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Comparison of Two Recent Solventless Methods for the Determination of Procymidone Residues in Wines: SPME/GC/MS and ELISA Tests

Keywords: *Procymidone; wine; SPME; ELISA; method comparison*

INTRODUCTION

Pesticides are now commonly used for crop production and harvest protection all over the world. Thus, pesticide residue determination in food has become a major field of investigation for analysts throughout all national and international food safety programs. Procymidone is a fungicide widely used against *Botrytis cinerea* on wine grapes. Resulting from unproper applications, undesirable residues at concentrations ranging from a few micrograms per liter (ppb, w/v) to several hundreds of micrograms per liter (U.S. EPA, 1991) can be found in wine after fermentation and even in old bottles because of its well-known persistence.

Procymidone residues are currently monitored by different chromatographic methods, which have been recently reviewed by Simal Gándara et al. (1993): all cited references mainly focus on the technical efficiency and accuracy of each method used and the separation and quantification of the analyte; some of these methods have been applied to wine. In contrast, very few are concerned with the extraction step and the sample preparation is generally carried out by extracting procymidone using liquid−liquid extraction (LLE) with hydrophobic organic solvents (Bertrand and Bertsch, 1990; Garcia-Cazorla and Xirau-Vayreda, 1994) or solid phase extraction (SPE) using polymeric bonded silica cartridges (Jeager et al., 1995). In both cases, analysis steps are carried out from organic solution samples, the concentrations of which are matched with the sensitivity level of the chromatographic system detector.

On the other hand, it has been recently pointed out by Bushway and Fan (1995) that the expense for chemical analyses is "skyrocketing" because of the need of trained personnel, expensive equipment, and solvent disposal cost. Then, the easy and cheap sampling method, so-called solid phase microextraction (SPME), which allows direct extraction of organics from aqueous solutions without using any organic solvent, appears of great interest. First described by Belardi and Pawliszyn (1989) and extensively developed for extraction of organics (Arthur et al., 1992a,b; Górecki and Pawliszyn, 1995; Louch et al., 1992; Potter and Pawliszyn, 1994; Zhang et al., 1994) and especially pesticide traces in natural waters (Boyd-Boland and Pawliszyn, 1995; Eisert and Levsen, 1995a,b; Magdic and Pawliszyn, 1996), the technique has been successfully applied to wines (Urruty and Montury, 1996). The extension of these results concerning procymidone extraction coupled with gas chromatography and mass spectrometry analysis (GC/MS) incited us to compare them with those given by immunochemical analysis of the same procymidone-spiked wine samples. Different immunoassay (IA) methods for procymidone analysis in wine have been recently proposed (Ferguson et al., 1993; Lawruk et al., 1994; Lucas et al., 1995), even one compared to LLE/GC/MS analysis (Wynn et al., 1993). A rapid kit for enzyme-linked immunosorbent assay (ELISA) is now commercially available from Ohmicron (Newton, PA) and Prolabo (Fontenay Sous Bois, France), constituting another solventless method that can be compared to SPME/GC/MS.

A collaborative study was undertaken to compare SPME/GC/MS and ELISA methodologies for analysis of procymidone residues in wines, in terms of repeatability, useful range, response linearity, and correlation between the two types of results.

MATERIALS AND METHODS

Apparatus. The GC used was a Varian 3400 equipped with a Finnigan ITS 40 ion trap mass spectrometer detector and a split/splitless injector. Separations were obtained with a Supelco PTE 5 column, 30 m × 0.32 mm, with a phase

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thickness of 0.25 μm. A manual SPME holder was used with a 100 μm polydimethylsiloxane fiber assembly (Supelco, France). A Ohmicron RPA-1 Rapid Analyser spectrophotometer and a two-piece magnetic separation rack with a test tube holder and a magnetic base containing rare earth magnets were furnished by Prolabo. A Sartorius balance Basic BA 110S and Gilson adjustable volume pipets P-1000, P-200, and P-20 were necessary for solution preparations.

