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Note

Determination of diethylene glycol in wine by high-performance liquid chromatography using anthracene-9-carbonyl chloride as a derivatising reagent

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Diethylene glycol (DEG) became well known as an illegal additive to white wine after its detection in 1985 in a number of Austrian wines. DEG has also been found in foods wrapped in regenerated cellulose which had been softened with the glycol[1]. Existing methods for DEG in wine have detection limits typically of 1–10 mg/l and are mainly based on gas chromatography, using either capillary[2–5] or packed columns[6–8]. Wines may be analysed without cleanup, either directly[2,5] or after reaction of a 10-μl aliquot with 100 μl bis(trimethylsilyl)-trifluoroacetamide[9]. However, selectivity is poor and confirmation by mass spectroscopy (MS) is required. High-performance liquid chromatographic (HPLC) methods have been developed[10] but these rely on refractive index detection, which is non-selective and insensitive. Thin-layer chromatographic[11] and ^{13}C NMR[12] methods are also available.

The development of anthracene-9-carbonyl chloride (ACC) as a derivatising reagent permitting fluorescence and UV detection of hydroxy compounds has been described elsewhere[13]. It was noted that the ratio of fluorescence to UV absorption was reduced for diol diesters and that this ratio was particularly low for DEG diester. Although this phenomenon indicated that absolute sensitivity would be considerably reduced compared to that generally expected with fluorescence derivatisation, it seemed to offer a useful confirmation of the identity of DEG, possibly avoiding the need for MS. Thus a method using ACC for derivatisation was devised for the determination of DEG in white wines, both because of the wider need for sensitive methods for glycols and also to demonstrate practically the utility of the ACC reagent.

EXPERIMENTAL

Materials and apparatus

Extrelut cartridges (Merck 11737 and 11738) were purchased from BDH (Poole, U.K.) and C_{18} Sep-Paks from Waters (Harrow, U.K.). Vials and PTFE-faced

septa were from Chromacol (London, U.K.). Water was purified using a Millipore Milli-Q system. All other solvents were glass distilled or HPLC grade from Rathburn (Walkerburn, U.K.). ACC reagent was prepared as before[13].

The HPLC gradient apparatus consisted of two 6000A pumps and a 660 controller (Waters), a Rheodyne injector with a 20-μl loop, a Pye UV detector and a Perkin-Elmer Model 3000 fluorimeter. A Kratos Model 773 UV detector and Perkin-Elmer Model LS-4 fluorimeter were used for one chromatogram (Fig. 1). A Spherisorb 5-μm silica column (250 × 4.9 mm I.D.) were supplied by Hichrom (Reading, U.K.). An Upchurch (Oak Harbor, WA, U.S.A.) precolumn filter was used, fitted with a 0.2-μm frit. Samples were filtered through Acro LC13 0.2-μm membranes (Gelman, Northampton, U.K.) before injection. The mobile phase was chloroform–hexane (20:80) at 1.0 ml/min, thermostatted at 35°C. UV detection was at 252 nm and fluorescence conditions were excitation at 360 nm and emission at 460 nm, both with 10 nm slit widths.

Methods

Wine (typically 1 ml; a maximum of 10 ml) was diluted with 12.5% aqueous ethanol to a total volume of 15 ml and the mixture poured into an Extrelut column[11]. The flask was rinsed with a further 5 ml aqueous ethanol and the washings added to the column, which was left for 15 min for the sample to equilibrate with the packing. The DEG was eluted with 120 ml dichloromethane–propan-2-ol (85:15) and the eluate evaporated to low volume in a rotary evaporator at 40°C. The residual solvent (*ca.* 5 ml) and washings were transferred to a 10-ml volumetric flask and made up to volume with acetonitrile. An aliquot (1 ml) of this solution in a 2-ml sample vial was evaporated just to dryness with gentle warming under a stream of nitrogen. The vial was then capped with a PTFE-lined silicone rubber septum and ACC (100 μl of a saturated solution in dry acetonitrile, *ca.* 0.25 *M*) added by syringe to the sample residue. The vial was shaken vigorously and left for 20 min at room temperature. The bulk of the solvent was then evaporated under dry nitrogen and 100 μl methanol added to react with excess reagent. After 5 min the remainder of the solvent was removed, the residue dissolved in 1.00 ml mobile phase which was filtered through a 0.2-μm membrane and analysed by HPLC.

Where necessary the following additional cleanup stage was incorporated. Solvent was removed completely from the derivatisation reaction mixture and the residue dissolved in acetonitrile–water (50:50, 5 ml) and passed through a 0.2-μm filter (to remove precipitated carboxylic acid and anhydride) into a pre-wetted C_{18} Sep-Pak. A further 5 ml of sample rinsings were loaded onto the cartridge, which was then washed twice with 5 ml with acetonitrile–water (60:40). The DEG diester was eluted with acetonitrile (5 ml), the solvent evaporated (taking care to remove all traces of water) and the residue redissolved in 1.00 ml mobile phase for HPLC analysis on the Spherisorb silica column.

