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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION AND DIODE-ARRAY SPECTROSCOPIC IDENTIFICATION OF DINITROPHENYLHYDRAZONE DERIVATIVES OF CARBONYL COMPOUNDS FROM WHISKIES

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SUMMARY

A liquid chromatographic method was developed to separate fourteen aldehydes and two ketones as their 2,4-dinitrophenylhydrazone (DNP-hydrazone) derivatives. Special attention was paid to the effects of the column materials and operating temperature. The identification of compounds in real samples was based on their UV spectra recorded with a diode-array detector. Although the shape of the UV spectra of the DNP-hydrazone compounds is similar, there are significant differences in the wavelengths of the absorption maxima. Several carbonyl compounds in whisky distillates were identified and the method was used to monitor the concentration changes of these compounds during whisky maturation.

INTRODUCTION

Carbonyl compounds are extremely important contributors to the aroma of alcoholic beverages. Their presence in beverages has generally been determined by thin-layer and gas chromatography[1]. In recent years, high-performance liquid chromatography (HPLC) has also been used for this purpose[2–5]. An appropriate chromophore is, however, needed to detect the compounds by spectrophotometry, and for this reason derivative formation is usually necessary. Some carbonyl compounds like acrolein and crotonaldehyde can be analyzed with good sensitivity without derivatization[2].

The most widely used derivative for carbonyl compounds is the corresponding 2,4-dinitrophenyl (DNP) hydrazone[6–13]. These compounds are formed by the addition of 2,4-dinitrophenylhydrazine in acidic solution; the resulting precipitate is filtered off, washed, then dissolved in a suitable solvent prior to liquid chromatography.

The identification of DNP-hydrazones is usually based upon their retention times, which may not be very reliable. In the present study a photodiode-array detector is employed to identify the compounds by recording the UV spectra during the analysis. The separation of DNP-hydrazones was optimized by use of RP-18 column materials from different manufacturers and by changing the column temperature.

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EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 1090 high-performance liquid chromatograph equipped with an HP 1040A photodiode-array detector, HP 85B personal computer, HP 3392A integrator and HP 9121 disc-drive unit for data storage were used.

Reagents and chemicals

2,4-Dinitrophenylhydrazine was from Fluka (Buchs, Switzerland). Hydrochloric acid, tetrahydrofuran and HPLC-grade acetonitrile were from Merck (Darmstadt, F.R.G.). Water was distilled and deionized. Aldehydes and ketones were purum or puriss grade.

Preparation of 2,4-dinitrophenylhydrazine (DNPH) reagent

A 5-g amount of DNPH was dissolved in 320 ml of 32% (w/v) hydrochloric acid and stirred overnight. Then 1680 ml water were added and the mixture was filtered.

Preparation of derivatives

A 100-ml volume of whisky and 150 ml DNPH solution were mixed and left overnight. The precipitate was filtered through a G4 glass filter and washed twice with 5 ml of 2.0 *M* hydrochloric acid and finally with 50 ml of water. The yellowish precipitate was dissolved in acetonitrile before injection.

Column

Five different ODS column materials (particle size 5 μm) were tested for the separation of DNP-hydrazone compounds: Nucleosil $5C_{18}$ (Macherey-Nagel), Spherisorb S5 ODS-2 (Phase Sep), LiChrosorb RP-18 (Merck), RP-8 (Merck) and Vydac RP-18 (Macherey-Nagel). The columns (200 × 4.6 mm) were packed in the laboratory by a slurry technique using acetone as the suspending medium and a 50-ml slurry reservoir. The packing pressure was 450 bar.

Chromatographic procedures

The eluent was pumped at a flow-rate of 1 ml/min and the UV detector was operated at 375 nm. The injection volume was 10 μl. During the first 7 min after injection the eluent was pumped isocratically at 45% acetonitrile in water, then gradient elution was used from 45% (v/v) acetonitrile in water to 80% acetonitrile over a period of 18 min and maintained at this concentration for a further 10 min. The analysis was completed in 35 min. Column temperatures of 40, 60 and 80°C were tested for the separation of DNP-hydrazone compounds and 60°C used for the quantitative analyses.

RESULTS AND DISCUSSION

The best separation of all the aldehydes and two diketones studied was obtained on Nucleosil $5C_{18}$ (Fig. 1) and Spherisorb S5 ODS-2 columns. Acrolein (5), furfural (6) and propionaldehyde (7) were difficult to separate at room temperature; it also was difficult to resolve isovaleraldehyde (11) and 2-methylbutyraldehyde (12).

