Multiresidue Method for Pesticide Determination in Wine by High-Performance Liquid Chromatography

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A new and rapid HPLC method which allows the simultaneous determination of 15 pesticides in wine has been developed. A satisfactory pesticide separation was achieved with an RP_{18} column and water–acetonitrile (50:50 v/v) as the mobile phase. The limit of detection ranged from 0.006 to 0.020 ppm. The method does not require extraction of active ingredients from wine but uses C_8 solid-phase extraction cartridges, which allow purification and concentration of the sample. With this extraction procedure, recoveries of pesticides at 0.01–0.02 and 1.00 ppm ranged from 85 to 108%.

Pesticide residues in food is of great importance in the evaluation of food quality. In several countries this has led to an increase in the control of pesticide residues in food, to evaluate whether the amounts found were within the limits established by national laws. Recently wine has been subjected to frequent quality control checks for pesticide residues used for the control of pests in vine. From a legal point of view, the maximum residue limits (MRL) for grapes have been established by the national guidelines of residues, but no limit has been set for wine. Switzerland is the only country to show different tolerance limits for grapes and wine. Even in Italy, for the new registered pesticides for vine, a limit is set for both grapes and wine. The European Community has considered the opportunity of fixing the MRL in wine, especially in view of the difficulties that may arise in commercialization (Marchese, 1990). The analytical methods available for the determination of pesticides in wine are numerous and use both GC (Lemperle et al., 1970, 1982; Gnaegi and Dufour, 1972; Gnaegi and Lipka, 1974; Brandolini et al., 1979; Barbina et al., 1980; Fabbrini et al., 1980; Flori et al., 1982, 1984) and HPLC (Lazzarini et al., 1980; Cabras et al., 1983, 1984a,b, 1986, 1988). Only few active ingredients can be detected simultaneously by these methods. These analytical methods first require extraction with different solvents, depending on the chemical and physical characteristics of the pesticide to be detected, followed by purification of the organic layer and finally chromatographic determination. The aim of this work was to set up a multiresidue method with a pesticide extraction procedure that should require neither different solvents in extracting active ingredients quantitatively nor purification. For the chromatographic determination HPLC was used.

EXPERIMENTAL PROCEDURES

Apparatus and Chromatography. A liquid chromatograph consisting of a Bischoff solvent delivery Model 2200 (Leonberg, Germany) equipped with a Valco loop injector (100 μL), an LC 235 diode array UV detector (Perkin-Elmer, Norwalk, CT) and an LC 100 integrator (Perkin-Elmer) was used. Pesticides were extracted from wine by means of a Bond Elut/Vac Elut system (Analytichem International, Harbor City, CA) connected with a water vacuum pump. A Spherisorb (Waddinxveen, Netherlands) S_5-ODS-1 (250 × 4.6 mm i.d.) column was employed with a 50:50 (v/v) mixture of water and acetonitrile as mobile phase, at a flow rate of 1.0 mL/min. The analyses were performed at the wavelength of 200 nm, which is best for the simultaneous determination of all pesticides. For most of the 15 pesticides, the limits of detection at this wavelength were not significantly different from those obtained at the absorbance maxima. Only for carbaryl and tetrachlorvinphos were the limits of detection substantially different (0.020 and 0.015 ppm, respectively). Therefore, in case of low concentrations it was convenient to operate at different wavelengths according to the absorbance maxima previously determined and reported in Table I.

Chemicals. Acetonitrile, methanol, ethanol, and methylene chloride were HPLC grade solvents (Carlo Erba, Milan). Eluting mixtures (H_2O/CH_3CN, 50:50 v/v, and H_2O/C_2H_5OH, 70:30 v/v) were prepared using HPLC grade water distilled twice and filtered through a Milli-Q apparatus (Millipore, Milan) before use. Pesticide analytical standards (≥99%) were obtained from the manufacturers or purchased from Ehrenstorfer (Augsburg, Germany). Stock standard solutions (≈500 ppm) were prepared by dissolution of pesticides in methanol and stored at 4 °C. Working standard solutions were prepared by dilution with the mobile phase. Bond Elut C_8 cartridges (500 mg/2.8 mL) (Analytichem International) were used to extract pesticides from wine.

