Journal of Chromatography, 445 (1988) 264–267
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 502

Note

Determination of 5-nitrofurylacrylic acid in wines by high-performance liquid chromatography

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(Received March 17th, 1988)

Since 1965, 5-nitrofurylacrylic acid (5-NFA) has been used as a preservative in wines[1]. Use of this additive is permitted in some East European countries for red and white wines containing sugar, but it has been banned in EEC countries because of claims that it may have an harmful effect on human health[2,3], therefore a reliable, rapid analytical method is needed for the determination of 5-NFA in wine.

Several methods have been proposed for determination of 5-NFA in wines, most involving previous extraction of the preservative. Thus Lafon-Lafourcade[4] proposed a demixing process with ammonium sulphate at 20°C, whereas with the Junge method[5] 5-NFA was extracted with *n*-butanol and the extract was shaken with Polyclar AT (PVPP) in order to make it colourless.

Pavlenco *et al.*[6] described a direct determination of 5-NFA in white wines but a sample of the same wine in the additive-free state is essential. Recently Kaniansky *et al.*[7] used photometric detection to find traces of 5-NFA by capillary isotachophoresis.

Only two studies deal with the determination of 5-NFA by high-performance liquid chromatography (HPLC) Debowski *et al.*[8] isolated and estimated several nitrofuran derivatives among other compounds using methanol–phosphate buffer as a mobile phase and a polarographic detector. Jeuring and Brands[9] determined 5-NFA in wines with methanol–acetate buffer as a mobile phase and spectrophotometric detection, but the retention time was long, approximately 15 min.

The aim of this work is to study several mobile phases in order to optimize a quick routine determination of 5-NFA in wines.

EXPERIMENTAL

Apparatus

A Shimadzu Model LC-6A liquid chromatograph equipped with a variable-wavelength UV detector (Shimadzu Model SPD-6A) and a computing integrator (Shimadzu Model C-R6A) was used. A glass column (150 mm × 3.2 mm I.D.) packed with LiChrosorb RP-18, particle size 5 μm, eluted with acetonitrile–water–glacial acetic acid (25:75:1.5) at the rate of 0.6 ml min^{-1} was used. The experiment was carried out at room temperature, and the detection wavelength was 360 nm.

0021-9673/88/$03.50

Millipore XX047 filtration equipment with a Millipore HVLP 04700 filter was used.

Reagents

Acetonitrile and glacial acetic acid. A stock solution of sodium 5-nitrofuryl-acrylate, 1000 mg l^{-1} in water, was prepared in our laboratory. This solution is stable for at least 3 months when stored at 4°C and protected from light. Working standard solutions of 5–50 mg l^{-1} were freshly prepared by appropriate dilution of the stock solution.

5-NFA-free wine was prepared by the Department of Agricultural Industries of the Polytechnic University of Valencia.

The reagents used as eluents in liquid chromatography were filtered through a Millipore HVLP 04700 filter immediately before use.

Procedure

A 20-μl volume of the filtered wine was injected in the chromatograph.

Standard solutions containing 5–50 mg l^{-1} of sodium 5-NFA salt were also injected for calibration.

RESULTS AND DISCUSSION

Determination of 5-NFA was achieved by optimization of the mobile phase pH by reversed phase HPLC. The mobile phases studied were 25% methanol or 25% acetonitrile, deionized water, alone or together with acetic acid–acetate buffer (pH 4.4) or glacial acetic acid. Table I shows the results obtained with the different mobile phases assayed for absolute and relative retention times, and the column efficiency at different pH values.

The retention time was higher at low pH values than at higher ones. The buffer had a significant effect on the column efficiency when the mobile phase was methanol, whereas the effect was negligible with acetonitrile.

Working conditions were established using the mixture of acetonitrile, deionized water and glacial acetic acid. Under the conditions established, the retention time at a flow-rate of 0.6 ml min^{-1} was 3.2 min. Figs. 1 and 2 shows the chromatograms of a red 5-NFA-free wine and in wine containing 20 ppm, respectively.

TABLE I

APPARENT pH, RETENTION TIMES (mm), RELATIVE RETENTION TIMES, k', AND EFFICIENCY, N, IN THE HPLC DETERMINATION OF 5-NFA IN WINE

	Apparent pH	t_R *(mm)*	k'	N
Methanol–water (25:75)	4.9	14	1.2	252
Methanol–water–buffer	4.4	28	2.9	4193
Methanol–water–acetic acid	3	69	4.5	2933
Acetonitrile–water (25:75)	5	9	0.7	100
Acetonitrile–water–buffer	4.4	22	2.4	5728
Acetonitrile–water–acetic acid	3	23	2.5	5987

Fig. 1. Chromatogram of a red wine free of 5-NFA.

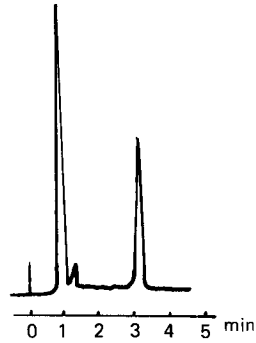

Fig. 2. Chromatogram of a red wine containing 20 mg l^{-1} of 5-NFA.

TABLE II

RECOVERIES OF ADDED 5-NFA

5-NFA added (*mg* l^{-1})	*5-NFA Found*	
	(*mg* l^{-1})	*%*
20	20.65	103.25
30	31.44	104.81
40	41.97	104.94

A linear response was obtained with a correlation coefficient of 0.9996 for 5-NFA concentrations of up to 50 mg l^{-1} in wines. The quantification was done by comparing peak areas with those of corresponding peaks of the standards. The detection limit determined as three times the coefficient of variation (C.V.) of the noise distribution was 0.1 mg l^{-1}.

The precision of the method was calculated from the peak heights of five samples containing 20 mg l^{-1} of 5-NFA (C.V. = 1.24) and by measuring a sample equivalent to five times the instrumental precision (C.V. = 1.04). The accuracy of the method was estimated by recovery assays in red wine. The results obtained are summarized in Table II.

It was shown that sorbic acid and sulphur dioxide, the only wine preservatives that are legal in Spain, do not interfere in the determination.

Wine samples from different Spanish areas were obtained from local supermarkets and analyzed by the proposed HPLC method. 5-NFA was not detected in any of these samples.

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