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Multiresidue method for the rapid determination of organophosphorus insecticides in grapes, must and wine

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Abstract

A rapid multiresidue gas chromatographic method for determining 12 insecticides in grapes, must and wine is described. A simple on-line microextraction method for isolating frequently applied insecticides on vineyard is used. The matrix, once extracted with an acetone–dichloromethane (1:1, v/v) mixture, was filtered and concentrated. Nitrogen–phosphorus detection (NPD) and electron-capture detection (ECD) were used to identify and quantify the insecticides, the findings being confirmed using mass spectrometric detection (MSD). No clean-up was necessary for either NPD or ECD. The regression coefficients relating to linearity were at least 0.99. Recoveries from spiked grape, must and wine samples ranged from 80 to 108% and relative standard deviations were no higher than 16% in the most unfavourable case. Individual detection limits were in the range 0.02–0.1 ng. Limits of quantification varied from 0.01 to 0.05 mg kg^{-1}, which are below the maximum residue limits set by the legislation of the main wine-producing countries of the European Union. Only in the case of methidathion and quinalphos were the limits of quantification equivalent to the maximum residue limits (0.05 mg kg^{-1}) established by Spanish and French legislation, respectively.

Keywords: Grapes; Wine; Food analysis; Must; Organophosphorus compounds; Pesticides

1. Introduction

Grapevine is subject to attack by numerous plant and animal parasites. Moths (*Lobesia botrana*, *Clysia ambiguella*) and mites (*Eotetranichus carpini*, *Panonichus ulmi*, *Tetranichus urticae*) are the most common phytophagous insects, although they do not usually cause lesions or other damage because timely identification and consequent pesticidal control are not difficult [1].

Although to a lesser extent than fungicides, insecticide residues on grapes can pass to the must and later to the wine, with a consequent toxicological risk for the consumer, despite the fact that wine-making processes (crushing, pressing, stabilization, etc.) may considerably reduce their presence in the wine [2–8].

There is therefore a need for rapid and reliable controls to ensure that the residual levels in grapes and wine are below the maximum residue limits permitted by different bodies of legislation.

Since the European Union has not yet established maximum residue limits (MRLs) for wine, the limits established for viniferous grapes must be used.

Routine methods used in pesticide residue analysis are often time and solvent consuming due to the steps involved in sample preparation before chromatographic analysis, although modern trends in analytical chemistry have led to the simplification and increasing automation of preliminary analytical operations, particularly as regards extraction steps [9–13].

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The latest technology used in the analysis of pesticide residues in vegetable and food processing includes more selective extraction methods involving water or supercritical fluid extraction (SFE) to reduce clean-up steps, on-line micro- or macroextraction, clean-up using solid-phase extraction (SPE) or solid-phase microextraction (SPME), and analytical techniques such as capillary gas chromatography (cGC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and immunoassay for the screening, detection and quantification of the full range of volatile, semivolatile, non-volatile, and thermolabile pesticides. Confirmation using mass spectrometry (MS) and the application of ion trap MS (IT-MS) as a single detector for all pesticide residues is considered fundamental at the present time [10,13–22].

In this paper, we describe a rapid and reliable multiresidue method, based on GLP (Good Laboratory Practice) criteria, for determining in grapes, must and wine the residues of 12 organophosphorus insecticides widely used in vineyard. The scope (range of analytes and sample matrix), specificity (interferences), LOD (minimum detectable concentration or mass), LOQ (minimum quantifiable concentration or mass), accuracy (agreement of results with the correct value), precision (repeatability of replicate analysis), sensitivity (slope of the curve relating response vs. concentration or mass) and practicality (cost, complexity, etc.) of the method were validated according to European norm EN-45000 [23–24]. On-line microextraction and GC with electron-capture detection (ECD), nitrogen–phosphorus detection (NPD) and MS detection were used.

