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Multiresidue Determination of Fungicides in Wine

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Botrytis cinerea (grey mould) is one of the most important fungal diseases in Galicia viticulture; its growth causes serious production losses and adversely affects wine quality. Many different fungicides have been developed and tested agains *B. cinerea,* t but the best results have been provided by some active derivatives of 3,5-dichloroaniline (3,5-DCBA) belonging to the dicarboximidic family such as vinclozolin, procymidone, iprodione and chlozolinate (Farris et al. 1992).

In Galicia (NW Spain) there are many different wines, which are classified according to type of grape or vine, climatology and properties soil. One of the most specific wines is the "Rías Baixas", variety known as “Albariño”, which is named for the grapes from which it is made. This is a white wine with a fruity taste and a low alcoholic grade. Its origin and quality are guaranteed by national law and, thus, it has a "Denominación de origen" title (DOG1988).

The use of these fungicides is increasing because their correct application does not produce a residue content higher than the legal limits for harvested grapes. However, although a loss of fungicides occurs during vinification, it is possible to find consistent residues of the fungicides in wines as a consequence of the frequent application of heavy dosages, together with or because of, a lack of respect for the statutory safety interval between treatment and harvest (Cabras et al. 1984; Holland et al. 1994).

Pesticide residues in food is of great importance in the evaluation of food quality.For example, we are also working on pesticide residues in honey (Garcia et al. 1995-6). Recently wine has been subjected to frequent quality control checks for pesticide residues used for the control of pests in grapes. From a legal point of view, the maximum residue limits (MRL) for grapes have been established by the Spanish 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 wine, a limit is set for both grapes and wine. The European Community has considered the opportunity of setting the MRL in wine especially in view of the difficulties that may arise in commercialization (Cabras et al. 1992). In 1990 the F.D.A. decided to stop the importation of wine which contains residues of procymidone.

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The analytical methods available for the determination of pesticides in wine are numerous and use Gas Chromatography (GC) (Bertrand and Bertsch 1990; Dugo et al. 1992; Simal et al. 1993; Holland et al. 1994).

The objective of this work is to set up a residue method with a pesticide extraction procedure for wine and and grapes and chromatographic determination of the levels of the pesticide residues in these products.

MATERIALS AND METHODS

The study was carried out on samples of Albariño wine and some samples of grapes, corresponding to province of Pontevedra, Galicia (NW Spain).

Each wine corresponds to a different tank of fermentation and each tank contains wine obtained from Albariño grapes grown in different vineyards, which have been treated with similar phytosanitary products.

For each sample of wine two analyses were performed; one with static wine and the other with centrifuged wine. The assays were done in triplicate. Analyses were conducted for three pesticides: chlozolinate, procymidone and vinclozolin (Ehrenstorfer , Ausburg, Germany, purity 99, 99.9 and 99.9 % respectively).

Stock standard solutions (300 µg/mL) for chlozolinate and vinclozolin, and 200 µg/mL for procymidone) were prepared by dissolution of pesticides in n-hexane and stored at 4 °C. Working standard solutions were prepared by dilution of stock standard solutions in n-hexane, obtaining the following range of concentrations: chlozolinate, 15-100 mg/mL for NPD, and 0.125-4 µg/mL for ECD; procymidone, 25-150 µg/mL for NPD, and 0.1-13.6 µg/mL for ECD; vinclozolin, 15-105 µg/mL for NPD, and 0.05-1 µg/mL for ECD. All solvents used were pesticide analytical grade reagents free of interfering residues as tested by gas chromatography.

50 mL aliquot of wine was extracted with 10 mL of n-hexane.It was then mixed and allowed to stand until phase separation. The aqueous phase was partitioned again by adding 5 mL of n-hexane. The aqueous phase was discarded and the organic phase was added to the organic phase collected by the initial extraction. The final extract (two organic phases) was evaporated to dryness under a stream of nitrogen at room temperature and redissolved in n-hexane to a fixed volume.

Ten g of unwashed grapes were ground in a mixer. They were extracted with 10 mL of n-hexane. They were mixed and allowed to stand until two phases had separated (aqueous phase and organic phase). The aqueous phase was partitioned again by adding 5 mL of n-hexane. It was mixed again and allowed to stand until the two phases had separated. The aqueous phase was discarded and the organic phase was added to the organic phase collected by the initial extraction. The final extract (two organic phases) was evaporated to dryness under a stream of nitrogen at room temperature and redissolved in n-hexane to a fixed volume (0.25-1 mL).

Gas chromatography analysis was performed using a Hewllett Packard 5890 equipped with a nitrogen-phosphorus detector and confirmed with a Perkin-Elmer Autosystem equipped with a ^{63}Ni electron capture detector. The columns used in each gas chromatograph were, a capillary column HP-101 containing methylsilicone fluid as non polar stationary phase (12 m length, 0.2 mm id. and 0.2 µm film thickness) (HP), and a capillary column HP-5 containing phenylmethylsilicone as stationary phase (25 m lenght, 0.2 mm id. and 0.33 µm film thickness) (PE). Chromatographic conditions for the NP detector were: detector, 250 °C, injector, 200 °C, the initial column temperature was 60 °C, to 240 °C at 30 °C min^{-1}, 1 min final hold. The mobile phase was nitrogen (N-50) purge flow, 1.05 mL min^{-1}, hydrogen flow, 3.5 mL min^{-1}, air flow, 100 mL min^{-1}, head column pressure, 9 psi, column flow, 1.4 mL min^{-1}, make-up flow, 36 mL min^{-1}, ratio split column, 53, injection volume, 2 µL.

