

Parameters Affecting Extraction of Selected Fungicides from Vineyard Soils

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This paper describes a sensitive method for the simultaneous quantification of eight commonly used grapevine fungicides in vineyard soils: cyprodinil, fludioxonil, metalaxyl, penconazole, pyrimethanil, procymidone, tebuconazole, and vinclozolin. The fungicides are extracted from the soil sample by sonication with water followed by shaking with ethyl acetate and are quantified by gas chromatography with mass spectrometry. Average extraction efficiencies in a sample of seven spiked, previously fungicide-free soils were $\geq 79\%$ for all of the analytes, method precisions were $\geq 17\%$, and quantification limits were $\leq 50 \mu\text{g}/\text{kg}$. However, because recoveries varied considerably from soil to soil, there is a need to control for soil matrix differences (mainly soil pH and exchangeable calcium content); as a consequence, soil fungicide contents must be quantified by the standard additions method. When the method was applied in this way to soil samples from vineyards belonging to the specified wine-growing region of Rías Baixas (Galicia, northwestern Spain) taken at the beginning of October (1 month after the crop's final treatment), levels of fludioxonil as high as $991 \mu\text{g}/\text{kg}$ were found, but at the start of the season (9 months after the previous crop's final treatment) only fludioxonil was detected at levels higher than its limit of quantification (45 and $52 \mu\text{g}/\text{kg}$).

KEYWORDS: Fungicide; soil; solid–liquid extraction; gas chromatography

INTRODUCTION

Fungicides are widely applied to grapevines to prevent the growth of gray mold (*Botrytis cinerea*), powdery mildew (*Uncinula necator*), and downy mildew (*Plasmopara viticola*) (1–6). Although in principle sprayed on the aerial parts of the grapevine, as the result of dripping, accidental spraying, and the actions of wind, rain, and dew, fungicide solutions can also enter the soil beneath, where they can alter fertility through their influence on chemical and microbiological properties (2, 7–11). Because a considerable variety of fungicides are now available for treatment of grapevines, it is desirable, for the purposes of monitoring the state of vineyard soils, to be able simultaneously to determine the levels of multiple fungicides in each soil sample. In this paper we describe a method for the simultaneous determination, in soil samples, of eight of the nine fungicides shown by surveys of local grapegrowers and phytochemical suppliers, and by exploratory analyses of a sample of local vineyard soils, to be those that are currently most commonly used in the specified wine-growing region of Rías Baixas (Galicia, northwestern Spain): cyprodinil, fludioxonil, metalaxyl, penconazole, pyrimethanil, procymidone, tebuconazole, and vinclozolin. No method for the simultaneous deter-

mination of these fungicides has previously been published. The ninth fungicide commonly used in this area, dichlofluanid, was also included in the study, but with unsatisfactory results.

In previous methods for the determination of fungicides or pesticides in soil, the analyte has been extracted from the sample in a Soxhlet or by means of microwaves, shaking, or sonication, using organic solvents or solvent mixtures: acetone (4, 12, 13), acetonitrile (1, 14, 15), benzene (2), cyclohexane (5), ethyl acetate (7), methanol (16–19), acetonitrile plus water (20), ethyl acetate plus acetone (21), or methanol plus water (3, 6, 22–24). The method described in this paper also uses an organic extractant (ethyl acetate), but, unlike many of the methods cited above, it does not require subsequent partition or chromatographic purification steps, which significantly lengthen the procedures in which they are employed. Given the volatility and thermal stability of the proposed analytes, in the developed method (as in several of those cited above) they are separated and quantified by gas chromatography with mass spectrometric detection (GC-MS). The method was developed using spiked samples of an uncontaminated soil, and the influence of soil variability on the efficiency with which fungicides were extracted was investigated using spiked samples of six other uncontaminated soils. Finally, we report the results of applying the method to samples from Rías Baixas vineyards.

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Table 1. Physical and Chemical Properties of the Soils Studied^a

soil	sand (%)	silt (%)	clay (%)	pH (H ₂ O)	FC (mL)	OM (%)	C/N ratio	C (%)	Ca (mequiv/100 g)	FAO classification
A	64.2	22.8	13.1	7.0	2.1	4.6	13.3	2.7	8.1	Umbric leptosol
B	73.4	16.2	10.4	4.4	1.5	5.2	15.6	3.0	2.2	Umbric leptosol or haplic umbrisol
C	38.3	36.0	25.7	5.1	2.9	11.4	8.7	6.6	4.3	Ferralic umbrisol or ferralic cambisol
D	72.0	15.1	13.0	4.5	1.0	10.7	12.3	6.2	1.0	Umbric leptosol or haplic umbrisol
E	73.3	16.0	10.7	4.3	1.6	4.1	7.3	2.4	1.2	Umbric leptosol or haplic umbrisol
F	40.5	45.0	14.5	4.7	2.6	12.1	9.7	7.0	1.7	Ferralic umbrisol or ferralic cambisol
G	16.8	53.2	30.0	4.5	3.9	19.1	9.8	11.1	1.3	Ferralic umbrisol or ferralic cambisol
1	68.5	17.5	14.0	7.4	1.7	5.1	12.3	2.9	8.6	Umbric leptosol
2	66.2	17.8	16.1	7.4	1.3	4.7	11.3	2.7	9.1	Umbric leptosol
3	57.2	25.5	17.3	7.5	2.0	4.9	16.8	2.9	8.8	Umbric leptosol
4	58.0	20.3	21.7	7.4	2.2	6.6	12.3	3.8	10.1	Umbric leptosol

^a FC, field capacity; OM, organic matter; C, carbon; N, nitrogen; Ca, exchangeable calcium.

