

Determination of Ethanol in Complex Liquid Media for Continuous Processing Control

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An at-line method for measurement of volatile ethanol concentrations ranging from 0.01 to 10% (v/v) in complex liquid products is described. A fraction of ethanol present in the liquid is transferred to a gas phase where it is analyzed. Two methods are compared: gas chromatography, which permits in 10-20 min identification of the constituents and measurement of their concentration; quantitative and continuous gas-phase composition analysis by means of a semiconductor sensor. The latter method is rapid and nonexpensive but unable to identify the constituents. Gas chromatography is useable over the whole working range, whereas the semiconductor sensor saturates for concentrations above 2% (v/v). Both methods require periodic monitoring. The developed method is easily automated for process control.

The control of processes requires implementation of selective sensors in order to perform real-time, reproducible, and automatic on-line measurements of the few parameters that are strictly necessary to survey the process in progress. In addition, measurements should be quick, realized in a duration remaining short compared to the processing time, and should not lead to significant losses of product.

The selection of parameters and measurement methods depends on the nature and the evolution of the process itself and to some degree even on the control equipment. Methods may be direct, like temperature or pressure determination, or indirect, with results in close relation with the evolution of a parameter representative of the process. Such a method is illustrated by the specific mass measurement in fermentation processes leading to the composition of the medium but without any indication about the nature or distribution of its constituents.

In another way, measurement can be made continuous or discrete but in sequences in close relation and adapted to the processing time. The rapid knowledge of a sensible parameter allows one to react quickly to the processing conditions, to correct them if necessary, and consequently to maintain the process under control. Often on-line measurements are not feasible, for example in the case where the analytical method requires separation of phases or constituents or needs to operate under conditions far from those existing in the medium to be analyzed. In that case an at-line measurement on a derivation proves to be a tractable solution.

This paper deals with an indirect method, an at-line automatic determination of ethanol concentration in liquids containing solid particles of various size in suspension. Results obtained from a detailed study of experimental aspects of ethanol concentration measurements in the range 0.01-10% (v/v) with a precision of 0.005-0.1% are reported. These ethanol concentrations are encountered in fermentation worts and distillation residues. It is also shown that a low-cost analysis combining a short response time with a small volume is feasible.

PRINCIPLE OF THE METHOD

Basically a gas bubbles through the alcoholic liquid phase and strips a small quantity of ethanol (Dumoulin

et al., 1986). The vapor phase is subsequently analyzed by chromatography and by an ethanol semiconductor sensor. The quantity of stripped ethanol, even if small, is representative of the ethanol concentration in the liquid phase. The transfer from liquid to gas phase depends on carrier gas contact surface and flow rate and on temperature. The determination of ethanol in the complex liquid medium becomes a determination of ethanol in a gas saturated with water at the exchange temperature.

Ethanol in the gas phase is analyzed by gas chromatography with automatic gas sampling and by a semiconductor sensor immersed in the continuous gas flow.

For the experimental trials, the ethanol-charged gas flows successively over the semiconductor and then into the sampling valve for chromatography.

The detection with n-type semiconductor SnO_2 relies on the exponential decrease of the conductivity of the oxide with increasing organic constituent concentration in the atmosphere in contact. This effect is related to the electron concentration in the conduction band of the semiconductor. Reaction mechanisms are not totally known in detail, but the presence of oxygen and water in the gas is important to consider (Morisson, 1982; Tischer and Pink, 1980; Boyle and Jones, 1977). The temperature of the semiconductor affects the response time and sensitivity (Lalauze and Pijolat, 1984; Pijolat, 1986).

Semiconducting SnO_2 is often used as a detector in fermentation processes. Ethanol can be detected in the gas at the exit of a fermentor, giving the concentrations in the wort from 0 to 0.3% (v/v) (Bach et al., 1978). Beyond this concentration, the semiconductor is saturated.