2. Experimental

2.1. Chemicals and reagents

Pesticide analytical standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and were certified at least >98% pure. Acetone, dichloromethane, isooctane and toluene were for pesticide residues (Scharlau, Barcelona, Spain). Sodium chloride was of analytical grade (Panreac). Two standard solutions containing different insecticides (ca. 200 $\mu g\,ml^{-1}$) were prepared in isooctane–toluene (1:1, v/v) (chlorpyrifos methyl, fenitrothion, parathion ethyl and parathion methyl for the GC–ECD system, and chlorpyrifos, diazinon, ethion, fenthion, malathion, methidathion, parathion methyl, pirimiphos methyl and quinalphos for the GC–NPD system). In both cases, different working standard solutions (0.01, 0.05, 0.1, 0.5 and 2 $\mu g\,ml^{-1}$) were prepared by dilution in the same solvent.

2.2. Apparatus and chromatography

GC–ECD system: A Perkin-Elmer Autosystem gas chromatograph was used to determine chlorpyrifos methyl, fenitrothion, parathion ethyl and parathion methyl. The chromatograph was fitted with an electron-capture detector, an autosampler (Perkin-Elmer) and split-splitless injector, connected to a Nelson 1020 (Perkin-Elmer) reporting integrator. A SPB-608 (Supelco) fused-silica column (30 m×0.25 mm I.D. and film thickness 0.25 μm) was used. 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, rising to 150°C (10°C min^{-1}) for 3 min and to 270°C (6°C min^{-1}).

GC–NPD system: A Hewlett-Packard 6890 system equipped with a nitrogen–phosphorus detector, an autosampler (Hewlett-Packard) and a split-splitless injector connected to a HP ChemStation (Hewlett-Packard) was used for the determination of chlorpyrifos, diazinon, ethion, fenthion, malathion, methidathion, parathion methyl, pirimiphos methyl and quinalphos. The capillary column was a HP-5 (30 m×0.32 mm I.D.) with 5% diphenyl–95% dimethylsiloxane (film thickness 0.25 μm) (Hewlett-Packard). The injector and detector were operated at 250°C and 300°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, rising to 180°C (10°C min^{-1}) for 1 min, to 205 (1°C min^{-1}) and finally to 250 (30°C min^{-1}). Nitrogen was used as the carrier and make-up gas at 1 ml min^{-1} and 9 ml min^{-1}, respectively. Hydrogen and air were used as detector gases at 3 ml min^{-1} and 60 ml min^{-1}, respectively.

GC–MSD system: A Hewlett-Packard 6890 gas

chromatograph was used to confirm the identity of all insecticides. It was fitted with a mass-selective detector 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% dimethylsiloxane liquid phase (film thickness 0.25 μm) (Hewlett-Packard). The injector and interface were operated at 250 and 280°C, respectively. The operation conditions were: acquisition-mode scan (mass range 50–450), voltage 1650 V, ionisation foil temperature 230°C, quadrupole temperature 150°C. 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, rising to 210°C (10°C min^{-1}), to 240°C (5°C min^{-1}), to 270°C (30°C min^{-1}), and held for 3 min. Table 1 shows the spectral characterization using GC–MS with electron impact ionization (EI) of the insecticides studied.

2.3. Extraction procedure

A micro on-line extraction method for the extraction of insecticide residues in grapes, must and wine, was used. The sample was extracted with an acetone–dichloromethane mixture, filtered and concentrated to obtain the extract.

Extraction on grapes: Grapes (5 g) were homogenised at 8000 rpm for 3 min in a high-speed electric mixer (Polytron-Aggregate, Kinematica, Germany) with 30 ml of the solvent mixture acetone–dichloromethane (1:1, v/v) and 2 g of anhydrous NaCl. The mixture was filtered through a porous plate funnel (pore size No. 4) and the filtrate was passed through Phase Separator Paper (Whatman 2100150 1PS), washing flask and filter with 10 ml of the solvent mixture. All the fractions were collected in a concentration flask and concentrated to dryness using rotary vacuum evaporation. The dry extract was dissolved in 5 ml of the isooctane–toluene (1:1, v/v) mixture.