RESULTS AND DISCUSSION

As reported elsewhere[13], base catalysis of the esterification reaction between alcohols and ACC could not be employed because of the stability of the intermediate. However, under the conditions used for derivatisation of wine residues the reaction

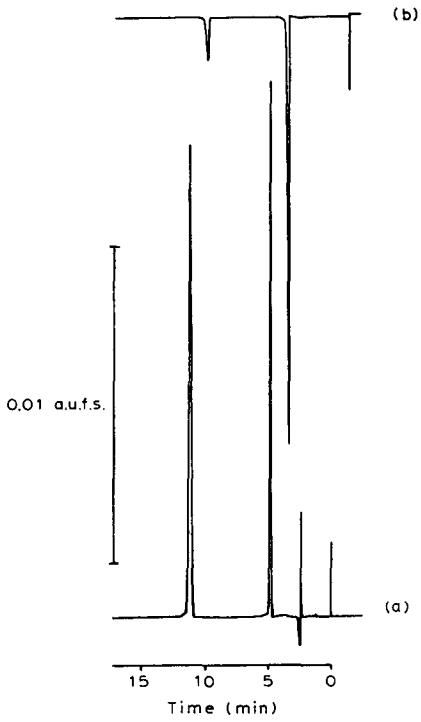

Fig. 1. Chromatogram showing UV and fluorescence detector responses to DEG anthracene-9-carboxylic acid monoester and diester. (a) UV detection (Kratos 773, 360 nm, 0.030 a.u.f.s.). (b) Fluorescence detection (Perkin-Elmer LS-4, excitation at 360 nm, emission at 460 nm; sensitivity, fix scale 0.500, 5-fold greater than minimal). Other conditions: Spherisorb 5 ODS-1 column (250 × 4.9 mm I.D.) eluted with acetonitrile–water (80:20) at 1.0 ml/min; retention times, monoester 4.8 min, diester 11.4 min; sample contained diester (262 ng, 0.51 nmol) and monoester (169 ng, 0.55 nmol); detectors were in-line, UV first, followed by the fluorimeter.

between ACC and DEG was essentially complete within 20 min at room temperature. The fluorescence to UV absorption ratio of DEG diester in a range of both normal and reversed-phase eluents is only 12% of that of monoderivatives due to interaction between the two anthracyl groups[13] (Fig. 1). Because of this, the sensitivities to the diester of the two detectors were very similar but in routine use fluorescence detection was used only for confirmation because the time constant characteristics (a minimum of 2 s) of the Model 3000 fluorimeter employed gave rise to poor peak profiles. Thus UV chromatograms are reproduced here for all wine samples. Apart from peak shape, fluorescence and UV chromatograms were very similar.

Initially an attempt was made to analyse wines directly, without any cleanup, by taking a 10-μl sample, evaporating off the water and ethanol and derivatising the residue. This approach was acceptable for samples containing more than about 500 mg/l DEG (Fig. 2) but at lower levels a large interference precluded quantitation. Thus cleanup was required. Classical liquid–liquid partition of DEG from aqueous solutions into non-polar organic solvents is inefficient but the Extralut column partition method of Lehmann and Ganz[11] was found to be convenient, rapid and effective at concentrations of DEG down to 1 mg/l.

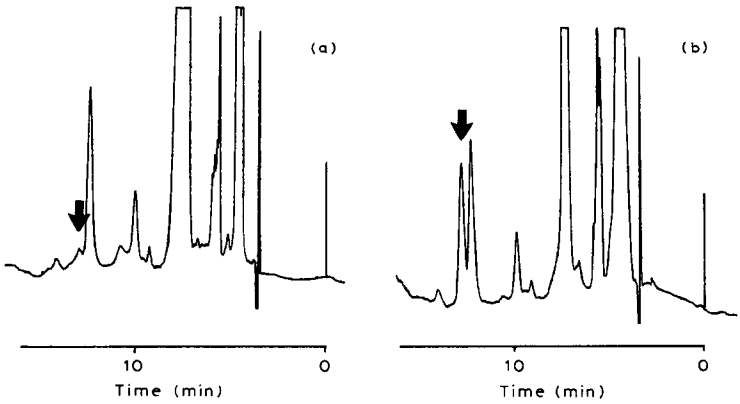

Fig. 2. Analysis of blank and spiked white wine without cleanup. (a) Blank wine, 50 μl equivalent injected. (b) Wine spiked with 93 mg/l DEG. Conditions: UV detection at 360 nm, 0.16 a.u.f.s.; 50 μl equivalent wine injected.