Chromatographic conditions for the EC detector were: detector, 300 °C, injector, 200 °C, the initial column temperature was 60 °C, held 1 min after the injection, programmed from 60 °C to 180 °C at 20 °C min^{-1}, held for 1 min, programmed from 180 °C to 240 °C at 10 °C min^{-1}, held for 1 min, programmed from 240 °C to 250 °C at 5 °C min^{-1}, held for 2 min. The mobile phase was nitrogen (N-50) split flow, 200 mL min^{-1}, head column pressure, 30 psi, column flow, 2 mL min^{-1}, make-up flow, 30 mL min^{-1}, ratio split column, 100, injection volume, 1 µL. The samples were injected in the splitless mode and the splitter was opened after 0.5 min.

Peaks were measured by area counts given by the integrator. Quantification was by comparing peak areas of the sample with those of corresponding standards.

RESULTS AND DISCUSSION

Chlozolinate, procymidone and vinclozolin were identified by comparison of their retention times to the standards. Table 1 shows the retention times of these pesticides.An external standard method was used to determinate the linearity of response of the two detectors, NPD and ECD, to calculate the percentages of the pesticides recovered and to evaluate the level of the residues in wine and grape samples. The estimation of pesticide residues was done with a NP detector. In order to avoid errors, the identities of pesticides were confirmed by GLC Perkin-Elmer with EC detector.

Table 1. Retention times of the studied pesticides

Pesticide	R_T (min) for HP-101	R_T (min) for HP-5
Chlozolinate	5.9	8.8
Procymidone	6.1	10.5
Vinclozolin	5.3	8.9

Subsamples of wines and grapes with no significant pesticide residues were fortified with standards at two different concentration levels for each pesticide (Table 2). Aliquots were analyzed in triplicate for the two levels. Recoveries were between 87 and 99 % for wine samples.

Table 2. Recoveries for pesticides in wine

Pesticide	µg added	µg recoveries	% recovery
Chlozolinate	25.00	21.75	87.0
	60.00	54.00	90.0
Procymidone	15.00	13.95	93.0
	40.00	38.00	95.0
Vinclozolin	17.25	17.10	99.1
	86.25	85.20	98.8

Quantification levels were estimated from pesticide concentration which produce a signal similar to the background signal and one ten times the standard deviation of the background (Table 3). The minimum detectable levels (MDL) given in table 3 were estimated from standard pesticide concentrations which produce a signal similar to the background signal and one three times the standard deviation of the background.

Table 3. Minimum detectable and quantification levels of the pesticides

Pesticide	Minimum Detectable Level (µg/mL)	Quantitation level (µg/mL)
Chlozolinate	7.38	24.62
Procymidone	4.00	10.60
Vinclozolin	9.00	28.00

During wine-making, a noteworthy reduction of residues present in grapes occurs due to hydrolysis and absorption on suspended matter. This reduction is already significant in the centrifuged wines, because of the reduction in particulate matter. In Figure 1 the reduction of the vinclozolin concentrations (µg/mL) as they go from grapes to static wine and centrifugated wine can be observed.

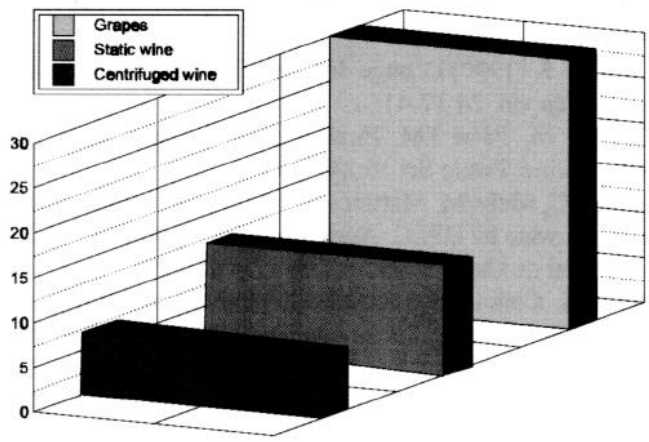

Figure 1. Reduction of the vinclozolin concentrations (µg/mL) in grapes and wines

Of the analyzed samples, only one sample of static wine did not contain residue. Higher values in the grape and static wine samples were observed in all samples. The highest concentration found was procymidone which is the most frequently fungicide used by vine growers, and the lowest was chlozolinate. Table 4 shows the mean values obtained with a NP detector, in static and centrifugated wines and grapes.

Table 4. Mean values (µg/L) of the studied pesticides

Sample	Chlozolinate	Procymidone	Vinclozolin
Static wine	1	19	12
Centrifugated wine	2	11	7
Grapes	76	208	30

None of the samples analyzed exceed the maximum residue levels (MRL) in wine and grapes. These MRL values for chlozolinate and procymidone in grapes are 3 ppm and 5 ppm, respectively (BOE 4/11/89). In wine they MRL are 5 ppm and 1.5 ppm, respectively (O.M. Italian 18/7/90). For vinclozolin MRL has not been stablished.The acceptable daily intake level (IDA) for procymidone by FAO/WHO is 0.0.1

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