Ethanol may also be transferred from the fermentation medium to a carrier gas through a gas-permeable membrane (tubing method) (Mandenius et al., 1984; Kempe and Schallenberger, 1983; Lee et al., 1981; Yamane et al., 1981; Puhar et al., 1980). For determination of higher concentrations, the carrier gas is first diluted (Mandenius et al., 1983). Semiconductor or gas chromatography (Pons et al., 1985) analysis is used for final detection. The semiconductor protected with a hydrophobic membrane may also be dipped in the liquid (Picque et al., 1988).

All these works concern fermentations: sugar juices, glucose, molasses, grapes, beer, vinegar. The semiconductors are mainly made of SnO_2 (Tagushi Gas Sensor (TGS), from Figaro Engineering).

In this work a gas flows at constant rate through a known volume of liquid at constant temperature; no membrane is used. Working at-line gives the opportunity to perform the analysis under precisely fixed conditions. These conditions are usually variable and are hard to stabilize inside the fermentation reactors.

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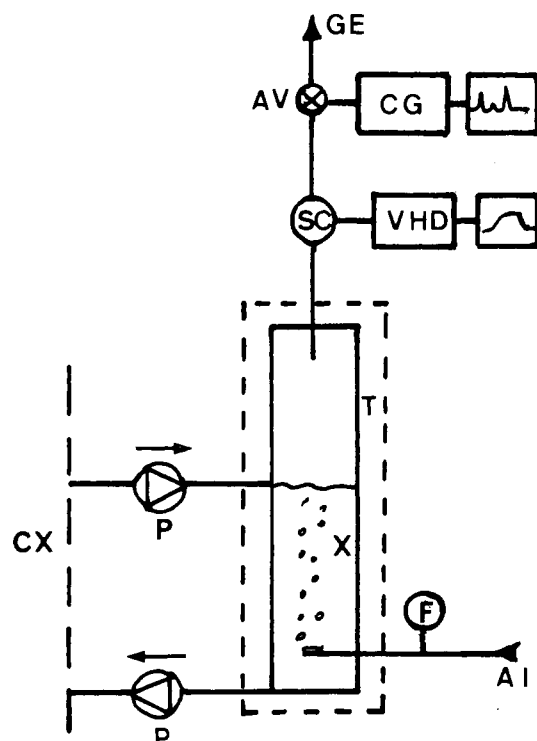


Figure 1. Stripping apparatus: X, medium for analysis; CX, open circuit or closed loop (pipe or reactor); AI, air inlet; GE, gas exit; AV, automatic sampling gas valve; T, constant temperature; F, gas flow meter; CG, chromatograph; SC, semiconductor sensor (VHD = voltage, heating, detection); P, peristaltic pump.

MATERIALS AND METHODS

Stripping Apparatus. The experimental setup is shown in Figure 1. A continuous flow of sample (dynamic processing), is obtained with use of a peristaltic pump (12 L/h) and a counterflow between liquid and gas. Circulation occurs in a closed loop or in an open circuit when cleaning is necessary. These operations can be automatically controlled. The sampling volume is 250 cm³. The stripping gas consists of air with a flow rate of 600 cm³/min.

Dynamic or static processing (liquid sample immobilized in the volume V) gives similar results. The effect of varying the carrier air flow rate and the exchange temperature is studied on trials using chromatographic detection.

Gas Chromatography Instruments. An Intersmat IGC 121 DFL chromatograph equipped with a flame ionization detector is fitted to a stainless steel column packed with Porapak Q (2 m × 0.32 cm; 80/100 mesh). The oven operates at 170 °C; the injector and the detector operate at 200 °C. The N₂, H₂, and air flow rates were 25, 20, and 300 cm³/min, respectively. A pneumatic valve and a loop of 300 μL are used for gas sampling.

The peak area is determined with Delsi ENICA 21 integrator. For each test, three gas samples are analyzed at different times, giving three different peaks: the first one (S₁) deals with the end of rinsing with the analyzed solution. The peak area after 260 s, the time corresponding to the area determination, is S₂. The third peak area (S₃) (13 min) is that after 17 min 15 s.

Semiconductor Detector. Sintered SnO₂ (n-doped) coming from two firms is used: Figaro, TGS 817 (1 and 2); Coreci.

Four electric contacts are used for heating and for measurement. In the Coreci set a temperature probe is incorporated.