Reversed-phase HPLC was not satisfactory for this analysis because of interferences encountered in all samples tested. Normal phase chromatography on silica gave good resolution of DEG diester, and at the DEG diester retention time reagent blanks showed no observable peaks, with a maximum signal equivalent to less than 1 μg/l. Recoveries of DEG added to aqueous ethanol and wines are given in Table I. Nine white wines were analysed by this method without evidence of interference; red wine was not studied. Fig. 3 shows the chromatograms obtained from a blank hock and from the same wine spiked with DEG at 93 mg/l. An additional solid phase extraction cleanup was required for lower levels of DEG, when a detection limit of

TABLE I

RECOVERY OF DEG ADDED TO WINES OR ETHANOL IN WATER (1:8)

Sample	*DEG added (mg/l)*	*Cleanup*	*Recovery (%)*
Aq. ethanol	22	None	95, 97
	19.6	None	96, 98, 95, 104
	19.6	Extrelut	92
	0.78	None	91, 90
Wine 1	1035	None	94, 94
	1035	Extrelut	94, 94
	93.9	None	100, 103
	93.9	Extrelut	73, 83
	19.3	Extrelut	71, 72
	1.75	Extrelut	72
	1.75	Extrelut + Sep-Pak	41
	0.37	Extrelut	78. 78
	0.44	Extrelut + Sep-Pak	44
Wine 2	93.9	Extrelut	77
Wine 3	84.5	Extrelut	71, 77
Wine 4	2.2	Extrelut	74

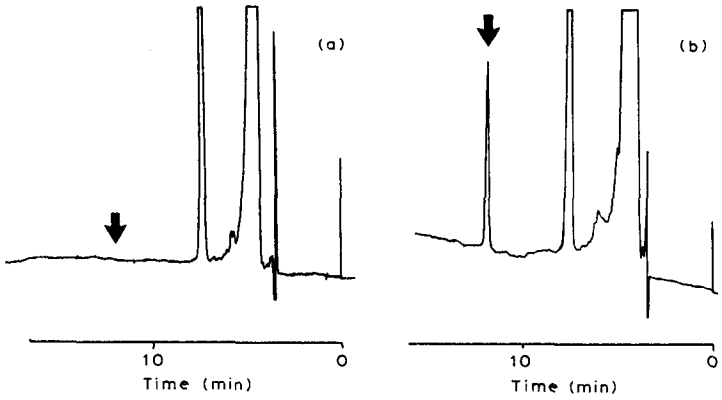

Fig. 3. Analysis of blank and spiked white wine with Extrelut cleanup. (a) Blank wine. (b) Wine spiked with 98 mg/l DEG. Conditions as for Fig. 1.

<0.1 mg/l could be attained, with however a low overall recovery of 40%. The detection limit was defined as that concentration of DEG giving a peak height five times the maximum observed for the unspiked wine in a retention window 2-min wide, centred about the DEG diester retention time. Fig. 4 shows UV chromatograms from a wine spiked at 1.75 mg/l and cleaned up by Extralut alone or in combination with the ODS cartridge. Recoveries from the ODS cartridge were not good, reflecting poor discrimination between the DEG diester and the interference. Alternative bonded phases were not explored.

The ratio of UV to fluorescence detector outputs for a range of ACC mono- and diesters was determined. This data is to be reported in detail elsewhere[14], but all monoderivatives tested had ratios similar to that observed for DEG monoester while diester ratios were typically in the range 0.2–0.4. Out of a total of 32 compounds

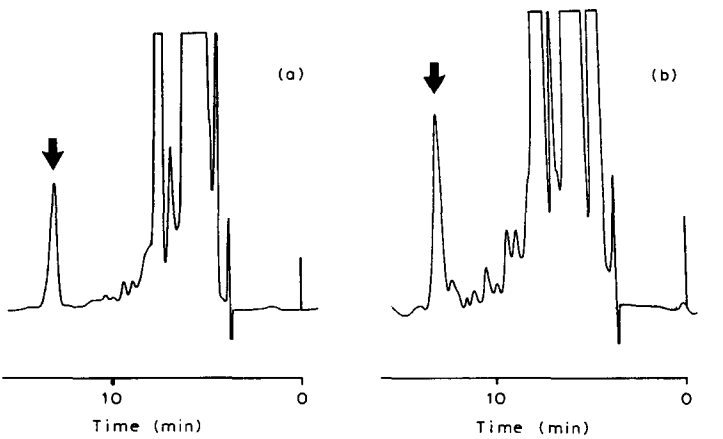

Fig. 4. Analysis of white wine spiked with 1.75 mg/l DEG. (a) Extrelut and Sep-Pak cleanup. (b) Extrelut cleanup only. Conditions: UV detection at 252 nm, 0.64 a.u.f.s.; 1.0 ml equivalent wine injected.

examined, only the diderivative of 1,4-dihydroxy-*cis*-but-2-ene exhibited a ratio as low as that for DEG diester (0.11). Thus measurement of this ratio affords a useful confirmation of the identity of DEG.

CONCLUSIONS

It has been shown that derivatisation with ACC provides a sensitive assay for DEG in white wine. The method permits rapid analysis (a batch of six samples in *ca.* 4 h) of the glycol at concentrations down to 1 mg/l and the detection limit may be further reduced if required. Determination of the fluorescence–UV adsorption ratio provides a measure of confirmation of identity of DEG. It may be possible to employ a similar approach for the determination of DEG in chocolate and other commodities wrapped in regenerated cellulose film.

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