Extraction in must and wine: Must or wine samples (5 ml) were placed in a 50-ml glass flask with 20 ml of the solvent acetone–dichloromethane (1:1, v/v) mixture and 2 g of anhydrous NaCl before being hermetically closed. The flasks were introduced into an ultrasonic bath (Ultrasons 613, Selecta) with distilled water for 10 min and the liquid was passed through Phase Separator Paper (Whatman 2100150 1PS), washing flask and filter with 10 ml of the solvent mixture. All organic fractions were evaporated using rotary vacuum evaporation and the residue dissolved in 5 ml of isooctane–toluene (1:1, v/v).

Table 1
Spectral characterization using GC–MS in EI mode of the insecticides studied

Insecticides	*m/z* (100%)	Other fragments
Chlorpyrifos	314	197, 258
Chlorpyrifos methyl	286	79, 125
Diazinon	179	137, 304
Ethion	231	153, 384
Fenitrothion	277	125, 260
Fenthion	278	109, 125
Malathion	173	93, 158
Methidathion	145	85, 93
Parathion ethyl	291	97, 109
Parathion methyl	109	125, 263
Pirimiphos methyl	290	125, 276
Qinalphos	146	157, 298

2.4. Recovery assays

Untreated grape, must and wine samples, once crushed and homogenised, were spiked with insecticides. Recovery assays were performed at 0.01–0.5 ppm. The samples were allowed to equilibrate for 60 min prior to extraction, and then processed according to the above procedure. Five replicates were analyzed at each fortification level.

3. Results and discussion

Scope and specificity: The insecticides determined using GC–ECD eluted between 26 and 28 min in the following order: chlorpyrifos methyl, parathion methyl, fenitrothion and parathion. For the insecticides analyzed using GC–NPD, the elution order was methidathion, diazinon, parathion methyl, pirimiphos methyl, malathion, fenthion, chlorpiryfos, quinalphos and ethion with retention times of between 10 and 35 min. In both cases, the chromatograms were very clean with no interfering peaks appearing in the areas of interest. No clean-up was

therefore necessary. Figs. 1 and 2 show chromatograms of standard solutions of insecticides and spiked untreated grape, must and wine samples for both GC–ECD and GC–NPD systems.

With the column used in the GC–NPD system, although oven temperature and carrier gas flow were varied, it was not possible to separate the following groups of compounds: parathion methyl–chorpyrifos methyl and chlorpyrifos–parathion ethyl–fenitrothion. Bearing in mind the length of the column used (30 m) and the mean velocity necessary to reach a maximum resolution when using N_2 as carrier gas (10 cm s^{-1}, according to the Van Deemter optimum), perhaps a flow-rate of 0.4 ml min^{-1} would have been the most appropriate. However, we found that this flow-rate did not significantly improve the separation obtained and prolonged the time needed for analysis to an unacceptable extent. The resolution would probably have been better if H_2 or He had been used as carrier gas, since the optimum flow for these gases is 2–3 times higher than the optimum flow for N_2. In the case of the GC–ECD system, there was a sufficient degree of separation between the pair fenitrothion–chlorpyrifos and parathion. For this