EXPERIMENTAL PROCEDURES

Chemicals and Apparatus. Standards of cyprodinil [CAS Registry No. 121552-61-2], dichlofluanid [1085-98-9], fludioxonil [131341-86-1], metalaxyl [057837-19-1], penconazole [66246-88-6], pyrimethanil [53122-28-0], procymidone [32809-16-8], tebuconazole [107534-96-3], and vinclozolin [050471-44-8], all >99% pure, were purchased from Riedel-de-Haën (Seelze, Germany), and lindane (97%) was purchased from Aldrich (Steinheim, Germany). Methanol (from Aldrich); ethyl acetate, *n*-hexane, and cyclohexane (from Merck, Darmstadt, Germany); and water and acetic acid (from Panreac, Barcelona, Spain) were analytical grade reagents with purities >98%. Anhydrous sodium sulfate (99%), sodium acetate 3-hydrate (99%), anhydrous sodium carbonate (99.8%), calcium carbonate (98.5%), and sodium polyphosphate (65%) were all purchased from Panreac.

Stock standard solutions of each fungicide, with concentrations of ~1 g/L, were prepared by weighing 0.025 g of the analyte into a 25 mL volumetric flask and making up to the mark with methanol. Intermediate standard mixtures were prepared by mixing aliquots of the stock standard solutions and diluting with methanol so that each fungicide was present at a concentration of ~100 mg/L. All stock and intermediate standard solutions were stored in the dark at 4 °C, under which conditions they proved to be stable for at least 3 months.

EPA vials (40 mL) with Teflon-faced silicone septa were purchased from Wheaton (Millville, NJ). Sonication was performed in an ultrasound water bath from Selecta (Barcelona, Spain) and shaking in a Promax 1020 reciprocating shaker from Heidolph (Schwabach, Germany). Extracts were condensed in round-bottom Duran flasks from Schott (Mainz, Germany) in a Heidolph WB 2000 vacuum rotary evaporator and were homogenized with a Heidolph Reax Top vortex homogenizer. Final samples were placed in 350 μ L inserts in 2 mL vials from Supelco (Bellefonte, PA).

Analytical Instruments and Operating Conditions. Gas chromatography was performed on a GC 8000 gas chromatograph (from Fisons, Ipswich, U.K.) equipped with a Fisons MSD 800 mass-selective detector linked to a PC running Masslab v. 1.4 (from ThermoQuest, Manchester, U.K.). Supelco MDN-5S fused silica capillary columns (30 m \times 0.25 mm i.d., film thickness = 0.25 μ m) were used with the following oven temperature program: 5 min at 100 °C, followed by a 10 °C/min ramp to 200 °C, 5 min at 200 °C, a 10 °C/min ramp to 278 °C, and 15 min at 278 °C. The carrier gas was helium at a constant column-head pressure of 100 kPa (so that flow rate fell as the oven temperature increased), and injection was performed in splitless mode, with a column/vent ratio of 10:1 when after 5 min the vent was opened; the injector and transfer line temperatures were 240 and 275 °C, respectively.

Electron-impact mass spectrometric detection was performed at an ionization energy of 70 eV in single-ion monitoring mode (actually, the spectrometer is programmed to sum the simultaneously monitored intensities of signals detected at multiple prespecified *m/z* values). The ions monitored were those combining high *m/z* and high intensity: those at *m/z* 198 for pyrimethanil (eluting at 16.4 min); 198 and 213 for vinclozolin (17.8 min); 132, 160, and 206 for metalaxyl (18.1 min); 123, 167, and 224 for dichlofluanid (19 min); 225 for cyprodinil (21.3

min); 161 and 250 for penconazole (21.5 min); 96 and 285 for procymidone (22 min); 248 for fludioxonil (24.2 min); 125 and 250 for tebuconazole (26.5 min); and 111, 183, and 219 for lindane (15.8 min), which was used as internal standard (lindane is appropriate for this purpose because, its phytochemical use having been banned, it should not be present in any vineyard soil; and on no occasion have we ever detected it in any vineyard soil).