Reagents were purchased as follows: ethanol, ethyl acetate, and ultrapure water, all gradient grade solvents Lichrosolv (Merck, Darmstadt, Germany); helium C (Air Liquid, Paris, France); L-(+)-tartaric acid and sodium hydroxide analypur quality (Osi, Elancourt, France); ELISA procymidone Ohmicron Rapid Assay kit (Prolabo); analytical grade procymidone (Alltech, Templeuve, France). A stock solution of procymidone was prepared by dissolving 100 mg of this product in 100 mL of ethyl acetate (1000 ppm, w/v).

Standard and Sample Preparations. A working solution containing 50 mg/L (50 ppm, w/v) of procymidone in ethyl acetate was prepared by diluting 500 μL of the stock solution to 10 mL with the same solvent. According to already published results (Urruty and Montury, 1996), a synthetic solution was obtained by dissolving 3 g of L-(+) tartaric acid into a mixture of 120 mL of ethanol and 850 mL of water, adjusting the pH to 3.5 with a 5 M sodium hydroxide solution, and completing to 1000 mL. An aliquot of 2 mL of the working solution at 50 ppm was diluted to 100 mL with the synthetic solution to give solution A (1000 ppb, w/v) used to prepare the calibration graph for SPME/GC/MS analysis: aliquots of 25, 5, 2.5, and 1 mL of solution A were diluted to 50, 50, 50, and 100 mL, respectively, with the synthetic solution affording standards at concentrations of 500, 100, 50, and 10 ppb, respectively. Aliquots of 25, 5, 2.5, and 1 mL of the standard at 10 ppb were diluted to 50, 50, 50, and 100 mL, respectively, with the synthetic solution, affording standards at 5 and 1 ppb and 500 and 100 ppt (ng/L, w/v), respectively. White and red wines were obtained from local producers of the Bergerac area (France); their ethanol concentrations were measured at 11.9% and 12.25%, respectively, and they were found free of procymidone.

Procymidone-spiked samples of white and red wines were obtained by applying the same dilution procedure to these wines as was used for synthetic solution. Spiked samples at concentration ranging from 100 ppt to 1000 ppb were immediately conditioned in 12 mL sealed ampules and stored at 4 °C until analysis. Procymidone-spiked samples of white and red wines at the concentration of 20 ppb were prepared by diluting 10 mL of the 100 ppb spiked sample to 50 mL with the corresponding wines and stored under identical conditions.

SPME/GC/MS Analysis. A 3 mL aliquot of the sample, warmed to room temperature, was transferred to a 5 mL Teflon-lined septum cap vial, equipped with a Teflon-coated magnetic bar. The SPME fiber was introduced and exposed into the liquid stirred at a regular speed of about 200 rpm. After an adsorption time of 30 min, the fiber was withdrawn into the holder and removed from the vial, immediately introduced into the injection port of the GC, and thermally desorbed in the injector used in the splitless mode for 3 min at 250 °C and in the split mode (60 mL/min helium) after 3 min. The temperature program of the oven was as follows: 50 °C for 3 min, increased at 10 °C/min to 300 °C, and held at 300 °C for 2 min. The ion trap was held at 220 °C and the transfer line at 250 °C. The mass spectrometer was tuned to FC 43 (perfluorotributylamine) and m/z ratios between 35 and 450 amu were scanned. Selected ion monitoring (SIM) mode was used, and the peak at 283 selected to characterize and quantify procymidone. Quantitation was performed by measuring peak areas. Each sample was run in triplicate for wines and duplicate for synthetic solution. Using these conditions, the linearity of the response of the mass detector was tested by injecting in triplicate 1 μL of ethyl acetate solutions of procymidone over the range 0.1−10 mg/L and the linear regression coefficient observed was $r = 0.9989$.

Immunoassay Analyses. A typical assay was run by adding at room temperature 100 μL of wine sample or standard, 250 μL of the procymidone conjugate enzyme, and 500 μL of the corresponding anti-procymidone-coupled particles into a test tube. After vortex agitation (2 s), the mixture was incubated for 30 min at room temperature. The magnetic particles were separated using the magnetic rack for 2 min. The liquid was decanted and the residue washed twice with 1 mL of deionized water. Bound enzymes were detected by addition of 500 μL of a mixture of hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine. The color reaction was stopped after 20 min of incubation at room temperature by the addition of 500 μL of sulfuric solution. The absorption (B) at 450 nm was determined within 15 min after the reaction was stopped, using the RPA Analyzer and compared to a zero concentration standard (B_0) to give the ratio B/B_0, which was plotted as the Logit B/B_0 against the Log of the concentration (Log C).