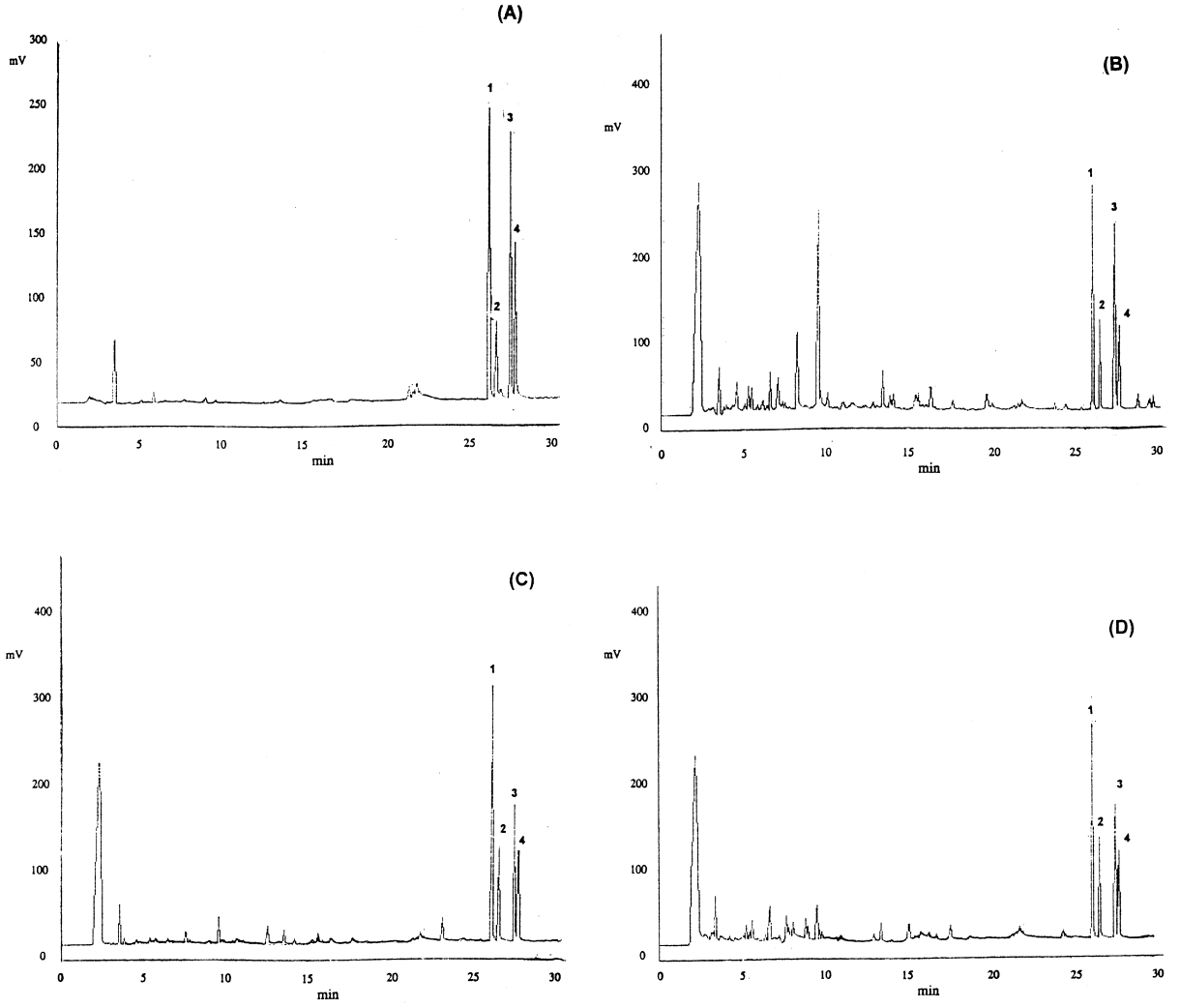

Fig. 1. GC–ECD chromatograms of standard solutions (0.5 ng μl^{-1}) of insecticides (A), and spiked extracts of untreated grape (B), must (C) and wine (D) samples at 0.01–0.05 mg kg^{-1}. 1, Chlorpyrifos methyl; 2, parathion methyl; 3, fenitrothion; 4, parathion ethyl.