Soil Samples and Spiking. Soil A (used in developing the method) and soils B–G (used to assess the effects of soil variability on fungicide extraction efficiency) belonged to a vineyard (A), potato or maize fields (B and C), and woodland (D–G) located in the province of Ourense (Galicia, northwestern Spain), that is, outside the Rías Baixas region, whereas soils 1–4 belonged to Rías Baixas vineyards growing white grapes of the variety Albariño. All soil samples were collected from the top 10 cm of soil and stored in small portions at –18 °C pending analysis; their main physical and chemical properties and FAO classifications (25) are listed in **Table 1**. Prior to extraction, soil samples were air-dried and sifted to remove particles >2 mm in diameter. Preliminary analyses (in which extraction was performed as described under Fungicide Extraction and quantitation as described under Analytical Instruments and Operating Conditions) failed to detect any of the targeted fungicides in soils A–G.

When required in the experiments and determinations described below, soil samples were spiked with fungicides. Following the method of Pérez et al. (26), fungicides were added to the soil in the volume of spiking solution required to bring the dry stored sample to field capacity [previously determined for each soil according to the method of Klute (27); see **Table 1**]. Fungicide recovery can be distorted if the water content of the sample is too low (which can cause inefficient adsorption of fungicides onto the sample) or too high (which can shift adsorption equilibria in favor of the solution) (7). Spiking solutions were prepared by diluting intermediate standard mixtures with the appropriate amount of water. To determine the time required for the adsorption of the fungicides to reach equilibrium, samples of soil A that had been spiked with 5 mg/kg of each fungicide were left at room temperature in closed 40 mL EPA vials for 0.5, 3, 6, 9, or 24 h and were then analyzed using the extraction parameters described under Fungicide Extraction (Preliminary Analyses); because there were no differences in the measured fungicide contents of the samples that had been left for 6, 9, and 24 h, in all subsequent work spiked samples were left to equilibrate overnight under the above conditions.

Fungicide Extraction. Final Optimized Procedure. The soil sample (10 g of dry weight; spiked if necessary as described above) was sonicated for 10 min at room temperature in 15 mL of a buffer solution of pH 8 consisting of anhydrous sodium carbonate (3.6% w/v) and sodium polyphosphate (0.8% w/v) in distilled water. Ethyl acetate was added (10 mL), and the mixture was shaken mechanically at 200 rpm for 45 min. The organic phase was drawn off with a Pasteur pipet, dried over anhydrous Na₂SO₄ (0.15 g), and filtered, and 8 mL of this solution was transferred to a 25 mL round-bottom flask and concentrated to dryness at 40 °C in a rotary evaporator. The resulting residue was redissolved in 0.5 mL of a 4 mg/L solution of lindane (the internal standard) in ethyl acetate, and this solution was vortexed, after which

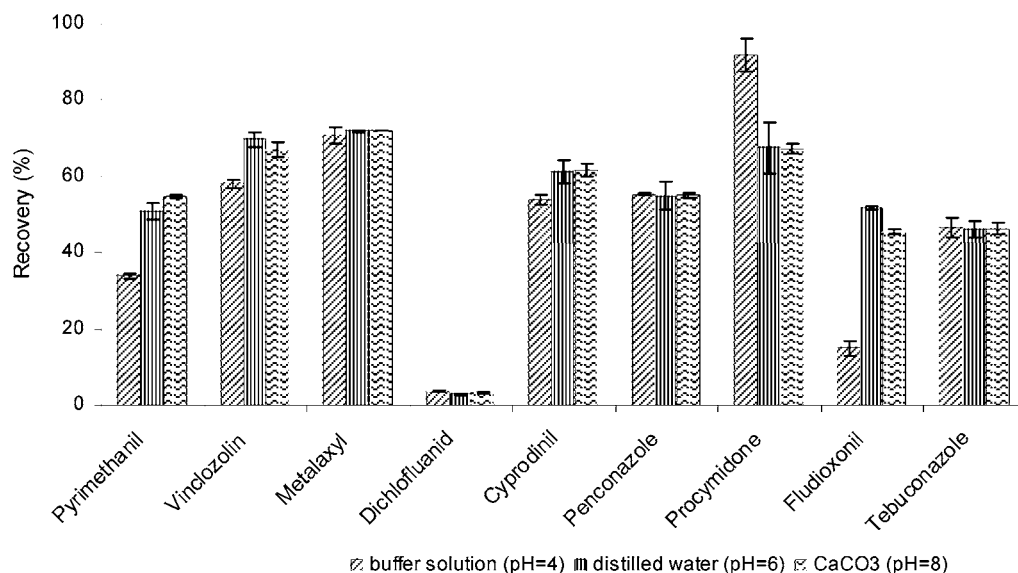


Figure 1. Influence of pH of the aqueous extractant on fungicide recovery (percent). Columns show mean recovery from three replicate soil samples; error bars show standard deviations.

it was transferred to an autosampler vial and 1 μ L injected into the GC-MS equipment for analysis.

Preliminary Analyses. In preliminary analyses, the aqueous solvent was distilled water (10 mL), the organic solvent was 3:1 (v/v) ethyl acetate/hexane (10 mL), and the shaking time was 10 min.

