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Note

High-performance liquid chromatographic determination of acetaldehyde in wine as its lutidine derivative

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Suzuki and Maruyama[1] reported on the high-performance liquid chromatography (HPLC) of acetaldehyde (Ac) in wine as its 2,4-dinitrophenylhydrazone derivative. This method was not successful in the determination Ac, because the high temperatures and high concentrations of acid required to transform Ac into hydrazone with the reaction system equipped with the specific apparatus, also caused corrosion of the stainless-steel columns in the HPLC system. In a previous paper[2], we considered the HPLC retention behaviour of low molecular weight aldehydes as their lutidine derivatives on 3-aminopropyltriethoxysilane (3APTS) treated silica gel. Therefore, we studied the determination by HPLC of Ac in wine as its lutidine derivative on the 3APTS-treated silica gel or the commercial NH_2-chemically bonded stationary phase.

EXPERIMENTAL

Reagents

Ac, propionaldehyde (PA) and acetylacetone (AA) were obtained from Wako (Osaka, Japan). 3APTS was purchased from Aldrich (Milwaukee, WI, U.S.A.). Nucleosil 5 NH_2 was purchased from Macherey, Nagel & Co. (Düren, G.F.R.). A highly microporous spherical silica gel (mean pore diameter 95 Å, surface area (BET) 380 m^2/g, particle size distribution 5.5 μm) was obtained from Fuji-Davison (Nagoya, Aichi, Japan). Hexane and ethanol were used after distillation. The other reagents and organic solvents were reagent grade.

Apparatus

Three liquid chromatographs were employed: a Hitachi 635 T equipped with a visible spectrophotometer and Hitachi 204 S fluorescence spectrophotometer; a Jasco Twincle equipped with a Jasco Uvidec 100-III visible spectrophotometer and a Jasco FP 110 fluorescence spectrophotometer; and a Type KHU 16 of Kyowa Seimitsu Mini Micro Pump equipped with a Type KLC 200 of Kyowa Seimitsu variable-wavelength detector.

Determination of the number of accessible NH_2 surface groups per gram (or 100 $Å^2$) of homemade gel

Dried silica gel (5 g) was added to 50 ml of a 2.5% benzene solution of 3APTS.

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After stirring for 24 h at room temperature, the silica gel was filtered with a glass filter (1 μm), washed several times with benzene and methanol, and then dried *in vacuo* at 70°C for 2 days. According to the assumption made in a previous paper[2,3], the number of accessible NH_2 surface groups per gram of silica gel was calculated to be $0.45 \cdot 10^{21}$, and the number of accessible NH_2 surface groups per 100 Å^2 of silica gel was calculated to be 1.76.

Preparation of Ac-AA and internal standard

According to a previous paper[2], the lutidine derivatives of Ac and PA (internal standard) used in this report were Ac-AA (m.p. 157–159°C; analysis for $C_{12}H_{17}NO_2$ (calculated): C, 69.53; H, 8.27; N, 6.76; (found): C, 69.47; H, 8.51; N, 6.73) and PA-AA (m.p. 162–164°C; analysis for $C_{13}H_{19}NO_2$ (calculated): C, 70.54; H, 8.66; N, 6.33; (found): C, 70.42; H, 8.82; N, 6.37).

Column preparation

Silica gel treated with 3APTS or Nucleosil $5NH_2$ was packed into stainless-steel columns (250 mm × 4 mm I.D.) using a balance density method and a 10-ml stainless-steel packer at a rate of 500 kg/cm^2 (Kyowa Seimitsu Type KHW-20 ultra-high-pressure pump).

Procedure

To a 15-ml aliquot of wine, 5 ml of a mixture[6] of 2 *M* ammonium acetate, 0.05 *M* acetic acid and 0.02 *M* AA was added. The reaction mixture was warmed in a water-bath at 60°C for 30 min as described by Suzuki and Tani[4]. After cooling, 5 ml of internal standard solution (15 μg of the lutidine derivative of PA in 1 ml of chloroform) were added. The mixture was shaken well and allowed to stand for some minutes. The aqueous phase was then discarded, and the organic phase dried over anhydrous sodium sulphate. A 50-μl volume of the resulting solution was subjected to HPLC. The operating conditions are given in the legend to Fig. 2.

When PA was omitted from the mixture, it was not detected as present in the wine. Therefore PA was selected as the internal standard.

RESULTS AND DISCUSSION

To determine the best extracting solvent for Ac-AA in wine, the variation of the peak shape to peak area ratio with Ac-AA/PA-AA was measured on the 3APTS-treated silica gel column and on the Nucleosil $5NH_2$ column with methylene chloride, ethyl acetate or chloroform. Of these, chloroform was chosen as the most suitable for extraction, as it clearly separated from the aqueous phase as a lower layer, which was convenient for the separation procedure.