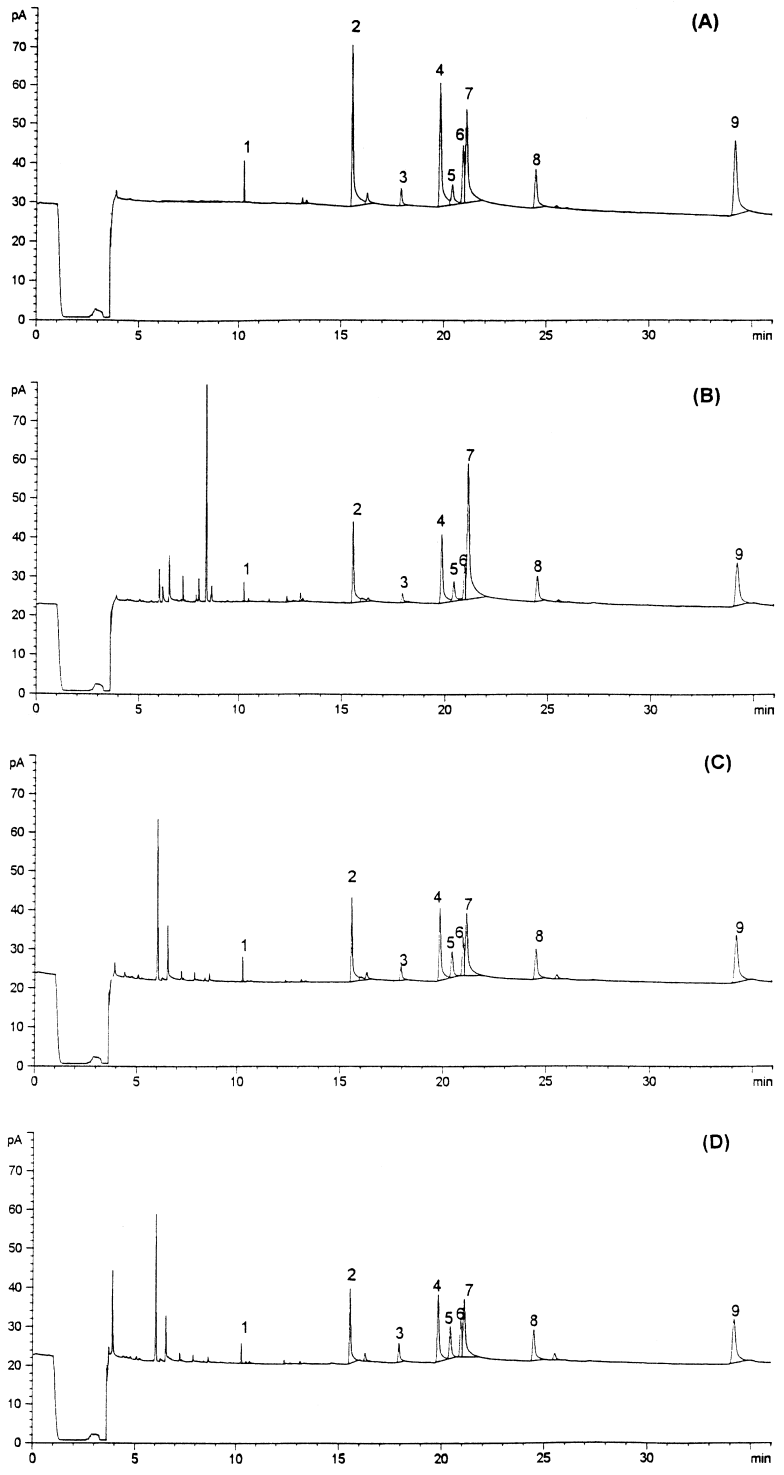

Fig. 2. GC–NPD chromatograms of standard solutions (0.5 ng μl^{-1}) of insecticides (A), and spiked extracts of untreated grape (B), must (C) and wine (D) samples at 0.01–0.05 mg kg^{-1}. 1, Methidathion; 2, diazinon; 3, parathion methyl; 4, pirimiphos methyl; 5, malathion; 6, fenthion; 7, chlorpyrifos; 8, quinalphos; 9, ethion.

reason, we determined chlorpyrifos methyl, parathion methyl, parathion ethyl and fenitrothion in the GC–ECD system. However, in this system, fenitrothion and chlorpyrifos have similar retention times and so they are not adequately separated, for which reason we suggest these two insecticides be identified using GC–MSD, bearing in mind the selected ions shown in Table 1.

