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Determination of chlorpyrifos, penconazole, fenarimol, vinclozolin and metalaxyl in grapes, must and wine by on-line microextraction and gas chromatography

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Abstract

A rapid gas chromatographic method for determination of residue levels of one insecticide (chlorpyrifos) and four fungicides (penconazole, fenarimol, vinclozolin and metalaxyl) in grapes, must and wine is described. An on-line microextraction method was used. The matrix, once extracted with a mixture of acetone–dichloromethane (1:1, v/v) was filtered and concentrated. Electron-capture detection for chlorpyrifos, penconazole, fenarimol and vinclozolin and mass-selective detection in the selected-ion monitoring mode for metalaxyl were utilised. No clean-up was necessary because there were no interferences in the area of interest of the chromatogram. Linearity of both detectors, in the range 0.02–2 ng/μl, was checked. In all cases, the correlation coefficient was the same or superior to 0.997. Recoveries from spiked grapes, must and wine ranged from 78% to 101% (fortification level, 0.1–1 mg/kg). Limits of determination were between 0.01 mg/kg for metalaxyl and 0.001 mg/kg for vinclozolin. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Grape (*Vitis vinifera*) production is widespread in the Mediterranean area, Spain, Italy and France principally. Grapes are used directly but are mainly destined for wine. The grapevine is subject to attack by numerous plant and animal parasites. Moths and mites are the most common phytophagous insects, but do not usually cause serious damage because timely identification and consequent pesticidal control are not difficult. The threat from cryptogams is far more serious and can lead to complete crop loss. The most frequent diseases caused by fungi are downy mildew, powdery mildew and gray mold.

Among the different products used for the control of these pests and diseases, the following are commonly utilised in the vineyards of the Jumilla wine-producing region, an area of great viticultural importance in the Region of Murcia (SE of Spain): chlorpyrifos (*Lobesia botrana*), fenarimol and penconazole (*Uncinula necator*), mancozeb and metalaxyl (*Plasmopara viticola*) and vinclozolin (*Botrytis cinerea*).

Vine growers need to protect their crops with pesticides, which can contaminate the wine obtained from treated berries. During the first steps in the wine-producing process (i.e., crushing, draining and pressing), pesticides on the grape berries can pass into the must or may remain in the wine, depending on the wine-making procedures. From a legal point

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of view, the maximum residue limits (MRLs) for grapes that have been established by the national guidelines of residues however, are the few wine-producing countries in the European Union that have established limits for wine. Furthermore, it is necessary to keep in mind that the concentrations in wines are usually very low and therefore it is necessary to develop very precise analytical methods. On some occasions, the extraction procedure is complex and expensive. Numerous and different methods dedicated to the isolation and extraction of pesticides in different vegetable substratums appear in the bibliography [1–4] and also, more concretely in grapes, must and wine [5–9]. In this paper, a rapid gas chromatographic method for simultaneous determination of residue levels of the compounds previously mentioned in grapes, must and wine is proposed.

2. Experimental

2.1. Chemicals and materials

Pesticide analytical standards were purchased from Basf (vinclozolin 99.4%), DowElanco (chlorpyrifos 99.8% and fenarimol 99.7%) and Novartis (metalaxyl 97.2% and penconazole 97.4%). β-Endosulfan (99.6%) was used as internal standard (I.S.) and was of analytical grade (Supelco). Acetone, dichloromethane, isooctane and toluene were for pesticide residues (SDS, France); anhydrous sodium sulphate was analytical grade (Panreac). Stock standard solution containing all pesticides (ca. 50 ng/μl each) were prepared in isooctane–toluene (1:1, v/v). An intermediate solution containing all pesticides was prepared by dilution in the same solvent with β-endosulfan (1.46 mg/l) as I.S.

2.2. Apparatus and chromatography

A Perkin-Elmer Autosystem gas chromatograph was used for determination of vinclozolin, chlorpyrifos, fenarimol and penconazole. It was fitted with an electron-capture detection (ECD) system, an autosampler (Perkin-Elmer) and split–splitless injector, connected to a Nelson 1020 (Perkin-Elmer) reporting integrator. A SPB-5 fused-silica column (30 m×0.25 mm I.D.) was employed, with 5% diphenyl 95% dimethyl siloxane as liquid-phase (film thickness 0.25 μm) (Supelco). The injector and detector were operated at 250 and 320°C, respectively. The sample (2 μl) was injected in the splitless mode (30 s), and the oven temperature was programmed as follows: 90°C for 1 min, raised to 210°C (30°C/min), to 240°C (10°C/min), to 280°C (5°C/min), and held for 7 min.