Method Development. Experiments were carried out to optimize the recovery of fungicides from soil A and the sensitivity of their determination. All of these experiments were performed on triplicate samples that were spiked as described above with 1 mg/kg of each fungicide.

Experiments were first carried out, using the extraction parameters specified in under Fungicide Extraction (Preliminary Analyses), to determine whether any advantage might be obtained by removing the soil from the aqueous extract before the addition of the organic extractant to the latter. Leaving the soil in would predictably increase the efficiency of the extraction procedure, but might also allow the organic extractant to extract compounds that diminished the performance of the GC-MS quantitation procedure and, hence, the overall observed recovery. In view of the results (see below), in all subsequent experiments the organic extractant was added without removal of soil.

The pH (4, 6, or 8) and volume (5, 10, or 15 mL) of the aqueous extractant (distilled water for pH 6, 0.1 M sodium acetate/acetic acid buffer for pH 4, and 1 mM aqueous calcium carbonate solution for pH 8) were then optimized in experiments in which the organic solvent was 3:1 (v/v) ethyl acetate/hexane (10 mL) and the shaking time 10 min.

The organic extractant (ethyl acetate, hexane, or a 3:1, 1:1, or 1:3 mixture of the two) and its volume (10, 15, or 20 mL) were optimized in experiments in which the aqueous extractant used was distilled water (5 mL).

The time for which shaking with organic extractant was performed was optimized using ethyl acetate (10 mL) and shaking times of 5, 10, 15, 30, 45, or 60 min following extraction with distilled water (5 mL) as aqueous extractant.

Following the above optimization experiments, experiments were carried out using soils A–G to determine the extent to which fungicide recovery varied with soil characteristics [it was in view of the results of these experiments that the aqueous extractant described under Fungicide Extraction (Final Optimized Procedure) was chosen; see Results]. These experiments involved triplicate samples that were spiked as described above with 0.25 mg/kg of each fungicide except dichlofluanid, for which the spike was 1 mg/kg, and the extraction conditions were those that had been developed for soil A. Significant differences among the fungicide recoveries in different soils were identified at the 95% confidence level by means of a multiple-range test based on

pairwise Student's *t* tests. These differences were also used to estimate the precision of the method.

Finally, limits of detection and quantitation were defined, in accordance with ACS recommendations (28), on the basis of the level of noise in the analytical signal, which was quantified using seven unspiked samples of soil A and the extraction procedure described under Fungicide Extraction (Final Optimized Procedure).

Fungicide Quantitation. Because fungicide recoveries varied significantly from soil to soil (see Results and Discussion), fungicides were quantified in soils 1–4 using the standard additions method (29). For each soil, five samples were analyzed as described above under Fungicide Extraction (two unspiked samples and samples spiked with 0.05, 0.1, and 0.15 mg/kg of each fungicide), and the fungicide content of the soil was then calculated as a/b , where $y = a + bx$ was the equation obtained for the regression of the analytical signal y (the ratio of the fungicide and internal standard peak areas) on the spike level x . Note that even when the standards addition method is employed to control for matrix effects, the internal standard is still necessary to control for between-sample variation in injection and instrument response.

RESULTS AND DISCUSSION

Method Development. *Influence of the Presence of Soil during Organic Extraction.* Recoveries lower than 32% were obtained when the soil was removed from the aqueous extractant prior to addition of the organic extractant, as against 50–72% when it was not removed. The higher recoveries in the presence of soil are attributable to the transfer of fungicides from the aqueous to the organic phase, resulting in more fungicide being desorbed from the soil into the aqueous phase so that overall transfer to the organic phase was raised.

Selection of Aqueous Phase pH and Volume. Because the fungicide recoveries obtained at pH 6 and 8 did not differ significantly for any fungicide and those for fludioxonil and pyrimethanil were both significantly higher than those obtained at pH 4 (Figure 1), distilled water was used as the aqueous extractant in subsequent experiments until the results of experiments with the whole set of uncontaminated soils (A–G) prompted a switch to a buffer of pH 8 (see Influence of Soil Matrix on Fungicide Recovery). Because the volume of water used did not significantly affect recovery, the volume used in these subsequent experiments was the smallest tested, 5 mL.

Selection of Organic Extractant and Volume. Ethyl acetate afforded the best recovery of all fungicides except vinclozolin