Wines. The two most important Sardinian wines, Nuragus di Cagliari (white wine) and Cannonau di Sardegna (red wine), were used. Their compositions were, respectively, 10.5 and 12.5% (v/v) ethanol, 0.18 and 0.36% reductive sugar, 6.10 and 5.60 g/L total acidity, 0.16 and 0.38 g/L volatile acidity, and pH 3.18 and 3.38. These wines were obtained from grapes from experimental vineyards that had never been treated with synthetic pesticides and in which only inorganic compounds (Cu and S) were used.

Extraction Procedure. The Bond Elut C_8 cartridge was first treated with methanol (2 mL × 3) and washed with HPLC grade water (2 mL × 3). Two milliliters of wine was then added and allowed to percolate slowly (1 mL/min). The cartridge was then washed with HPLC grade water (2 mL × 3) and with 30% ethanol (2 mL × 3) and allowed to dry in a vacuum. The pesticides were eluted with 2 mL of methylene chloride, allowed to percolate (percolation rate, 1 mL/min) through the cartridge under positive pressure, and collected in a 3-mL vial. The solvent was evaporated to dryness at room temperature under a nitrogen stream. The dry sample was then taken up with an appropriate volume (0.5–2 mL, depending on the concentration of the compounds to be determined) of eluting mixture and injected for HPLC analyses. Each cartridge could be used again after it was washed with 25 mL of methanol.

Recovery Assays. White and red wine samples (10 mL) were fortified with 0.01 or 0.02 and 1.00 ppm of each pesticide by adding 100 μL of methanolic solution. The samples were then taken through the extraction procedure. The recovery assays were replicated four times.

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Table I. Analytic Characteristics of the Pesticides

pesticide	λ max, nm	tr, min	limit of detection, ppm
(2) carbaryl	220	7.81	0.006
(8) metalaxyl	195	8.66	0.006
(9) methidathion	195	12.15	0.020
(14) triadimenol	195	12.42	0.010
(3) captan	195	14.14	0.020
(13) triadimefon	195	15.18	0.010
(6) fenarimol	195	15.24	0.010
(7) iprodione	200	16.90	0.010
(11) procymidone	204	17.86	0.010
(12) tetrachlorvinphos	210	19.28	0.010
(15) vinclozolin	200	21.05	0.010
(1) benalaxyl	195	22.36	0.010
(5) dichlofluanid	195	23.89	0.010
(4) chlozolinate	200	26.29	0.010
(10) penconazole	199	34.88	0.010

RESULTS AND DISCUSSION

For the chromatographic determination, different ratios of an acetonitrile–water mixture (ranging between 40:60 and 60:40 v/v) were tested. The best separation of all 15 pesticides in a reasonable time was achieved with a 50:50 (v/v) acetonitrile–water mobile phase. However, the separation of some peaks, such as those referring to triadimenol with methidathion and fenarimol with triadimefon, was critical owing to their very close retention times (Table I). Even a slight loss in the efficiency of the column (due to wear) made these peaks overlap. Pesticide separation was then achieved by increasing the water content of the mobile phase by 5%. This raised the retention times and improved separation. Using a diode array detector, it was possible to check the purity of the peaks and by means of the UV spectra to confirm the nature of the pesticide. In this way it was possible to decide whether to modify the eluting mixture. The calibration graphs were built on six points by plotting peak areas (external standard method) vs concentration. Data were processed by a statistical package for least-squares regression; correlation coefficients, standard deviations of slope, and intercept showed a good linearity in the range 0–1.5 ppm. Under optimum conditions, the limits of detection (Thier and Zeumer, 1987) ranged from 0.006 to 0.020 ppm.

Generally, for most of the analytical methods used to determine pesticides in wine, the procedure requires extraction with a suitable extracting solvent, cleanup, evaporation of the extracting solvent to dryness, and chromatographic analysis. The extracting solvent is chosen according to the chemical and physical characteristics of the active ingredient to be detected. In a multiresidue method, extraction must be carried out with different solvents, each specific for a group of pesticides. We thought of using a solid-phase method with a reversed-phase cartridge starting with the assumption that since most pesticides are nonpolar compounds, they were retained on the cartridge in presence of water as mobile phase. Therefore, with suitable washing it should be possible to eliminate a large quantity of interfering compounds. A synthetic solution of wine, obtained from a 10% (v/v) ethanol mixture in water with 5 g/L tartaric acid and 5 g/L glycerol, was used to set up the extracting method. In this way it was possible to find that methylene chloride was the best organic solvent to elute pesticides quantitatively. Subsequently, using wine samples, we tried to find the best way to wash the cartridge so as to eliminate as many interfering compounds as possible without affecting pesticide quantitative recovery. After making several attempts with different combinations