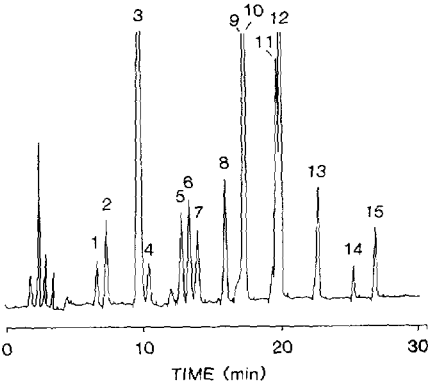

Fig. 1. Chromatogram of the standard DNP mixture on Nucleosil $5C_{18}$ at 60°C. Flow-rate, 1 ml/min. During the first 7 min after injection the eluent was pumped isocratically at 45% acetonitrile in water, then gradient elution was used from 45% acetonitrile in water to 80% acetonitrile in 18 min. Peaks: 1 = 5-hydroxymethylfurfural; 2 = formaldehyde; 3 = acetaldehyde; 4 = diacetyl; 5 = acrolein; 6 = furfural; 7 = propionaldehyde; 8 = crotonaldehyde; 9 = isobutyraldehyde; 10 = *n*-butyraldehyde; 11 = isovaleraldehyde; 12 = 2-methylbutyraldehyde; 13 = caproaldehyde; 14 = 2,4-pentanedione; 15 = caprylaldehyde.

Acetonitrile–water gradient elution generally gave better separations than the equivalent methanol–water system. No significant improvements in separation resulted from the addition of tetrahydrofuran to the eluents.

Elevated column temperatures improved the separation and reduced the analysis time considerably. Acrolein (5), furfural (6) and propionaldehyde (7) are best separated from each other at 60°C (Fig. 1) and isovaleraldehyde (11) and 2-methylbutyraldehyde (12) at 80°C. *Iso-* (9) and *n*-butyraldehyde (10) could not be separated at any conditions. Changes in column temperature did not affect the separation of the other aldehyde derivatives.

Identification of carbonyl compounds

With a diode-array detector the UV spectrum of a zone can be recorded during elution, thus facilitating the identification of compounds. The wavelength of the absorption maxima of DNP-hydrazone compounds is dependent on the compound type (Table I). The wavelengths vary from 352 nm (formaldehyde) to 436 nm (glyoxal). A mean value of 375 nm was selected for quantitative analysis.

The UV spectrum recorded for a peak in the whisky sample was compared to that recorded for a DNP-hydrazone derivative having the same retention time. Fig. 2 shows a chromatogram from a whisky sample and some UV spectra obtained for peaks eluted with the same retention times as the DNP-hydrazone derivatives in a standard mixture. The UV spectra of the pure DNP-hydrazone derivatives are represented as a solid line and those from the whisky sample as a dotted line. The UV spectra of peaks 1 and 3 coincide very well with those of the pure compounds (5-hydroxymethylfurfural and acetaldehyde). A good match is also found for peaks 5 and 16 (acrolein and glyoxal, respectively). The small differences in the spectra of the latter peaks relative to the pure compounds may be due to the background reference selected or to a small impurity. The UV spectrum of peak 8 is somewhat different