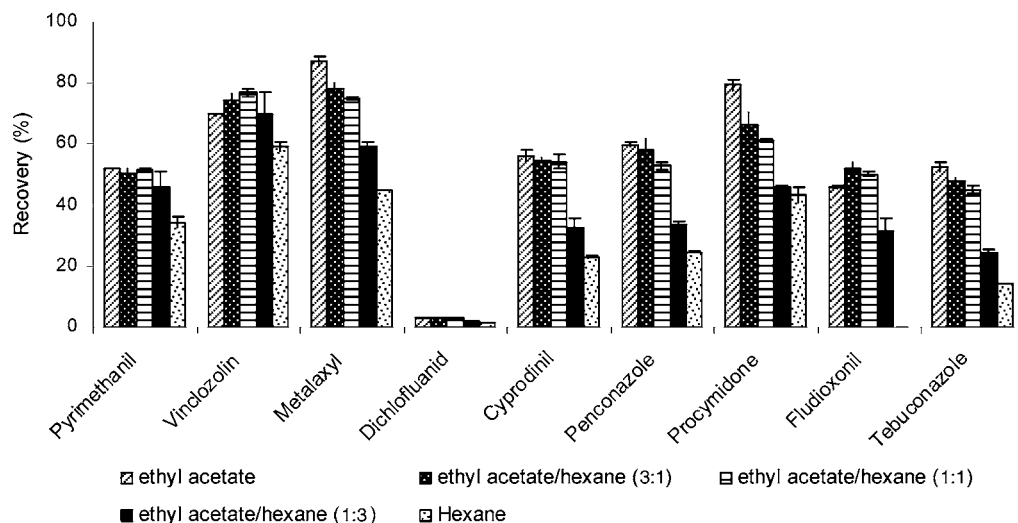


Figure 2. Influence of organic extractant on fungicide recovery (percent). Columns show mean recovery from three replicate soil samples; bars show standard deviations.

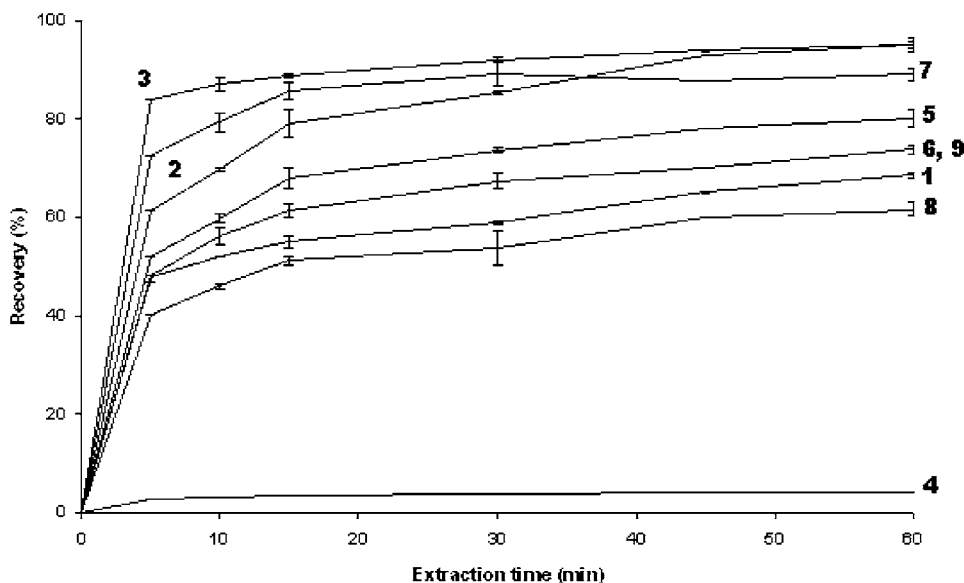


Figure 3. Kinetics of fungicide recovery (percent) (means of three replicate experiments; bars show standard deviations): 1, pyrimethanil; 2, vinclozolin; 3, metalaxyl; 4, dichlofluanid; 5, cyprodinil; 6, penconazole; 7, procymidone; 8, fludioxonil; 9, tebuconazole.

and fludioxonil, and for these two it was only slightly suboptimal (**Figure 2**). It was therefore chosen as organic extractant. Because no significant differences among the recoveries achieved with different volumes of ethyl acetate were observed, the volume used in subsequent work was the smallest tested, 10 mL.

Influence of the Duration of Extraction with Ethyl Acetate. In view of the recovery versus extraction time profiles obtained for the various fungicides (**Figure 3**), 45 min was chosen as a suitable compromise between the need for desorption to be as complete as possible and the need for the method not to be too lengthy.

Influence of Soil Matrix on Fungicide Recovery. The significant between-soil differences in fungicide recovery observed, for all of the fungicides, when soils B–G were analyzed according to the method initially developed for soil A (**Table 2**) may be attributed to the physical and chemical differences among these soils. In particular, the fact that recoveries were generally lower from soils B–G than from soil A is attributable to soil buffering capacity, which will have led to aqueous

extraction having taken place at the pH of the soil in water; because the pH values of soils B–G (pH 4.3–5.1; see **Table 1**) are lower than that of soil A (pH 7.0), the lower recovery from the former is in keeping with the results obtained previously for soil A and shown in **Figure 1**.

When carbonate/polyphosphate buffer of pH 8 was used as aqueous extractant instead of distilled water, recoveries were higher and varied less from soil to soil, although both between-soil and between-fungicide differences were still evident (**Table 3**). In particular, the very low recovery of dichlofluanid [probably attributable to its hydrolysis during extraction (6, 11)] led to our excluding it from the study at this point. The average recoveries of the other fungicides in soils A–G did not differ significantly from 100%, although that of fludioxonil was borderline, which may be due to fludioxonil—the only phenylpyrrole among these fungicides—being planar and/or having a $pK_a > 13$ (whereas the other fungicides used are not planar and have pK_a values of between 5 and 6; see **Table 4**).