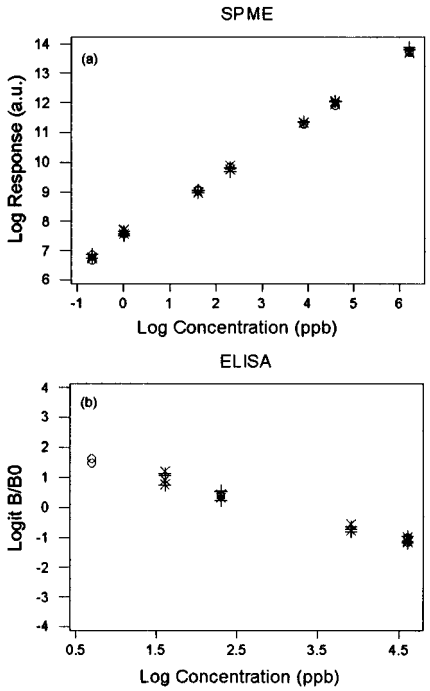

Figure 1. Calibration points obtained by SPME/GC/MS [SIM mode, arbitrary units (au)] between 0.5 and 500 ppb (a) and by ELISA between 2 and 100 ppb (b) in spiked samples [(○) standard solution, (+) white wine, (×) red wine)]. For the calibration line equations, see parameters in Table 1.

RESULTS AND DISCUSSION

Calibration Range and Linearity. The two diagrams of Figure 1 present the observed responses for the calibration graphs obtained by SPME, on the one hand, from the synthetic solution and the spiked red and white wines and by ELISA, on the other hand, from the standard solution and the spiked red and white wines. Natural logarithmic scales (Log) are used in both cases to make results easier to compare. All experiments were performed by the same operator with no preliminary training for the ELISA technology.

As described by Lawruck et al. (1994) and the supplier, responses for the ELISA were found linear in the range 2−100 ppb for the three matrices (Table 1). At the level of 1 μg/L in white wine, procymidone was detected but the mean value observed was far from the corresponding regression line. At levels <1 ppb, no significant differences were observed for Logit B/B_0 between the sample and the zero standard.

For the SPME/GC/MS method the observed linearity parameters are also indicated in Table 1 and show standard deviations slightly smaller than those of the other method for every matrix. In contrast, on the basis of measured values with a signal to noise ratio (S/N)

Table 1. Regression Parameters of the Calibration Lines Obtained for Procymidone Determination by SPME and ELISA Methods

technique	sample	intercept	slope	r	no. of calibrtn points
SPME	synthetic solutn	7.5008	0.984302	0.9989	14
	white wine	7.49836	1.00216	0.9995	21
	red wine	7.50156	0.988956	0.9990	21
ELISA	standard solutn	1.997126	−0.664815	0.9975	6
	white wine	2.04893	−0.691971	0.9864	12
	red wine	1.97939	−0.669539	0.9874	12

Table 2. Repeatability of the Responses Calculated from 10 Replicates in Wines Spiked at 20 μg/L

sample	technique	mean[a]	standard deviation	RSD (%)
white wine	SPME	35326	2142	6.1
	ELISA	0.619	0.062	10.0
red wine	SPME	38213	1190	3.1
	ELISA	0.667	0.030	4.6

[a] Peak area for SPME and absorbance B for ELISA.

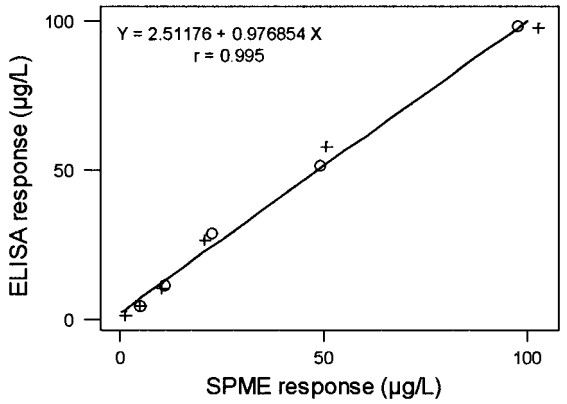

Figure 2. Comparison of procymidone concentrations determined by SPME and ELISA in white wine (+) and red wine (○).