Sensitivity and LOD: Calibration curves were prepared for the insecticides by plotting peak areas vs. concentrations for both ECD and NPD. Good linearity was achieved in the 0.01–2 $\mu g\,ml^{-1}$ range with correlation coefficients ranging between 0.993 for parathion ethyl and 0.999 for methidathion. The repeatability of peak areas was also good with coefficients of variation ranging from 1.1 for fenthion to 12.7 for fenitrothion. Table 2 summarises the statistical parameters calculated when carrying out the linear regression and repeatabilities of peak area for each active ingredient. The values expressed in Table 2 show a high degree of correlation between concentration and area for the 12 compounds studied. The detection limits obtained (signal to bottom-noise ratio=3) ranged from 0.02 ng to 0.1 ng.

Accuracy and precision. Table 3 shows recoveries of 12 insecticides at two concentration levels. For grapes, recoveries ranged from 80 to 100% with RSD values of 6 and 4% for pirimiphos methyl and malathion, respectively. Recoveries for must varied between 82% (ethion) and 108% (chlorpyrifos methyl) with RSD values of 11.5 and 10.5%, respectively. In the case of wine, recoveries ranged from 82 (pirimiphos methyl) to 105% (chlorpyrifos methyl); RSD values were not higher than 16.5% in the most unfavourable case (fenthion).

LOQ: The corresponding limits of quantification for each insecticide, taking into account the detection limit for each compound, mass of sample, volume of extract and volume injected, are shown in Table 4. This table also shows the real limit of quantification (theoretical limit of quantification multiplied by the mean recovery of the extraction method), which was calculated using the mean recovery at the lowest fortification level in grapes, must and wine for each insecticide. As can be seen, the values calculated are in all cases lower than the maximum residue limits established by the Spanish, French and Italian legislation (the main wine-producing countries of the European Union) for viniferous grapes, no MRLs for wine having been established [25]. Only in the case of methidathion and quinalphos did the limits of quantification correspond with the maximum residue limits permitted in Spain and France, respectively.

Table 2
Linearity [peak area=b (ng)$\pm a$] and repeatability [RSD (%), $n=7$] of peak areas for both NPD and ECD

Insecticides	Linearity				Repeatability
	r	SEE[a]	$a\pm$(95%) CI[b]	$b\pm$(95%) CI	
Chlorpyrifos[d]	0.9972	30.21	−25.93±45.13	465.12±48.18[e]	2.3
Chlorpyrifos methyl[c]	0.9980	1572.54	−1272.25±3185.33	26505.28±3105.28[e]	4.8
Diazinon[d]	0.9998	10.21	−11.74±15.25	656.57±16.28[e]	8.1
Ethion[d]	0.9985	19.42	−17.85±29.01	411.50±30.98[e]	2.7
Fenitrothion[c]	0.9986	727.12	−150.97±1472.86	15007.9±1437.9[e]	12.7
Fenthion[d]	0.9986	10.86	−15.73±21.99	219.35±21.44[e]	1.1
Malathion[d]	0.9972	9.36	−12.22±18.96	134.46±18.48[e]	7.4
Methidathion[d]	0.9999	0.09	0.19±0.32	8.56±0.28[e]	2.1
Parathion ethyl[c]	0.9934	1230.35	−629.27±2492.21	11444.48±2429.25[e]	5.9
Parathion methyl[c,d]	0.9985	1.72	−2.02±3.48	34.19±3.39[e]	3.7
Pirimiphos methyl[d]	0.9963	29.01	12.94±43.33	386.59±46.27[e]	2.4
Quinalphos[d]	0.9991	2.75	−2.96±5.57	68.08±5.43[e]	2.9

[a] SEE, Standard Error of Estimation.
[b] CI, Confidence Interval.
[c] ECD.
[d] NPD.
[e] $P<0.001$.

