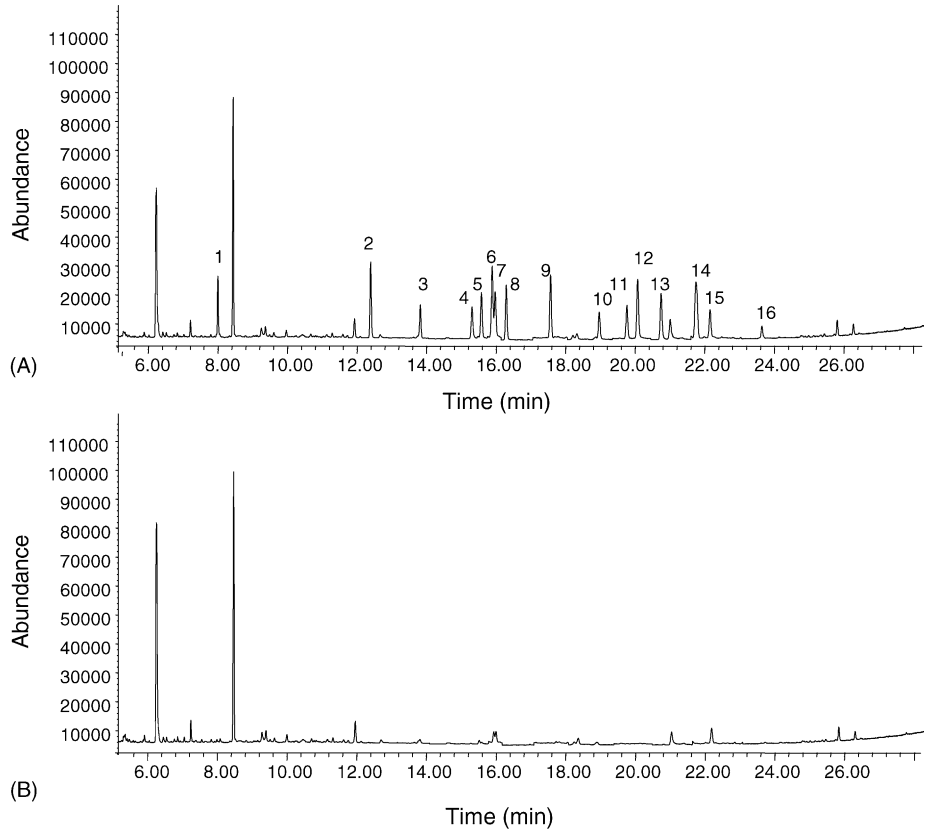

Fig. 2. GC–MS-SIM chromatograms of a grape juice sample fortified at 0.01 μg/ml (A) and a blank grape juice sample (B). See Table 1 for peak identification.

Table 6
Limits of detection (LOD) and quantification (LOQ) of the studied herbicides

	LOD (μg/l)	LOQ (μg/l)
EPTC	0.7	2.3
Propachlor	0.1	0.3
Trifluralin	0.3	1.0
Simazine	0.3	1.0
Atrazine	0.2	0.7
Terbumeton	1.6	5.3
Terbuthylazine	0.2	0.7
Triallate	0.1	0.3
Metribuzin	0.7	2.3
Alachlor	0.3	1.0
Prometryn	0.1	0.3
Terbutryn	0.2	0.7
Metolachlor	0.2	0.7
Cyanazine	0.8	2.7
Pendimethalin	0.8	2.7

in local supermarkets and analysed following the proposed method. No residues, above the detection limit, of the herbicides studied were found in these samples.

4. Conclusions

A rapid method, based on MSPD, has been developed for the simultaneous determination of 15 herbicides in different juices by GC–MS with selected ion monitoring. With the proposed analytical procedure, the extraction and cleanup can be performed in a single step requiring a low volume consumption of organic solvents. Good recoveries and low detection limits were achieved with this procedure. The developed MSPD method was applied to determine herbicide residue levels in carrot, grape and multivegetable juices sold in Spain and no residues above the detection limits were found.

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References

[1] R.S. Sheridan, J.R. Meola, J. AOAC Int. 82 (1999) 982.
[2] W. Liao, T. Joe, W.S. Cusick, J Assoc. Off. Anal. Chem. 74 (1991) 554.
[3] A. Sannino, J. AOAC Int. 81 (1998) 1048.
[4] C. Sorce, N. Ceccarelli, R. Lorenzi, Agric. Med. 124 (1994) 132.
[5] J. Fillion, R. Hindle, M. Lacroix, J. Selwyn, J. AOAC Int. 78 (1995) 1252.
[6] J. Engebretson, G. Hall, M. Hengel, T. Shibamoto, J. Agric. Food Chem. 49 (2001) 2198.

[7] M. Volante, M. Cattaneo, M. Bianchi, G. Zoccola, J. Environ. Sci. Health B33 (1998) 279.
[8] K. Yoshii, M. Okada, Y. Tsumura, Y. Nakamura, S. Ishimitsu, Y. Tonogai, J. AOAC Int. 82 (1999) 1239.
[9] A. Kaihara, K. Yoshii, Y. Tsumura, Y. Nakamura, S. Ishimitsu, Y. Tonogai, J. Health Sci. 46 (2000) 336.
[10] S.J. Lehotay, K.I. Eller, J. AOAC Int. 78 (1995) 821.
[11] K. Adou, W.R. Bontoyan, P.J. Sweeney, J. Agric. Food Chem. 49 (2001) 4153.
[12] A. Bouald, A. Martín-Esteban, P. Fernández, C. Cámara, Fresen. J. Anal. Chem. 367 (2000) 291.
[13] L. Kadenczki, Z. Arpad, I. Gardi, A. Ambrus, L. Gyorfi, G. Reese, W. Ebing, J. AOAC Int. 75 (1992) 53.
[14] J.L. Tadeo, C. Sánchez-Brunete, Chromatographia 57 (2003) 793.
[15] B. Albero, C. Sánchez-Brunete, J.L. Tadeo, J. Agric. Food Chem. 51 (2003) 6915.
[16] M. Khrolenko, P. Dżygiel, P. Wieczorek, J. Chromatogr. A 975 (2002) 219.
[17] M.Y. Selisker, D.P. Herzog, R.D. Erber, J.R. Fleeker, J.A. Itak, J. Agric. Food Chem. 43 (1995) 544.
[18] J.J. Jiménez, J.L. Bernal, M.J. del Nozal, L. Toribio, E. Arias, J. Chromatogr. A 919 (2001) 147.
[19] P.L. Wylie, K. Uchiyama, J AOAC Int. 79 (1996) 571.