The heating voltage V_c (dc voltage) is 5 V for the Figaro semiconductor and 6–8 V for Coreci, corresponding to 350 ± 2 °C for ethanol detection. The measurement voltage V_{el} is 5 V for Figaro and 2.5 V for Coreci.

Reagents. Other Analytical Methods. For calibration, we used standard solutions prepared with absolute ethanol and distilled water.

A sugar beet wort has been tested. Calibration is performed with ethanol added to the same sample matrix, obtained as a residue of the distillation of a part of the wort. Thiomersal is used as a stabilizer (100 mg/L).

Table I. Detection by Gas Chromatography: Relationship between Ethanol Concentrations in the Solution and Chromatographic Peak Areas Obtained from Stripping Gas^a

ethanol concn range, % (v/v)	experimental relationship					
	ethanol concn = a + bS ₁			ethanol concn = c + dS ₃		
	a	b × 10 ⁻⁵	r	c	d × 10 ⁻⁵	r
0.01–10	-0.015	+0.782	0.998	-0.004	+0.780	1
0.01–0.1	+0.001	+0.871	0.982		+0.911	0.994
0.1–1				+0.011	+0.783	
1–10	-0.112	+0.793	0.994	-0.049	+0.785	0.999

^a Key: S₁, S₃, peaks areas after 9, 17 min; r, correlation coefficient.

Table II. Detection by Gas Chromatography: Repeatability

ethanol concn range, % (v/v)	analyzed sol, % (v/v)	ethanol concn	
		calcd from peak areas	
		S ₁	S ₃
0.01–0.05	0.01	0.010 ± 0.002	0.010 ± 0.001
0.01–10	3.6	3.61 ± 0.06	3.60 ± 0.06
0.01–10	8.2	8.14 ± 0.30	8.20 ± 0.10

Table III. Detection by Gas Chromatography: Precision and Response Time for Different Ethanol Concentration Ranges

ethanol concn range, % (v/v)	precision, % (v/v)	response time, min
0.01–0.05	±0.005	9
2.5–5	±0.05	15
5–10	±0.1	20

Fermentation of molasses (50% (w/w)) with baker's yeast has been followed with stripping apparatus. The medium contained 1 g/L ammonium phosphate, 133 g/L sugar, and 20 g/L yeast; pH was settled to 5 with sulfuric acid.

Standard analytic methods were used: pycnometry and areometry for pure water/ethanol solutions and distillates obtained from complex products; chemical oxidation of ethanol for low contents (<2%).

RESULTS AND DISCUSSION

Correlations have been established between the ethanol concentration in the solution and the detector information, peak area or voltage.

The limits and response times for both gas chromatography and semiconductor detections are given. Their application to continuous process control are described.

Gas Chromatography Detection. Correlations. The reported results are obtained with dynamic processing. The peak area varies linearly with the ethanol concentration, over the full range 0.01–10% (v/v) (see Figure 2). Basically, the calibration is established in the same range as the samples to be analyzed. Table I gives the established correlations between ethanol concentrations in the solution and the peak areas S₁ and S₃, with three different ethanol concentration ranges, 0.01–0.1, 0.1–1, and 1–10% (v/v). For lower concentrations, results are preciser.

Reproducibility. In the ethanol range 0.01–0.5% (v/v), calibrations with five concentrations lead to reasonable error inferior to 0.005% (v/v). The results are identical for S₁ and S₃.

A run of 10 measures performed in time span of 15 days on a calibration solution containing 1% (v/v) ethanol gives a concentration of 1.03 ± 0.05% (v/v) by using the correlation established for the full range from 0.01 to 10% (v/v).