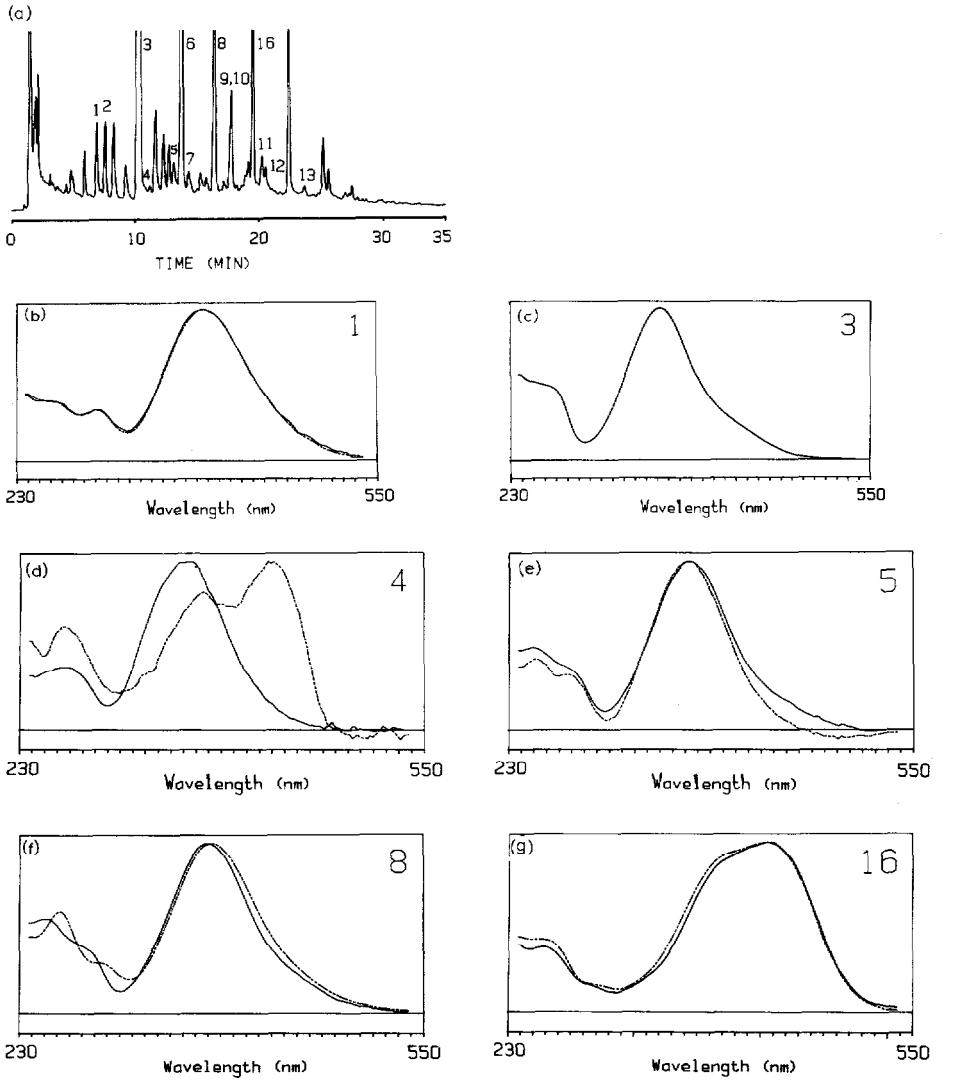

Fig. 2. Chromatogram of a Scotch whisky and some UV spectra obtained with the diode-array detector. The UV spectra of the pure DNP-hydrazones of carbonyl compounds are represented for comparison as solid lines. Chromatographic conditions as in Fig. 1. Peaks as in Fig. 1, except 16 = glyoxal.

from that of the pure compound (crotonaldehyde), so this peak is probably only partly pure. The UV spectrum of peak 4 is totally different from that recorded from the pure compound, diacetyl, which had the same retention time, thus demonstrating the unreliability of using only retention times for identifying compounds. The peak purity was confirmed in cases when quantitative values are given (Table I).

Proper selection of the reference spectrum (background correction) is essential for obtaining reliable results, as shown in Fig. 3 where the reference has been taken

TABLE I

WAVELENGTHS OF ABSORPTION MAXIMA AND CONCENTRATIONS OF DNP-HYDRAZONES OF CARBONYL COMPOUNDS BEFORE AND AFTER 12 MONTHS ACCELERATED MATURATION OF WHISKY

−, Identification could not be confirmed; tr = trace; i, could not be separated from background; +, qualitative identification.

no.	*Compound*	$\lambda_{max.}$ *(nm)*	*Concentration (mg/l)*	
			0	*12 months*
1	5-Hydroxymethylfurfural	396	−	−
2	Formaldehyde	352	2.4	2.8
3	Acetaldehyde	362	78.5	123
4	Diacetyl	360	tr	20.1
5	Acrolein	374	9.1	8.8
6	Furfural	390	116	105
7	Propionaldehyde	368	1.9	3.9
8	Crotonaldehyde	378	i	i
9	Isobutyraldehyde *n*-Butyraldehyde	364	14.3	20.9
11	Isovaleraldehyde	364	4.8	7.7
12	2-Methylbutyraldehyde	364	4.1	8.5
13	Caproaldehyde	362	4.9	8.2
14	2,4-Pentanedione	400	3.8	5.3
15	Caprylaldehyde	362	−	−
16	Glyoxal	436	+	+

from different stages of the analysis. The reference should be taken from the baseline where no absorption occurs.

Changes in carbonyl compound content during maturation of whisky

The method was used to follow the concentration of fourteen aldehydes and two ketones during accelerated maturation of whisky. The external standard method was used in quantitations. The concentrations of all the compounds studied, except acrolein and furfural, increased during maturation (Table I).

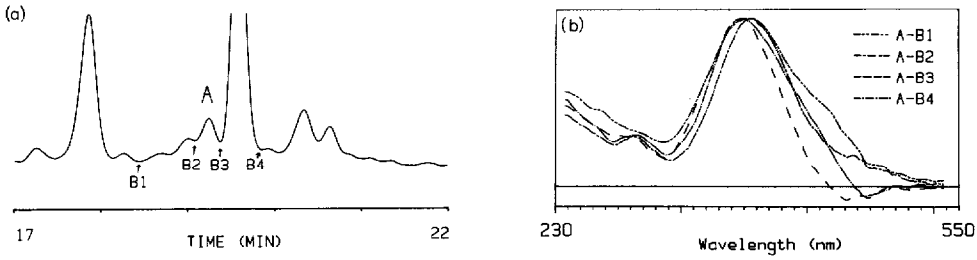

Fig. 3. Part of the chromatogram represented in Fig. 2 and the UV spectra obtained by taking references from points B1, B2, B3 and B4.

CONCLUSION

Retention times are not sufficient for identification of carbonyl compounds from whisky samples, as shown with a few examples in Fig. 2. However, the new method can be used successfully to determine the carbonyl compounds, and the identification can be confirmed by recording UV spectra of peaks with a diode-array detector.

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