A Hewlett-Packard 6890 gas chromatograph was employed for determination of metalaxyl. It was fitted with a mass-selective detection (MS) system HP 5971 (Hewlett-Packard), a split–splitless injector, connected to a HP Vectra 500 integrator (Hewlett-Packard). A HP-5MS fused-silica column (30 m×0.25 mm I.D.) was used, with 5% diphenyl 95% dimethyl siloxane liquid-phase (film thickness 0.25 μm) (Hewlett-Packard). The injector and interface were operated at 250 and 280°C, respectively. The operations condition were: acquisition mode selected-ion monitoring (SIM), voltage 1247 V, ionisation foil temperature 230°C, quadrupole temperature 150°C and selected ions of m/z: 160, 206, 234, 249 and 279. The sample (2 μl) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 90°C for 1 min, raised to 210°C (10°C/min), to 240°C (5°C/min), to 270°C (30°C/min), and held for 3 min.

The identity of chlorpyrifos, fenarimol, penconazole and vinclozolin residues in grapes, must and wine extracts was confirmed by GC–MS under the same conditions mentioned previously. Scan mass range, 50–290; SIM: β-endosulfan, m/z: 121, 159, 195, 237, 267, 339; chlorpyrifos, m/z: 97, 197, 258, 286, 314; fenarimol, m/z: 107, 139, 219, 251, 330; penconazole, m/z: 115, 159, 213, 248; and vinclozolin, m/z: 53, 124, 178, 212, 285.

2.3. Extraction procedure

For the extraction of penconazole, fenarimol, metalaxyl, vinclozolin and chlorpyrifos residues in grapes, must and wine, a micro on-line extraction method, based on the one proposed by Steinwandter [6] with some modifications, has been used. The vegetable material is extracted with an acetone–dichloromethane mixture, and then filtered and concentrated.

(a) Extraction in grapes: 5 g of grapes are

homogenised at 3000 rpm during 10 min in a high-speed electric mixer (Omni-Mixer, Sorvall) with 30 ml of acetone–dichloromethane (1:1, v/v), 2 g of Celite and 1 g of anhydrous NaCl. The mixture is filtered through a funnel of porous plate No. 4 and the filtrate is passed through 1 PS Phase Separator Paper (Whatman 2100150), washing flask and filter with 10 ml of the mixture solvent. All the fractions are picked up in a concentration flask and concentrated to dryness by rotary vacuum evaporation. The dry extract was dissolved in 5 ml of isooctane–toluene (1:1, v/v) that contains β-endosulfan (1.46 mg/l) as I.S.

(b) Extraction in must and wine: 5 ml of must or wine are placed in a 30-ml glass tube with hermetic closing, with 20 ml of acetone–dichloromethane (1:1, v/v) and 2 g of anhydrous NaCl. The tube is agitated smoothly, during 20 min, in a shaker (Unite-Mixer Lab Line 1306, Biomedical Prod.) and the liquid is passed through 1 PS Phase Separator Paper (Whatman 2100150), washing tube and filter with 10 ml of the mixture solvent. All organic fractions are evaporated by rotary vacuum evaporation and the residue dissolved in 5 ml of isooctane–toluene (1:1, v/v) that contains β-endosulfan (1.46 mg/l) as I.S.

2.4. Recovery assays

Untreated grape, must and wine samples, once crushed and homogenised, were spiked with 200 μl of two solutions containing 24 and 2.4 ng/μl of each one of the studied pesticides. The concentrations thus obtained were 1 and 0.1 mg/kg, respectively. The samples were allowed to equilibrate for 60 min prior to extraction, and were processed according to the above procedure. The recovery assays were replicated five times.

3. Results and discussion

3.1. Gas chromatographic determination

The identification of metalaxyl, penconazole, fenarimol, vinclozolin and chlorpyrifos was realized by the retention times obtained when standard solutions of concentrations between 0.02 and 2 ng/μl were injected into the gas chromatograph. In the case of metalaxyl the identification was carried out, also, for comparison with the corresponding mass spectra. In Table 1, absolute and relative retention times to β-endosulfan, used as I.S., are shown. For the quantification one kept in mind the area of each chromatographic peak.