Fludioxonil was also the only fungicide for which correlation analysis showed recovery to depend significantly on any of the

Table 2. Mean Fungicide Recoveries \pm Relative Standard Deviations (Percent) Determined Using Distilled Water (pH 6) as Aqueous Extractant

	soil A	soil B	soil C	soil D	soil E	soil F	soil G	overall
pyrimethanil	65 \pm 2ab ^a	68 \pm 2a	61 \pm 2c	60 \pm 3c	63 \pm 2bc	23 \pm 2e	34 \pm 2d	53
vinclozolin	93 \pm 3a	92 \pm 4a	55 \pm 5b	93 \pm 3a	96 \pm 2a	25 \pm 2d	36 \pm 2c	70
metalaxyl	94 \pm 2a	83 \pm 2b	89 \pm 2ab	84 \pm 6b	88 \pm 2ab	35 \pm 6d	50 \pm 2c	75
dichlofluanid	4 \pm 2e	29 \pm 2b	4 \pm 2e	40 \pm 2a	27 \pm 2b	12 \pm 2d	17 \pm 2c	19
cyprodinil	78 \pm 3a	63 \pm 5bc	56 \pm 9cd	71 \pm 9ab	68 \pm 2ab	30 \pm 2e	46 \pm 2d	59
penconazole	70 \pm 2a	16 \pm 6c	27 \pm 2bc	47 \pm 10ab	33 \pm 2bc	12 \pm 3c	27 \pm 8c	33
procymidone	88 \pm 3a	44 \pm 2d	44 \pm 4d	70 \pm 5b	56 \pm 3c	14 \pm 2 f	27 \pm 3e	49
fludioxonil	60 \pm 2a	29 \pm 8cd	32 \pm 4cd	39 \pm 2bc	47 \pm 4b	26 \pm 2d	14 \pm 2e	35
tebuconazole	68 \pm 2a	14 \pm 2cd	26 \pm 7bc	30 \pm 11b	35 \pm 6b	11 \pm 2d	10 \pm 2d	28

^a Soils are grouped from high to low recovery for a given fungicide by a multiple-range test based on Student's *t* pairwise comparisons.