Hexane was used as a component of the mobile phase, and the effect of an alcohol on the retention behaviour of Ac-AA was studied. According to the polarities of alcohols[5] methanol is the most suitable, but it has a very low solubility in hexane. Therefore, ethanol seems to be best from the viewpoint of the separation. The dependence of capacity factor on the alcohol concentration was studied with ethanol. The optimal separation for Ac and internal standard was attained for a hexane:ethanol ratio of 25:1 (Fig. 1). Elution with this mobile phase on the 3APTS-treated silica gel

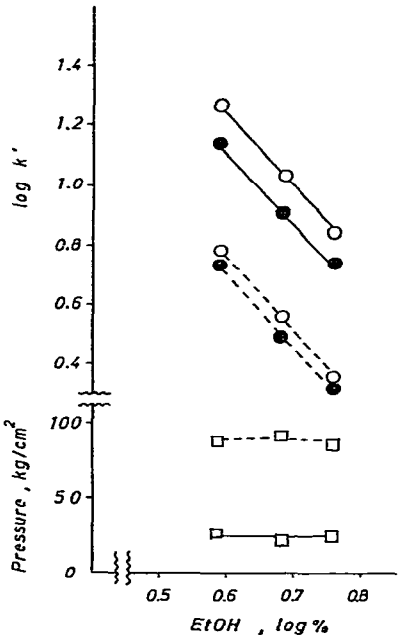

Fig. 1. Relationship of capacity factor, k', and column pressure to the concentration of ethanol (EtOH) in the mobile phase. ———, 25% 3APTS-treated silica gel column; – – – –, Nucleosil 5NH_2 column; ○, acetaldehyde, ●, propionaldehyde (i.s.); flow-rate, 1.2 ml/min.

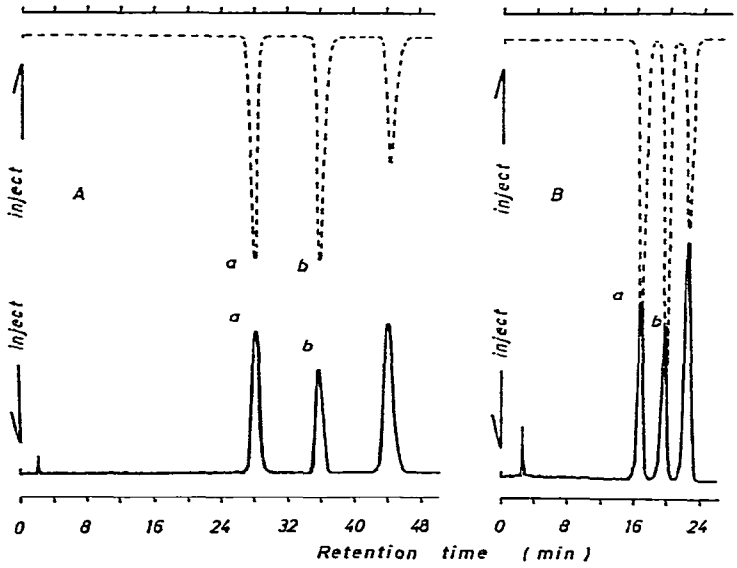

Fig. 2. Comparative liquid chromatograms of acetaldehyde in wine and propionaldehyde (internal standard). Stationary phase: A, 25% 3APTS-treated silica gel column; B, Nucleosil 5NH_2 column. Mobile phase, hexane–ethanol (25:1); flow-rate, 1.2 ml/min. Detection: ———, 375 nm; – – – –, 395 nm (Ex) 460 nm (Em). Peaks: a = propionaldehyde; b = acetaldehyde.

or the Nucleosil $5NH_2$, resulted in a resolution between the Ac and PA peaks of 4.00 or 1.88, respectively.

Fig. 2 shows the comparative chromatograms obtained from Ac in wine and PA as internal standard on the 3APTS-treated silica gel column or the Nucleosil $5NH_2$ column. No interfering peaks were observed at the retention times of the compounds of interest.

A calibration graph constructed by plotting the ratio of the peak area of Ac to that of the internal standard was linear and passed though the origin, in the ranges 0.01–0.5 and 0.1–6 μg using fluorescence and visible spectrophotometers, respectively. Nine replicate determinations on a test solution containing Ac (0.2 μg) gave a standard deviation of 3.38% and 1.98%, using fluorescence and visible spectrophotometers, respectively; the limit of detection was 0.01 μg.

Table I shows the acetaldehyde concentration in the samples commercial wines tested.

TABLE I

ACETALDEHYDE CONCENTRATION IN WINE

Wine sample	*Acetaldehyde (μg/ml)*
A	1.2
B	0.7
C	0.4
D	1.0
E	0.2

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