Table 1
Retention times ($n=5$), absolute and relative to the internal standard (β-endosulfan)

Pesticide	t_R (min)	t'_R (min)	R.S.D. (%)
Vinclozolin	10.794	0.624	0.53
Chlorpyrifos	12.264	0.709	0.61
Penconazole	13.480	0.779	0.39
Metalaxyl	13.720	–	0.31
β-Endosulfan (I.S.)	17.286	1	0.18
Fenarimol	21.621	1.250	0.66

Fig. 1 shows chromatograms of standard solution of chlorpyrifos, fenarimol, penconazole and vinclozolin and spiked samples of grapes, must and wine with ECD. A chromatogram, in the SIM mode, and mass spectrum of metalaxyl are shown in Fig. 2. ECD and MS showed high sensitivity and selectivity. All chromatograms were very clean without interfering peaks in the areas of interest.

3.2. Linearity of response and detection limit

Standard solutions of 2, 1, 0.4, 0.2 and 0.02 ng/μl were injected to obtain the graphic representation of peak areas vs. concentrations and to estimate detection limits for penconazole, chlorpyrifos, vinclozolin, fenarimol and metalaxyl. Tables 2 and 3 summarise the statistical parameters obtained when carrying out the lineal regression for each one of the studied products. The values exposed in those tables show a great correlation among concentration–area for the five studied compounds.

For the calculation of detection limits, the following approach has been applied: the area of the chromatographic peak is, as a minimum, three-times the bottom noise, considering for ECD the area reject in 25 000 area counts and for MS in 2000. According to the previous premise, the calculated limits were 0.025, 0.0047, 0.0049, 0.0076 and 0.0033 ng for metalaxyl, chlorpyrifos, fenarimol, penconazole and vinclozolin, respectively.