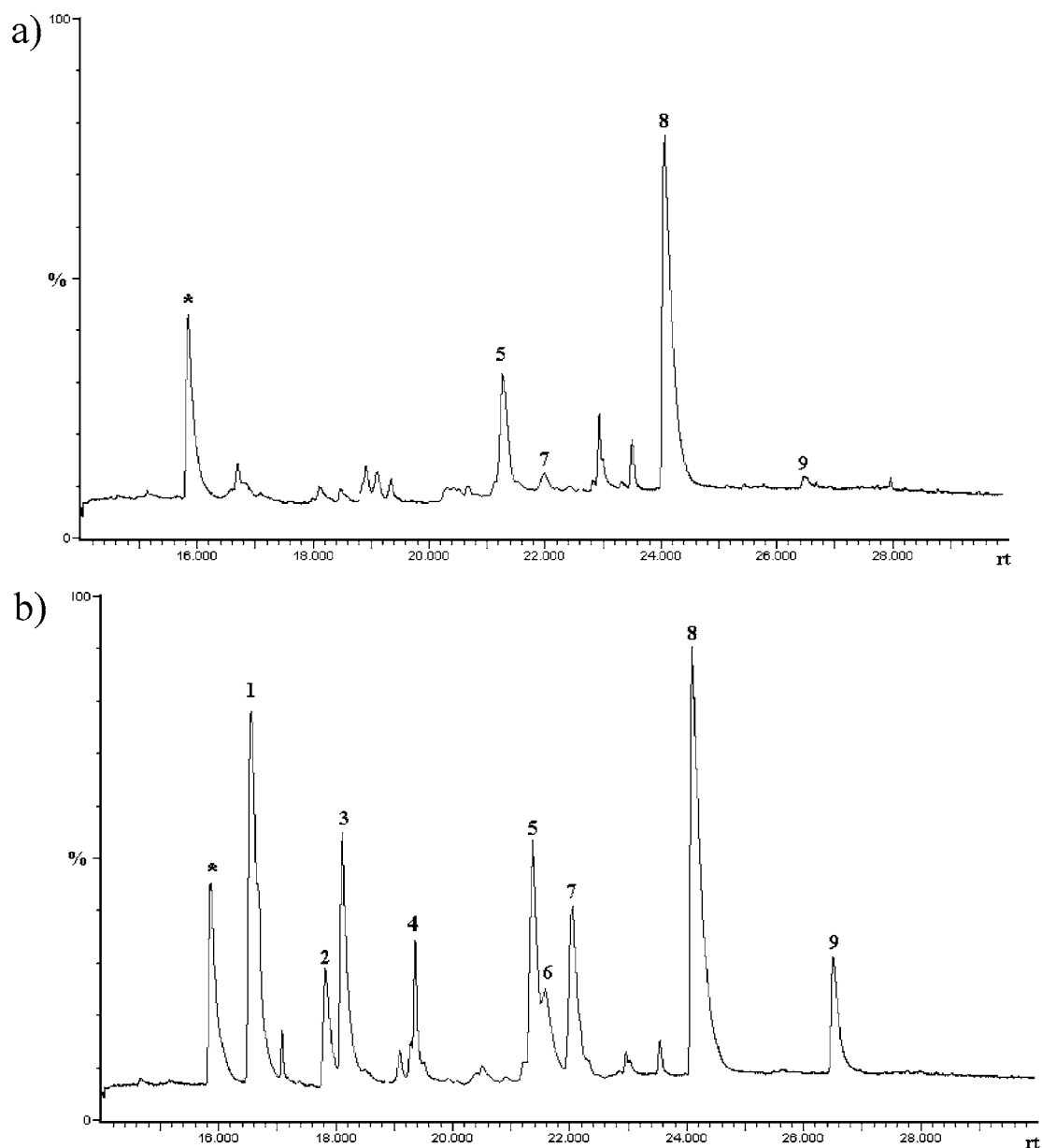


Figure 4. GC-MS chromatograms of (a) a subsample of vineyard soil 1 taken in October 2003 and (b) a subsample of the same sample spiked with 0.15 mg/kg of each of the fungicides considered except dichlofluanid, which was added at a level of 1 mg/kg. Peaks: *, lindane (internal standard); 1, pyrimethanil; 2, vinclozolin; 3, metalaxyl; 4, dichlofluanid; 5, cyprodinil; 6, penconazole; 7, procymidone; 8, fludioxonil; 9, tebuconazole.

physical and chemical properties listed in **Table 1**. Recovery of fludioxonil increased with exchangeable calcium content ($r = 0.7528$, $p < 0.05$), which is reminiscent of Mortland's (30) finding that desorption of the herbicide 3-aminotriazole

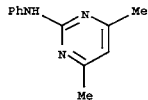
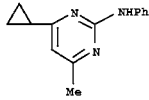
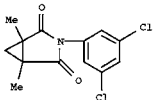
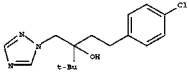
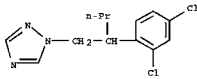
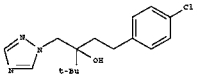
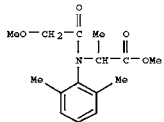
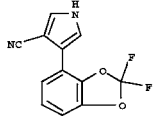
from clay was greatest when the dominant exchangeable cation was calcium, presumably due to the coordination of the herbicide to the cation being weakest for this metal. In the present case, our data imply that the liming of soils containing fludioxonil

Table 3. Mean Fungicide Recoveries \pm Relative Standard Deviations (Percent) Determined Using Carbonate/Polyphosphate Buffer (pH 8) as Aqueous Extractant

	soil A	soil B	soil C	soil D	soil E	soil F	soil G	overall
pyrimethanil	105 \pm 11bcd ^a	115 \pm 9ab	119 \pm 3a	110 \pm 2abc	101 \pm 5cd	95 \pm 3de	86 \pm 3e	104
vinclozolin	95 \pm 5b	115 \pm 10a	76 \pm 9c	108 \pm 2ab	114 \pm 9a	60 \pm 13d	76 \pm 8c	92
metalaxyl	121 \pm 5bc	110 \pm 8d	127 \pm 4ab	132 \pm 2a	111 \pm 3cd	105 \pm 3d	96 \pm 3e	115
dichlofluanid	14 \pm 13d	35 \pm 3a	3 \pm 26e	19 \pm 9c	23 \pm 3b	24 \pm 2b	24 \pm 3b	20
cyprodinil	113 \pm 15a	108 \pm 16ab	112 \pm 3ab	124 \pm 6a	108 \pm 7ab	92 \pm 2bc	80 \pm 5c	105
penconazole	116 \pm 2a	104 \pm 3b	100 \pm 7b	100 \pm 5b	107 \pm 2b	84 \pm 3c	74 \pm 7d	98
procymidone	98 \pm 16a	95 \pm 17a	97 \pm 11a	86 \pm 4ab	90 \pm 3ab	73 \pm 3bc	63 \pm 3c	86
fludioxonil	94 \pm 6a	74 \pm 10bd	88 \pm 9ab	82 \pm 2bc	79 \pm 4bcd	72 \pm 3de	62 \pm 5e	79
tebuconazole	109 \pm 2bc	100 \pm 7c	114 \pm 9ab	120 \pm 2a	101 \pm 2c	91 \pm 2d	75 \pm 3e	101

^a Soils are grouped from high to low recovery for a given fungicide by a multiple-range test based on Student's *t* pairwise comparisons.