>3, procymidone can be detected at the level of 100 ng/L (ppt, w/v) as well in the synthetic solution as in white or red wines. With a S/N ratio >10, quantitation can be performed as soon as the spiking level reaches 500 ppt and the corresponding RSD for triplicates is <10%. On the other side, at the top of the range, concentration levels >500 ppb induce saturation of the ion trap detector and an important decrease of repeatability.

From these results, SPME/GC/MS appears as a slightly more sensitive method for procymidone residue determination than ELISA, but both exceed the HPLC method sensitivity published by Cabras et al. (1992), the GC/MS method using Sep-Pak C_{18} extraction cartridges reported by Simal Gándara et al. (1992), or the LLE/GC/FID method developed by Bertrand (1990), with detection limits at 10, 10, and 3 ppb, respectively. Concerning the range width, the method using SPME allows determination upon at least 3 orders of magnitude against only 2 for the IA method before any dilution of the sample (Figure 1).

Precision. Two series of 10 samples of white and red wines, respectively, all spiked at the same concentration of 20 μg/L (ppb, w/v) were analyzed using both methodologies. Results are indicated in Table 2 and show that RSDs are ≤10% for the two matrices whatever the technique used. The slight differences observed between RSDs in this table are not significant since only two wine samples were used.

Method Comparison. Figure 2 displays the correlation between procymidone concentrations as determined by ELISA (y-axis) and SPME/GC/MS (x-axis) for the six samples spiked at 1, 5, 10, 50, and 100 ppb (triplicates) and 20 ppb (10 replicates) of white and red wines. Plotted values on both axes are those obtained by comparing the observed Logit B/B_0 with the regression line from the calibration standard solution (ELISA) and the observed Log response with the regression line from the calibration synthetic solution (SPME) for each sample studied.

The two technologies are highly correlated ($r = 0.995$), indicating a strong linear relationship between the two procymidone determination methods. The slope (0.977 ± 0.032), not significantly different from 1, suggests no differences in response to procymidone. The intercept (2.5 ± 1.5 μg/L), not equal to 0, may reflect minor matrix effects leading to significant overestimation of the analyte residues by ELISA, especially at low concentrations, as recently pointed out by Krotzky and Zeeh (1995).

On the other hand, on the basis of all determinations performed from spiked wine samples by both methods (11 results between 1 and 100 ppb for ELISA and 16 results between 0.5 and 500 ppb for SPME) averaged recoveries were found at the level of 109 ± 15% for ELISA and 103 ± 13% for SPME.

Conclusion. This comparative study indicates a good agreement between SPME/GC/MS and ELISA techniques for procymidone residue analysis in wines. Estimated concentrations by both methods were found very close to each other for spiked samples of two particular wines of the Bergerac area (France). This result can be reasonably extended to most wine types since the ELISA method has been largely validated for more than 50 different wines coming from all over the world (Lawruck et al., 1994) and the SPME method relative to other pesticide molecules for more than 20 other wines (Boyd-Boland and Pawliszyn, 1995), showing that corresponding efficiencies are not wine dependent as soon as, in the case of SPME, their alcoholic concentrations are of the same order (Urruty and Montury, 1996).

The advantage of immunoassays for pesticide residue analysis in food has recently been discussed by Bushway and Fan (1995), Hock et al. (1995), and Lucas et al. (1995), and procymidone monitoring is included: tests are rapid and allow economic savings, especially if large numbers of determinations must be done at the same time. On the other hand, SPME appears as a very promising tool for the same type of analysis: it has the same rapidity if only a few samples have to be treated, and the technique affords a slightly better precision but necessitates more expensive investments even if extraction and desorption steps can be automated (Arthur at al., 1992c; Barnabas et al., 1995). Moreover, as largely described in many other publications, the SPME is well-known to be a multiresidue method. However, the most important comparison point is surely that both methods allow high-performance analysis without using any organic solvents, which are becoming the main factors in price and pollution increases.

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