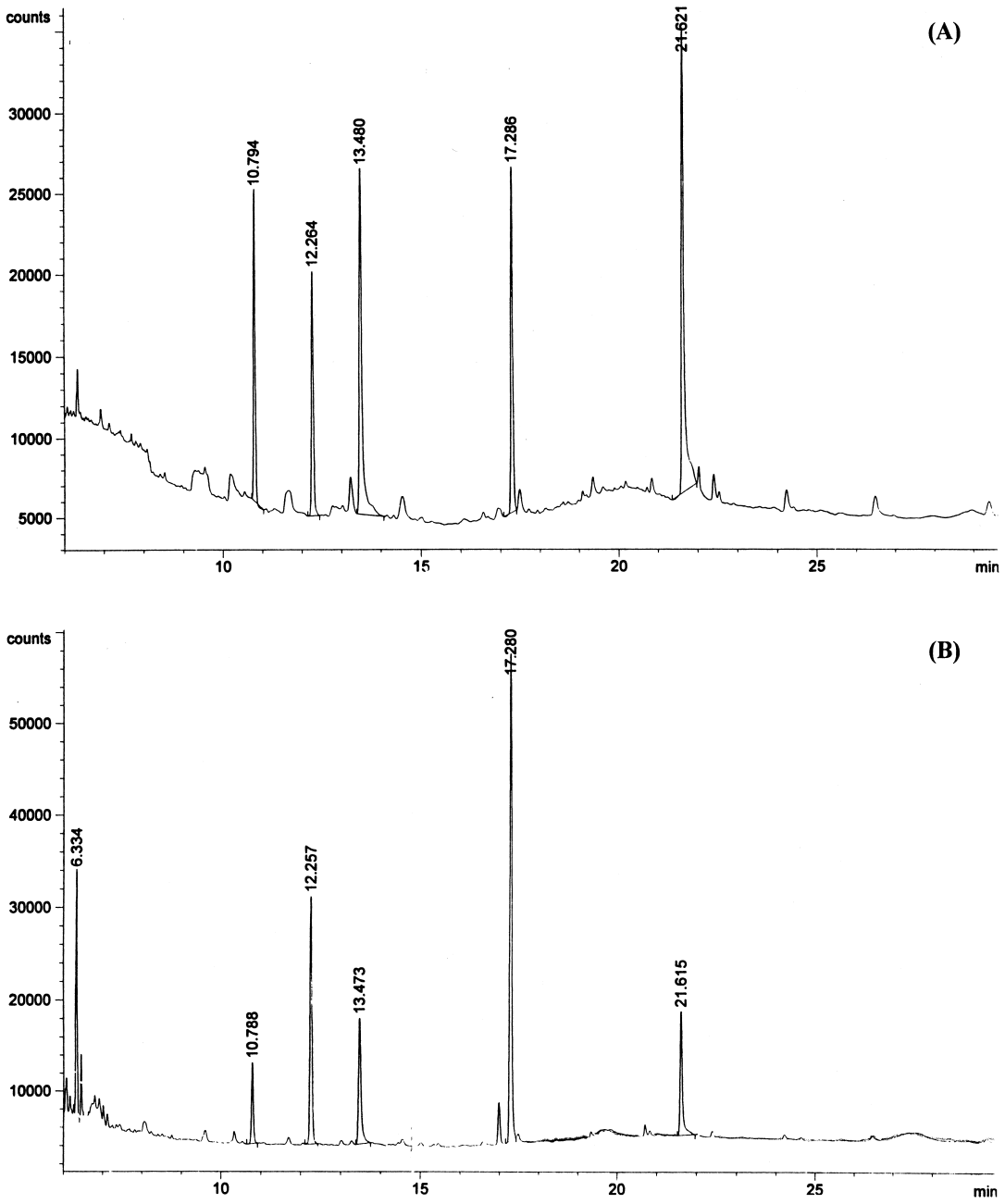

Fig. 1. GC–ECD chromatograms of standard solutions (4 ng) (A) and spiked extracts of grapes (B), must (C) and wine (D) (0.1 mg/kg of each compound). Identification by retention times (min): Vinclozlin 10.7; chlorpyrifos, 12.2; penconazole, 13.4; I.S., 17.2 and fenarimol, 21.6.