Table 4. Characteristics of the Fungicides Studied

Chemical family	Fungicide	Structure	Planar	pKa ^b
Anilinopyrimidine	Pyrimethanil		No	5.17 \pm 0.17
	Cyprodinil		No	5.25 \pm 0.17
Dicarboximide	Procymidone		No	-
	Vinclozolin		No	-
Triazole	Penconazole		No	5.20 \pm 0.41
	Tebuconazole		No	5.76 \pm 0.41
Phenylamide	Metalaxyl		No	-
Phenylpyrrol	Fludioxonil		Yes	13.57 \pm 0.5

^b From SciFinder Scholar (2004 version, American Chemical Society). —, not known.

probably favors its release into the soil solution, with the corresponding implications for soil-dwelling microorganisms and water pollution.

Precision and Limits of Detection and Quantitation. The relative standard deviations listed in **Table 3** for the fungicides other than dichlofluanid range from 2 to 17% (mean = 6%). For metalaxyl, cyprodinil, and tebuconazole, the limit of

detection determined using soil A was in the range of 2–10 $\mu\text{g}/\text{kg}$ and the limit of quantitation was in the range of 5–25 $\mu\text{g}/\text{kg}$. For pyrimethanil, vinclozolin, penconazole, procymidone, and fludioxonil, the limit of detection was in the range of 10–20 $\mu\text{g}/\text{kg}$ and the limit of quantitation was in the range of 30–50 $\mu\text{g}/\text{kg}$. In the cases of cyprodinil and penconazole, which had overlapping GC-MS peaks (**Figure 4b**), this performance

Table 5. Fungicide Concentrations Determined in Rías Baixas Vineyard Soils (Micrograms per Kilogram)^a

fungicide	soil 1		soil 2	soil 3	soil 4
	Oct 2003	May 2003	May 2003	May 2003	May 2003
pyrimethanil	—	—	—	—	—
vinclozolin	—	—	—	—	—
metalaxyl	—	—	LOD	—	LOD
dichlofluanid	—	—	—	—	—
cyprodinil	260	LOD	LOQ	LOQ	LOD
penconazole	—	—	—	—	—
procymidone	20	LOD	—	—	LOD
fludioxonil	991	LOQ	45	52	LOQ
tebuconazole	12	—	LOD	—	LOQ

^a —, not detected; LOD, detected at the limit of detection; LOQ, detected at the limit of quantitation.

was achieved by separately quantifying the signal at m/z 225 (cyprodinil) and the combined signal of the ions at m/z 161 and 250.

Fungicide Levels in Rías Baixas Vineyard Soils. In May 2003 (9 months after the latest fungicide application), samples were taken from all four of the Rías Baixas vineyards studied, and in October 2003 (1 month after the last application to the 2003 crop) another sample was taken from soil 1. In this last sample the fungicides tebuconazole, procymidone, cyprodinil, and fludioxonil were detected, in order of increasing concentration (see **Figure 4a** and **Table 5**); the predominance of the antibiolytic fludioxonil and cyprodinil over procymidone reflects the popularity of the local commercial product Switch (37.5% cyprodinil, 25% fludioxonil). Cyprodinil and fludioxonil were also detected in all four samples taken in May 2003, but only fludioxonil was detected at levels higher than its limit of quantification (45 $\mu\text{g}/\text{kg}$ in soil 2 and 52 $\mu\text{g}/\text{kg}$ in soil 3). Metalaxyl, procymidone, and tebuconazole were also detected at subquantitation levels in some of these samples.

Conclusions. The multiresidue method described above is suitable for the simultaneous determination of the fungicides cyprodinil, fludioxonil, metalaxyl, penconazole, pyrimethanil, procymidone, tebuconazole, and vinclozolin in vineyard soils. The extraction method employed, sonication of the soil sample in pH 8 buffer followed by extraction with ethyl acetate, allows quantitative determination without further purification. The method has adequate precision (2–17%, mean = 6%) and is highly sensitive (limits of detection < 20 $\mu\text{g}/\text{kg}$ and limits of quantitation > 50 $\mu\text{g}/\text{kg}$ for all of the fungicides included). It requires the use of both an internal standard (to control for between-sample variation in injection and instrument response) and the standard additions method (to control for matrix effects). Analysis of soil samples from Rías Baixas vineyards showed levels of individual fungicides as high as 991 $\mu\text{g}/\text{kg}$ 1 month after the crop's final treatment, but at the start of the season (9 months after the previous crop's final treatment) no fungicide was detected at a concentration > 52 $\mu\text{g}/\text{kg}$.

ACKNOWLEDGMENT

We thank the soil science group of the Faculty of Food Science and Technology for taking soil samples and determining the characteristics listed in **Table 1**.

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Received for review May 3, 2004. Revised manuscript received September 15, 2004. Accepted September 15, 2004. We are grateful to Xunta de Galicia for the research contract to B.C.-G. under the program "Parga-Pondal". This work was supported by the Spanish Ministry of Science and Technology under Project AGL2003-02244.

JF0493019