Repeatability. The same solution is analyzed five times, including between the determinations other solutions in

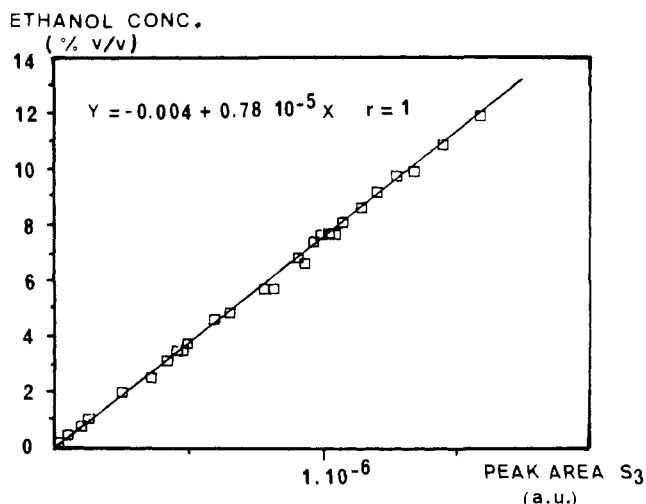


Figure 2. Detection with gas chromatography. Calibration with water/ethanol solutions.

Table IV. Detection with Semiconductor: Reproducibility

	ethanol concn range, % (v/v)	dev on whole range
Figaro	0.01-4	≤1.3% for ethanol concn inferior to 0.2% (v/v) ($V_{rl} = 2.25$ V)
Coreci	0.01-8	≤1% for ethanol concn inferior to 1.8% (v/v) ($V_{rl} = 450$ mV)

the same range (see Table II). The value calculated with the first peak area S_1 is in agreement with the true value. For the higher concentration (5-10% (v/v)) it may be better to wait for peak S_3 (Tables II and III).

Effect of Carrier Gas Flow Rate. A variation of the carrier gas flow rate, from 250 to 750 cm^3/min , does not give a significantly different signal, with water/ethanol solutions containing less than 1% (v/v) ethanol. For higher concentrations (1-10% (v/v) ethanol), the same variation of the gas flow rate gives a small variation of the signal, which can be interpreted as a difference of 0.06% in the ethanol concentration in the solution.

For signal stabilization in a few minutes, we chose an air flow rate of 600 cm^3/min .

Effect of Temperature. From 16 to 20 $^\circ\text{C}$, a 0.1 $^\circ\text{C}$ increase for stripping involves a variation in the response that can be interpreted as a 0.05% (v/v) increase of the ethanol concentration in the solution. It is necessary to regulate the temperature at ± 0.1 $^\circ\text{C}$.

Semiconductor Detection. We obtain correlations between ethanol concentration in the liquid sample and voltage in the range 0.01-10% (v/v) (see Figures 3 and 4). Figaro: $\log(\text{ethanol concentration}) = f(V_{rl})$, linear and similar for TGS 1 and 2. Coreci: $\log(\text{ethanol concentration}) = f(\log V_{rl})$ or $\log(\text{ethanol concentration}) = f(V_{rl})$ for the smaller range 0.01-4% (v/v).

With the previously described conditions and a liquid temperature of 17.3 $^\circ\text{C}$, the ranges for V_{rl} are as follows: TGS sensor, $0.5 < V_{rl} < 4.5$ V for $0.01 < \text{ethanol concn-}$

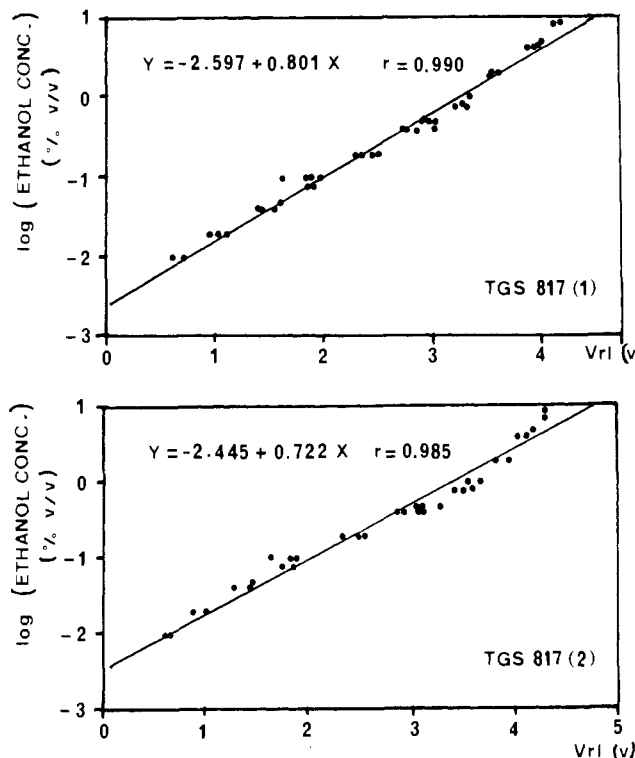


Figure 3. Detection with semiconductor sensor Figaro TGS 817 (1 and 2). Calibration with water/ethanol solutions.