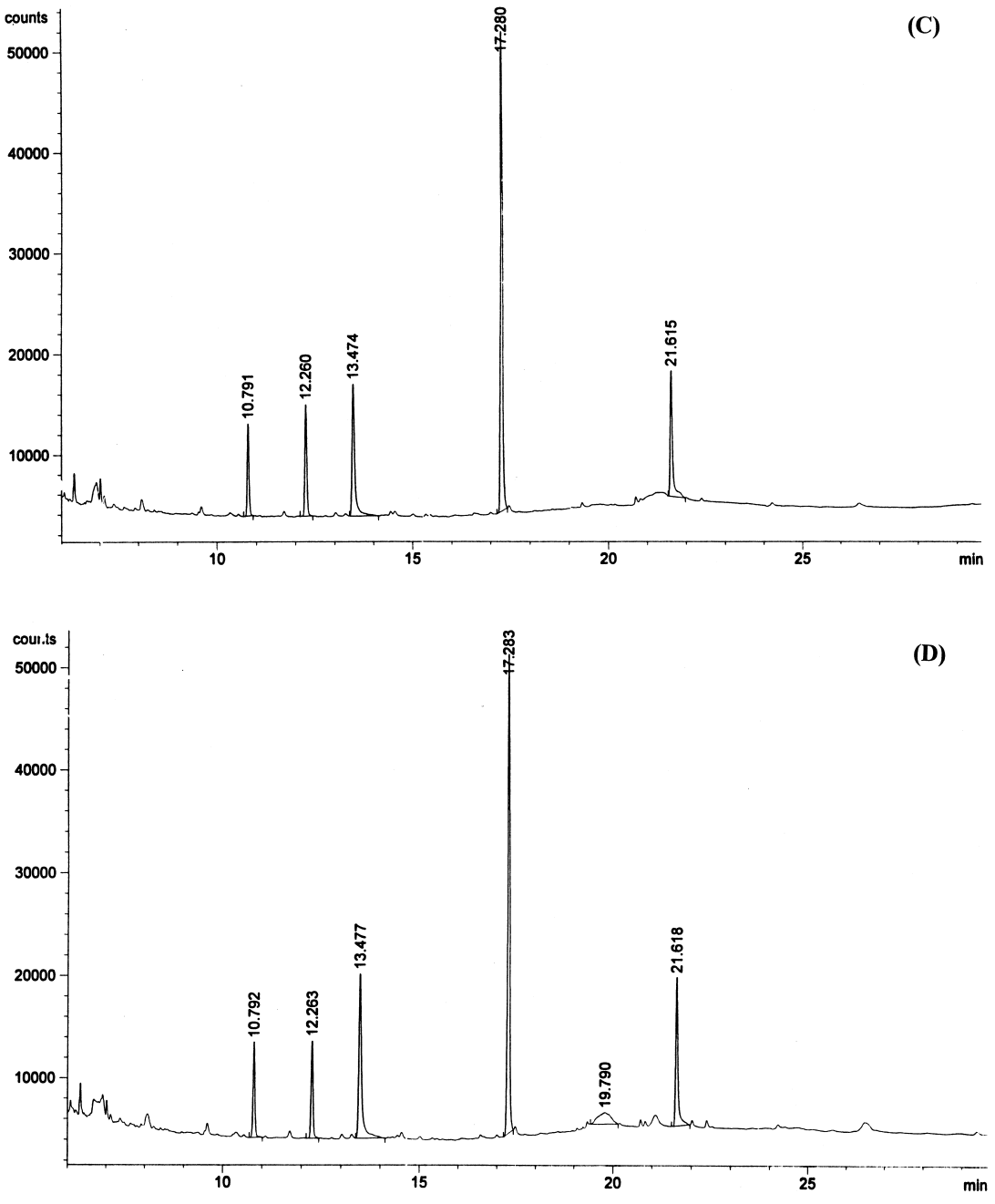

Fig. 1. (*continued*)

3.3. Recovery and repeatability of the extraction method

In order to check the reliability of our method we have carried out a study to determine its reproducibility. Previously, a blank assay was employed to check for the absence of residuals in grapes, must and wine. Tables 4–6 show descriptive statistical parameters.