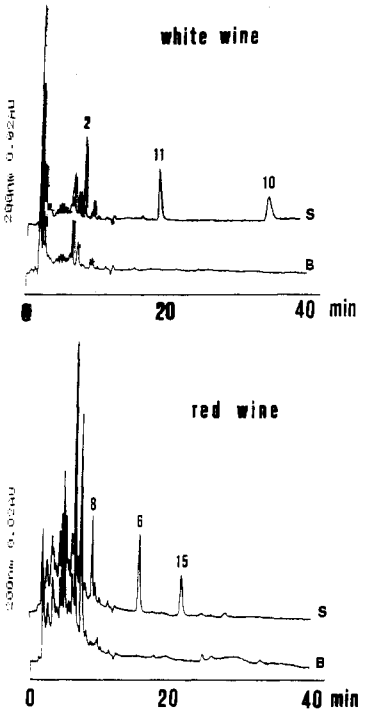

Figure 1. Chromatography of some pesticides in wine. Column, S_5-ODS-1; mobile phase, acetonitrile–water (50:50 v/v); flow rate, 1 mL/min; detection, UV at 200 nm; injected sample, 100 μL. B, control; S, sample spiked with 0.02 ppm of each pesticide. Peaks: 2, carbaryl; 11, procymidone; 10, penconazole; 8, metalaxyl; 6, fenarimol; 15, vinclozolin.

Table II. Pesticide Recoveries from Wine by Solid-Phase Extraction

pesticide	level, ppm	recovery,[a] % ± SD
(1) benalaxyl	1.00	102 ± 2
	0.01	100 ± 1
(2) carbaryl	1.00	96 ± 3
	0.01	105 ± 9
(3) captan	1.00	98 ± 1
	0.02	106 ± 2
(4) chlozolinate	1.00	85 ± 4
	0.01	93 ± 6
(5) dichlofluanid	1.00	88 ± 1
	0.01	97 ± 8
(6) fenarimol	1.00	95 ± 8
	0.01	88 ± 8
(7) iprodione	1.00	96 ± 2
	0.01	89 ± 2
(8) metalaxyl	1.00	95 ± 4
	0.01	99 ± 3
(9) methidathion	1.00	107 ± 1
	0.02	91 ± 7
(10) penconazole	1.00	102 ± 2
	0.01	95 ± 7
(11) procymidone	1.00	99 ± 2
	0.01	94 ± 1
(12) tetrachlorvinphos	1.00	105 ± 1
	0.01	100 ± 8
(13) triadimefon	1.00	108 ± 1
	0.01	87 ± 2
(14) triadimenol	1.00	103 ± 2
	0.01	88 ± 8
(15) vinclozolin	1.00	92 ± 5
	0.01	100 ± 3

[a] Mean values of duplicate analyses from four replicates.

of methanol, ethanol, acetonitrile, and water mixtures, we found that the method here described is an excellent way of obtaining chromatograms without interfering peaks (Figure 1) or quantitative recoveries (Table II). Owing to the scarce presence of interfering peaks, it was possible to concentrate the sample without problems. In fact, if we

recover the dried extract with 0.5 mL of eluting mixture, the sample is concentrated four times, and therefore very low pesticide concentrations (≈0.01 ppm) can be detected easily.

CONCLUSIONS

The described method allows quantitative extraction of the most important pesticides used to control pests and diseases in vines, easily and rapidly. This method may be suitable to detect many other nonpolar pesticides. The possibility of concentrating samples allows the use of a diode array detector at better sensitivities (0.01 ppm). This method also has the advantage of measuring peak purity and confirming the isolated pesticide by UV spectra.

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Registry No. Benalaxyl, 71626-11-4; carbaryl, 63-25-2; captan, 133-06-2; chlozolinate, 72391-46-9; dichlofluanid, 1085-98-9; fenarimol, 60168-88-9; iprodione, 36734-19-7; metalaxyl, 57837-19-1; methidathion, 950-37-8; penconazole, 66246-88-6; procymidone, 32809-16-8; tetrachlorvinphos, 22248-79-9; triadimefon, 43121-43-3; triadimenol, 55219-65-3; vinclozolin, 50471-44-8.