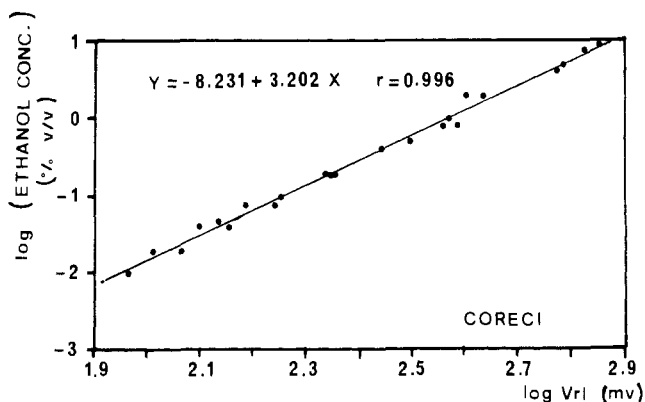


Figure 4. Detection with semiconductor sensor Coreci. Calibration with water/ethanol solutions.

tration in liquid $< 3.1\%$ (v/v); Coreci sensor, $100 < V_{rl} < 700$ mV for $0.013 < \text{ethanol concentration in liquid} < 8.2\%$ (v/v).

Beyond 4% (v/v) ethanol in liquid, with a resulting concentration of ethanol in gas of 3400 ppm, the Figaro response is unusable because of the semiconductor saturation.

Reproducibility. The deviation for measurements made with two calibration sets on two different days becomes more important for the higher concentrations. For usual practice, we recommend detection of only ethanol con-

Table V. Detection with Semiconductor: Repeatability

ethanol concn in medium, % (v/v) (no. ^a)	V_{rl} (min value - max value)			calcd ethanol concn, % (v/v)		
	TGS 1, V	TGS 2, V	Coreci, mV	TGS 1	TGS 2	Coreci
0.05 (4)	1.55-1.64	1.44-1.48	134.4-137.9	0.05 ± 0.01	0.040 ± 0.002	0.040 ± 0.004
0.483 (4) ^b	3.03-3.155	3.25-3.30	302.1-329.4	0.73 ± 0.16	0.60 ± 0.04	0.58 ± 0.15
0.485 (5) ^b	2.91-3.01	3.06-3.105		0.59 ± 0.08	0.84 ± 0.06	
4.83 (4)	3.97-4.045	4.18-4.21	568.4-643.5	4.15 ± 0.60	3.80 ± 0.17	4.86 ± 1.70

^a Number of experiences with the same solution. ^b Ten days between the two experiments.

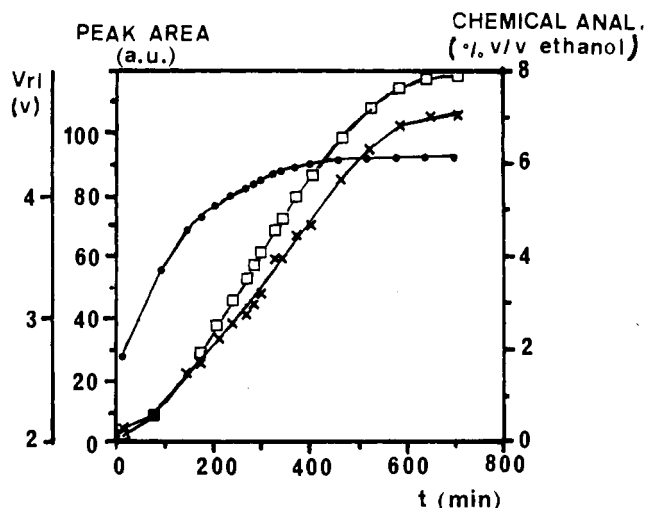


Figure 5. Batch fermentation of molasses with baker's yeast: (□) gas chromatography; (●) semiconductor Figaro TGS 817; (×) chemical oxidation.

concentrations inferior to 0.2% (v/v) with Figaro semiconductor (TGS 817) and inferior to 2% (v/v) with the Coreci semiconductor (Table IV).