Table 3
Recoveries (%±RSD, $n=5$) of insecticides from grapes, must and wine samples

Insecticides	Fortification level (mg kg^{-1})	Mean recovery (%)±RSD		
		Grapes	Must	Wine
Chlorpyrifos	0.01	91.4±5.3	100.2±4.9	96.8±4.7
	0.1	99.1±4.3	97.4±5.6	98.2±4.7
Chlorpyrifos methyl	0.05	84.2±6.6	107.8±10.5	104.8±14.4
	0.5	94.7±13.2	102.3±5.9	85.3±6.0
Diazinon	0.01	81.2±7.1	86.2±8.7	88.2±8.5
	0.1	89.0±4.0	87.3±11.7	91.3±5.2
Ethion	0.01	93.6±4.1	82.0±11.5	91.4±7.9
	0.1	99.0±5.0	91.0±9.5	101.3±4.9
Fenitrothion	0.05	97.8±6.1	100.0±4.3	99.0±5.5
	0.5	95.7±7.4	100.3±2.1	89.3±5.6
Fenthion	0.01	92.0±6.4	90.8±15.6	91.0±16.5
	0.1	90.3±14.3	82.6±6.6	104.0±8.4
Malathion	0.05	100.2±9.3	86.0±8.5	99.2±6.1
	0.5	100.3±3.7	83.0±5.5	99.7±3.2
Methidathion	0.05	82.2±5.1	100.4±7.6	99.8±5.6
	0.5	82.3±4.3	82.0±11.7	97.3±7.8
Parathion ethyl	0.05	93.4±16.3	99.0±3.9	98.2±10.4
	0.5	96.3±8.4	97.3±5.6	87.3±7.0
Parathion methyl	0.05	91.4±10.0	103.6±11.2	102.4±6.7
	0.5	98.7±7.3	106.0±6.2	101.6±3.4
Pirimiphos methyl	0.01	80.0±6.1	82.8±9.6	82.6±9.2
	0.1	84.6±0.7	87.0±10.0	88.3±2.9
Quinalphos	0.05	96.4±6.4	85.2±9.1	96.4±8.5
	0.5	91.3±2.3	86.3±12.7	101.0±6.2

Table 4
Theoretical (TLOQ) and real (RLOQ) limit of quantification (mg kg^{-1}) calculated and maximum residue limit (mg kg^{-1}) permitted in different countries of the European Union for each insecticide[a]

Insecticides	TLOQ (mg kg^{-1})	RLOQ (mg kg^{-1})			MRLs (mg kg^{-1})		
		Grapes	Must	Wine	Spain	France	Italy
Chlorpyrifos	0.01	0.0091	0.0100	0.0097	0.50	0.50	0.50
Chlorpyrifos methyl	0.05	0.0421	0.0539	0.0524	0.20	0.20	0.20
Diazinon	0.01	0.0081	0.0086	0.0088	0.50	0.50	0.50
Ethion	0.01	0.0093	0.0082	0.0091	0.50	N.E.	0.50
Fenitrothion	0.05	0.0489	0.0500	0.0495	0.50	0.50	0.50
Fenthion	0.01	0.0092	0.0091	0.0091	0.50	0.02	N.E.
Malathion	0.05	0.0501	0.0430	0.0496	0.50	0.50	0.50
Methidathion	0.05	0.0411	0.0502	0.0499	0.05	0.50	0.50
Parathion ethyl	0.05	0.0467	0.0495	0.0491	0.50	0.50	0.50
Parathion methyl	0.05	0.0457	0.0518	0.0512	0.20	0.20	0.20
Pirimiphos methyl	0.01	0.0080	0.0083	0.0083	0.50	0.05	0.50
Quinalphos	0.05	0.0482	0.0426	0.0482	0.30	0.05	0.10

[a] N.E., Not established.

Practicality: The proposed method provides satisfactory results at a minimum cost. It is simple and does not involve a great deal of sample manipulation. It is, we feel, a valid method for use in the control of the insecticides studied in grapes, must and wine.

4. Conclusions

The proposed method permits the rapid determination of 12 insecticides widely used on vine in grapes, must and wine after a simple extraction of the sample and follows Quality Control (QC) and Good Laboratory Practice (GLP) criteria. No clean-up is necessary, because the chromatograms of untreated grape, must and wine samples are free of interfering peaks. The method provides good recoveries and repeatabilities. The limits of quantification are much lower than the maximum residue limits set by the legislation of the main wine-producing countries of the European Union for insecticide residues in viniferous grapes.

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