In the case of grapes, the recovery values are

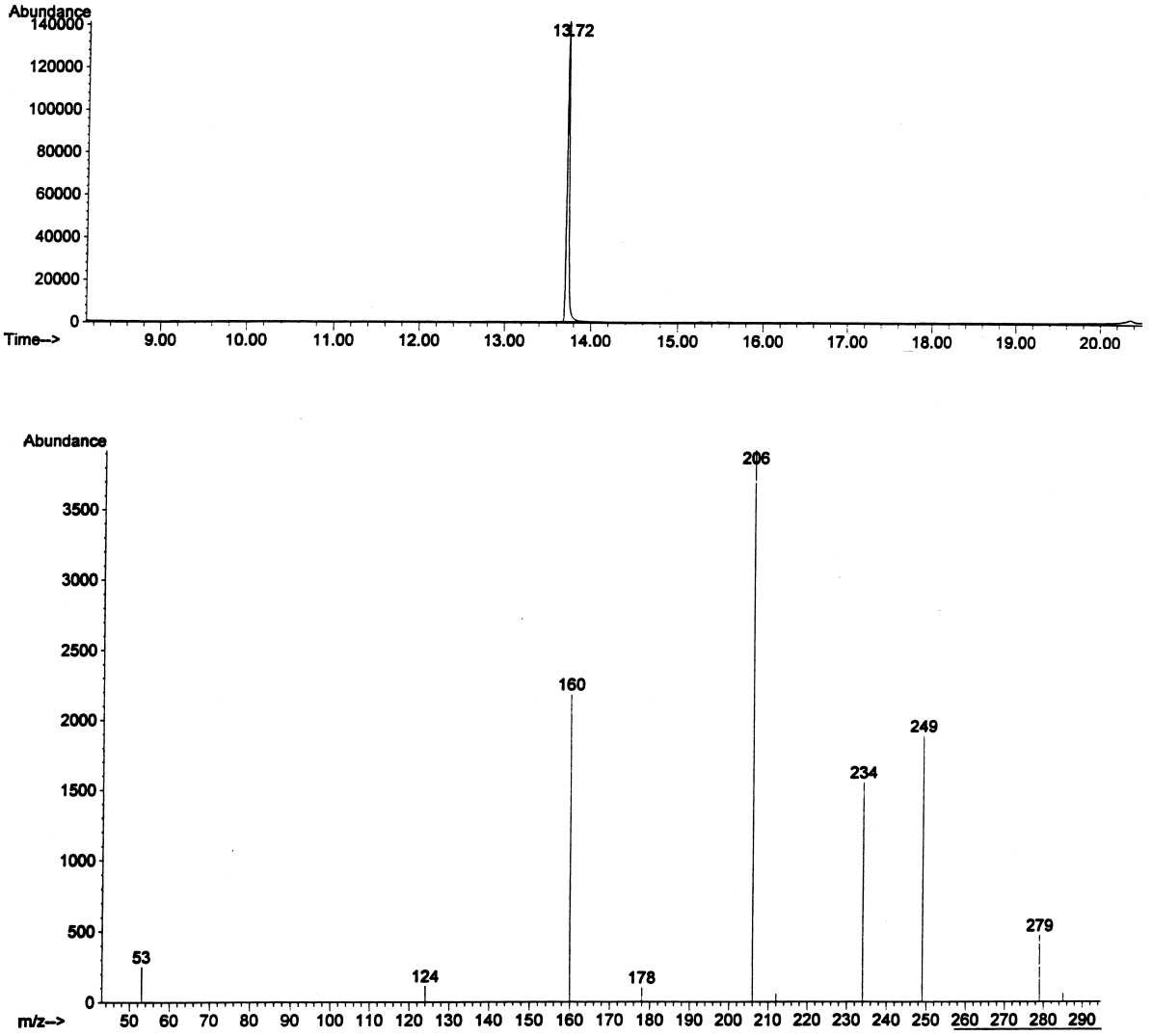

Fig. 2. GC–MS chromatogram (in SIM mode), and mass spectrum of metalaxyl. Time scale of chromatogram in min.

Table 2
Statistical data of the lineal fit for vinclozolin, chlorpyrifos and fenarimol

Parameter	Pesticides		
	Vinclozolin	Chlorpyrifos	Fenarimol
r	0.9979	0.9977	0.9985
r^2	0.9958	0.9954	0.9970
S.E.E.[a]	$8.8 \cdot 10^5$	$8.3 \cdot 10^4$	$1.1 \cdot 10^6$
$a \pm$(95%) CI[b]	$1.1 \cdot 10^6 \pm 1.3 \cdot 10^6$ *	$9.5 \cdot 10^4 \pm 1.6 \cdot 10^5$	$6.6 \cdot 10^4 \pm 2.1 \cdot 10^5$
$b \pm$(95%) CI	$1.6 \cdot 10^7 \pm 0.14 \cdot 10^7$ ***	$1.3 \cdot 10^6 \pm 1.7 \cdot 10^5$ ***	$2.1 \cdot 10^6 \pm 1.8 \cdot 10^5$ ***

[a] Standard error of estimation.
[b] CI = Confidence interval.
* ($P<0.05$); ** ($P<0.01$); *** ($P<0.001$).

Table 3
Statistical data of the lineal adjustment for metalaxil and penconazole

Parameter	Pesticide	
	Metalaxyl	Penconazole
r	0.9985	0.9986
r^2	0.9970	0.9972
S.E.E.[a]	$1.1 \cdot 10^3$	$1.1 \cdot 10^6$
$a \pm$(95%) CI[b]	$-7.8 \cdot 10^2 \pm 2.1 \cdot 10^3$	$5.9 \cdot 10^4 \pm 1.5 \cdot 10^5$
$b \pm$(95%) CI	$2.1 \cdot 10^4 \pm 1.8 \cdot 10^3$***	$1.3 \cdot 10^6 \pm 2.0 \cdot 10^5$***

[a] Standard error of estimation.
[b] CI = Confidence interval.
* ($P<0.05$); ** ($P<0.01$); *** ($P<0.001$).

superior to 85% in all cases, except for fenarimol, where the values are located around 80%. The relative standard deviation (R.S.D.) is not greater than 8% in the most unfavourable case. For must and wine, the lowest value of recovery is 87% for vinclozolin and the highest R.S.D., also for the same compound, is 7.4%.

Based on the exposed data, we can affirm that, when obtaining recoveries greater than 80% and R.S.D.s less than 10%, the used method is appropriate to extract these compounds in the studied range of concentrations.

Table 4
Percentage of mean recovery in grapes and representative statistical values

Pesticides	Fortification	Parameters ($n=5$)			
	level (mg/kg)	Mean	S.D.	S.E.M.	R.S.D. (%)
Vinclozolin	0.91	87.8	3.5	1.5	3.9
	0.09	86.0	6.1	2.7	7.0
Chlorpyrifos	1.02	101.0	4.1	1.8	4.1
	0.10	89.6	5.3	2.4	5.9
Fenarimol	0.97	80.4	5.4	2.4	6.7
	0.09	77.4	5.1	2.3	6.5
Metalaxyl	0.95	89.8	7.2	3.2	8.0
	0.09	89.0	5.5	2.5	6.2
Penconazole	0.93	95.2	5.6	2.5	5.9
	0.09	90.0	6.1	2.7	6.7

S.D. = Standard deviation; S.E.M. = Standard error of mean; R.S.D. = relative standard deviation.