Repeatability. Four determinations are performed on each sample in runs comprised of other samples with different ethanol concentrations. Ethanol concentrations are calculated from the correlations established for the whole range 0.01–10% (v/v). Results are reported in Table V.

Response Time. For stripping and detection, we have a mean response time (60% of the signal) in 1 min. When different concentrations are successively determined, response times depend on the previous analyzed solution. The response time is shorter with an increasing concentration (30–60 s) than with a decreasing one (1.5–2 min). The signal decreases more slowly than it increases.

Return to zero, base line, is obtained by rinsing with water during several minutes or by increasing the semiconductor heating voltage, but thermal stress may degrade the semiconductor characteristics.

Tests with Complex Products. Molasses Wort. The three semiconductors Figaro TGS 1 and 2 and Coreci were used for ethanol determination in a stabilized molasses wort containing 5.85% (v/v) ethanol. Calibration was made with samples containing the same matrix and known added ethanol quantities, in the same range. The Figaro 1 and 2 indicate 5 and 5.8% (v/v); the Coreci indicates 5.1% (v/v). It was an interesting result considering the bad range for semiconductors.

Fermentation of Molasses with Baker's Yeast. We have tested the experimental setup by means of samples intended to survey ethanol production. A part of the medium flows continuously through the experiment cell, maintained at constant temperature.

Three determinations are made: chemical oxidation on samples; chromatography and TGS semiconductor detections on air circulating through the liquid in the experiment cell. Results are shown in Figure 5. We note the limit response of the semiconductor beyond 4% (v/v).

CONCLUSION

The proposed apparatus permits ethanol stripping from a complex liquid phase, associated with gas chromatography or semiconductor detection. In the analysis cell separated from the fermentation reactor, the operating conditions are well fixed. Especially, the temperature is

not affected by the fermentation temperature fluctuations in the reactor.

Gas chromatography detection is well adapted to determine ethanol concentrations in the liquids in the range 0.01–10% (v/v). The described experimental conditions lead to a response time depending on concentration level of 10–20 min. The measurements are specific, are discontinuous, and give absolute values.

The semiconductor detection leads to a short response time of 2 min. The signal is continuous or discrete and therefore may even account for unusual fluctuations of ethanol concentrations into the mean fermentation reactor. The semiconductor sensor is well adapted for the low concentration level, below 2% (v/v). For higher concentrations, it is envisaged to decrease the exchange temperature or to dilute either the liquid medium before analysis or the gas containing ethanol. For every process, we will have to identify other volatile constituents, which could lead to a response with the semiconductor.

Considering the stripping of volatile ethanol from a medium containing salts, it is necessary to conduct the calibration of the method with products containing the same matrix as the medium to be analyzed, under well-known temperature and experimental conditions.

The semiconductor detection brings continuous information in a nonexpensive way, but chromatography is necessary to identify the different constituents.

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HPLC Method for Simultaneous Determination of Fungicides: Carbendazim, Metalaxyl, Folpet, and Propiconazole in Must and Wine

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A simple high-performance liquid chromatography method for simultaneous determination of fungicides, carbendazim, metalaxyl, folpet, and propiconazole, in must and wine has been developed. Analyses are carried out on a reversed-phase column (Spherisorb ODS-1 C₁₈) with an acetonitrile-water gradient as mobile phase. Absorbance at 220 nm is recorded as compounds are separated. The method provides minimum detectable levels, between 4 and 8 µg/L, for the four above-mentioned fungicides.

Botrytis cinerea, *Uncinula necator*, and *Plasmopara viticola* are the most common fungi encountered in viticultural pests. Several different fungicides are widely used to control them including carbendazim (methyl 1*H*-benzimidazol-2-ylcarbamate), metalaxyl (methyl *N*-(2-methoxyacetyl)-*N*-2,6-xylyl-DL-alaninate), folpet (2-[(trichloromethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione), and propiconazole ((±)-1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole).

Studies on individual determinations of some pesticides in grape must and wine at residue level have been published (Austin et al., 1976; Cabras et al., 1982; Chiba and Sing, 1986). Other studies on the effect of folpet and metalaxyl fungicides on the yeast flora during must fermentation and on yeast metabolism have been published in recent years (Benda, 1978; Minarik and Ragala, 1979; Guerzoni et al., 1982; Gnaegy et al., 1983; Conner, 1983).

Our method will permit the simultaneous determination of the four fungicides in must and wine and, thus, we will carry out studies on the influence of vinification technology on pesticide degradation.

EXPERIMENTAL SECTION

Chemicals. Water was distilled twice and filtered through a Millipore apparatus before use. Solvents (benzene, acetonitrile) were high-performance liquid chromatography grade (Chrom. HPLC; Mallinckrodt Inc.). Analytical samples of propiconazole (≥99.0%) and metalaxyl (≥99.6%) were donated by Ciba Geigy S. A. (Barcelona, Spain); and carbendazim (≥97.5%) and folpet (≥99.5%), by Basf Española S. A. (Barcelona, Spain) and Ind. Quim. Valles S. A. (Barcelona, Spain), respectively.

Preparation of Standard Solutions. Standard solutions of carbendazim, metalaxyl, folpet, and propiconazole were prepared at concentrations between 0.2 and 4 mg/L in methanol. These solutions can be stored at low temperature (-20 °C) for 1 week without appreciable degradation.

HPLC Apparatus. A Waters Associates model equipped with

Table I. Chromatographic Characteristics of Carbendazim, Metalaxyl, Folpet, and Propiconazole under Experimental Conditions

fungicide	retention time, min	correln ^a	MDL, ^b mg/L
carbendazim	7.50 ± 0.50	0.9912	0.2
metalaxyl	13.25 ± 0.05	0.9996	0.2
folpet	17.45 ± 0.55	0.9998	0.05
propiconazole	19.45 ± 0.10	0.9996	0.2

^a Concentrations of corresponding fungicides were prepared in methanol at 4, 2, 1, 0.4, and 0.2 µg/mL levels. Results are the mean of three injections. ^b MDL were those concentrations giving a signal 2 times higher than the block signal at the studied pesticides retention time when 80 µL of sample was injected.

Table II. Recoveries of Fungicides in Water and HAS

fungicide	fortificn level, µg/mL	% recovery ± RSD ^a	
		water	HAS
carbendazim	1.0	63.1 ± 7.1	54.9 ± 4.3
metalaxyl	1.0	85.1 ± 9.7	80.0 ± 9.6
folpet	1.0	94.9 ± 5.1	93.6 ± 8.4
propiconazole	1.0	99.0 ± 6.0	96.0 ± 2.2

^a Results are the mean of four extractions.

a TCM-002011 temperature control system, a Waters U6K injector (loop 200 µL), a Waters 490 variable-wavelength UV/vis detector, and a Data Module 730 reporting integrator was used.

HPLC Chromatography. The column employed was Spherisorb ODS-1 (250 × 4 mm (i.d.), 10 µm; Teknokroma SCL, Barcelona, Spain). A linear gradient from 20 to 80% acetonitrile in water in 20 min was applied at a flow rate of 2 mL/min. The best wavelength for the simultaneous determination of the studied fungicides was 220 nm according to their UV spectrum. The analyses were carried out at 25 °C.

A good linear response in the range 0-4 mg/L was achieved for all four fungicides. The minimum detectable levels for each were between 0.2 and 0.05 mg/L according to the results shown in Table I.

Preparation of Samples. Water and hydroalcoholic solution (10:100, v/v) were fortified with about 1 mg/mL of the four fungicides.

Must and white wine samples were obtained from Chardonnay vineyards and red wine samples from Cabernet Sauvignon.

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