Table 5
Percentage of mean recovery in must and representative statistical values

Products	Fortification	Parameters ($n=5$)			
	level (mg/kg)	Mean	S.D.	S.E.M.	R.S.D. (%)
Vinclozolin	0.91	87.4	6.5	2.9	7.4
	0.09	87.4	5.1	2.3	5.8
Chlorpyrifos	1.02	95.0	4.9	2.1	5.2
	0.10	100.6	4.3	1.9	4.2
Fenarimol	0.97	97.0	4.0	1.8	4.1
	0.09	92.2	5.1	2.3	5.5
Metalaxyl	0.95	97.8	7.2	3.2	7.3
	0.09	96.0	3.5	1.6	3.7
Penconazole	0.93	93.2	3.8	1.7	4.1
	0.09	96.6	3.8	1.7	3.9

Table 6
Percentage of means recovery in wine and representative statistical values

Products	Fortification level (mg/kg)	Parameters ($n=5$)			
		Mean	S.D.	S.E.M.	R.S.D. (%)
Vinclozolin	0.91	93.3	2.7	1.2	2.9
	0.09	91.5	3.7	1.6	4.0
Chlorpyrifos	1.02	96.2	5.0	2.2	5.1
	0.10	99.3	3.3	1.5	3.3
Fenarimol	0.97	98.0	3.8	1.7	3.9
	0.09	93.3	4.4	1.9	4.8
Metalaxyl	0.95	97.4	6.2	2.7	6.3
	0.09	96.2	3.5	1.6	3.7
Penconazole	0.93	96.4	2.5	1.1	2.6
	0.09	96.8	3.7	1.6	3.7

3.4. Limit of sensibility of the analytical method

The limit of sensibility of an analytical method, applied to the determination of pesticide residues, can be defined as the minimum value detectable with accuracy for a certain substance, expressed in ppm [10]. For its determination it is necessary to keep in mind the detection limit obtained for each compound, the quantity of initial sample, the volume of the obtained extract and the volume injected in the chromatographic determination. Its mathematical calculation is carried out from the following formula:

$$\mathrm{TLS} = \frac{V_e \mathrm{DL}}{V_i W} \quad (1)$$

where V_e = volume of the extract (ml), DL = detection limit (ng), V_i = injection volume (μl) and W = mass of sample (g).

If the theoretical limit of sensibility (TLS) is multiplied for the global efficiency of method, we obtain the real limit of sensibility (RLS). For its calculation, the mean value of recovery of the two fortification levels studied for each product, grape, must and wine, are used. In Table 7 the calculated values are shown. The data shown in the previous table show that the limit of determination is in all cases, very inferior to the MRL established by the different legislations [11,12]. The ratio between MRL (more frequent) and TLS is 3030, 213, 122, 160, and 53 for vinclozolin, chlorpyrifos, fenarimol, metalaxyl and penconazole, respectively. These values are much higher than 1, which shows that the used extraction method is adapted for the determination of residuals of the studied compounds.

4. Conclusions

The proposed method allows a simple and rapid determination of the five studied pesticides in grapes, must and wine. The method yields recoveries that ranged between 78–101%. No clean-up is necessary.

Table 7
Theoretical and real limit of sensibility (mg/kg) calculated and maximal residue limit (mg/kg) legislated for each one of the products

Pesticide	TLS	RLS			MRL[a]		
		Grapes	Must	Wine	A	B	C
Vinclozolin	0.00165	0.00143	0.00144	0.00153	5	5	5
Chlorpyrifos	0.00235	0.00235	0.00229	0.00229	0.5	0.5	0.5
Fenarimol	0.00245	0.00193	0.00233	0.00234	0.2	0.3	0.3
Metalaxyl	0.01250	0.01110	0.01211	0.01210	0.5	2	2
Penconazole	0.00380	0.00352	0.00359	0.00340	0.2	0.2	0.2

[a] A = Spanish legislation; B = EU legislation; C = more frequent in different legislations.

Limits of determination were, in all cases, lower than MRLs established by different legislations, which is indicative that the used method is valid for determination of residual levels of chlorpyrifos, fenarimol, metalaxyl, penconazole and vinclozolin in those matrices.

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