Journal of Chromatography, 350 (1985) 381–387
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 155

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF CARBOHYDRATES, ALCOHOLS AND DIETHYLENE GLYCOL ON ION-EXCHANGE RESINS

G. BONN
Institute of Radiochemistry, University of Innsbruck, A-6020 Innsbruck (Austria)
(Received September 4th, 1985)

SUMMARY

The separation of carbohydrates, alcohols and diethylene glycol (DEG) using individual and coupled columns of ion-exchange resins with different counter ions and water as the mobile phase was examined. The detection limit for DEG was found to be 20 ppm. The simultaneous detection of saccharose, glucose, fructose, the fermentation alcohols such as ethanol, methanol and glycerol as well as DEG in wine samples is examined for routine analysis.

INTRODUCTION

For the determination of food additives and for quality control, analyses of carbohydrates and alcohols are of importance. In particular, carbohydrates such as saccharose, glucose or fructose, aldehydes, *e.g.*, acetaldehyde, and alcohols (ethanol, methanol, glycerol) must be analyzed routinely in countries where the quality of wine and non-alcoholic beverages produced from grape juice is subject to strict supervision prescribed by the law. These compounds have been determined mainly by means of thin-layer chromatography (TLC)[1,2], gas chromatography (GC)[3,4] and high-performance liquid chromatography (HPLC) using octadecyl- and amino-bonded materials[5–11]. In addition, variously loaded ion-exchange resins have been employed as HPLC stationary phases for the separation of monosaccharides, carboxylic acids, alcohols, aldehydes and ketones[12–24]. Furthermore, the electrophoretic methods have received increasing attention[25,26].

The basis of wine production is anaerobic glycolysis within yeast cells, Embden–Meyerhof–Parnas (EMP) pathway, which leads to ethanol and can be accompanied to different extents by side reactions yielding glycerol[27]. The possible adulteration of wine with artificial additives in order to change its flavour poses a challenge to the analyst: the need to separate the natural substances as well as the adulterants. Following the discovery of diethylene glycol (DEG) in Austrian wine and grape-juice products, a fast and simple method for both qualitative and quantitative determination is desirable.

For the structural analysis of such products, gas chromatography–mass spec-

0021-9673/85/$03.30

trometry (GC–MS) appears suitable as a routine system, since it is only after identifying the nature of the additives that an analysis by HPLC or GC becomes useful[28]. In earlier studies[29], the connection of ion-exchange columns in series yielded good results in the separation of oligo- and monosaccharides. The concept has therefore been applied in the present work to include DEG and alcohols by coupling lead- and calcium-loaded columns.

EXPERIMENTAL

Apparatus

A high-performance liquid chromatograph (Model SP 8000 B; Spectra Physics, Santa Clara, CA, U.S.A.), equipped with an integrated data system and column-oven compartment, column-switching valve (Whitey, OH, U.S.A. and Scientific Systems, PA, U.S.A.), was employed with a differential refractive index detector (Altex, Berkeley, CA, U.S.A.). The samples were injected by a valve fitted with a 20-μl loop.

The following columns were employed: 300 × 7.8 mm I.D., HPX-87 H ion-exchange resin, hydrogen form (Bio-Rad Labs., Richmond, CA, U.S.A.); A, 100 × 7.8 mm I.D., cation-exchange resin, calcium form (Bio-Rad Labs.); B, 300 × 7.8 mm I.D., HPX-87P cation-exchange resin, lead form (Bio-Rad Labs.); C, 300 × 7.8 mm I.D., calcium form μSpherogel Carbohydrate N; sulphonated polystyrene–divinylbenzene resin (Beckman, Berkeley, CA, U.S.A.). For the pre-column, ion-exclusion Micro-Guard cartridges (Bio-Rad Labs.) were used.

Samples

All standard reference solutions were prepared from analytical reagent grade chemicals (Merck, Darmstadt, F.R.G.; Fluka, Buchs, Switzerland; Sigma, St. Louis, MO, U.S.A.). The investigated compounds are listed in Table I.

Samples of Austrian red and white wines were injected directly.

RESULTS AND DISCUSSION

Depending on the different functional groups present in the compounds under investigation, the separation of reference substances was studied on ion-exchange resins, hydrogen or lead forms, either individually or coupled in series.

TABLE I

PEAK IDENTIFICATION

Peak No.	*Compound*
1	D-Glucose
2	D-Fructose
3	Glycerol
4	Bis(2-hydroxyethyl) ether, "diethylene glycol" (DEG)
5	Acetaldehyde
6	Methanol
7	Ethanol
8	Saccharose

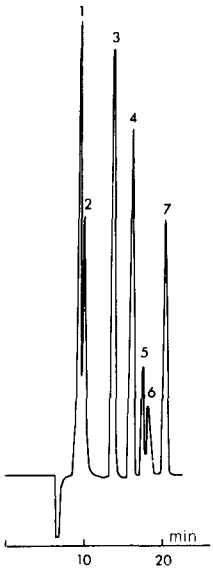

Fig. 1. Separation of a reference mixture. Chromatographic conditions: stationary phase, ion-exchange resin (hydrogen form); column temperature, 70°C; mobile phase, water; flow-rate, 0.7 ml/min; detection, refractive index. For peak identification see Table I.

Separation on ion-exchange resin in the hydrogen form

Since the retention times of the compounds studied were strongly dependent on the column temperature, the latter parameter was optimized, and found to be 70°C.

Experiments at different flow-rates and corresponding plots of H/u *vs.* linear velocity, u, showed the minimum height equivalent to a theoretical plate, H, to be achieved at 0.7 ml/min when this resin was used with water as the mobile phase. The chromatogram given in Fig. 1 was obtained under these optimized conditions.

The use of 0.01 N sulphuric acid as the mobile phase did not result in any significant advantage for the separation of the examined compounds.

Separation on ion-exchange resins (Pb^{2+} or Ca^{2+}) singly and coupled

Because of their high selectivity towards carbohydrates and alcohols, ion-exchange resins in the lead and calcium forms were investigated. The reference mixtures were applied to the individual columns as well as to columns coupled in series as shown in Fig. 2. In order to maintain the counter-ion concentration as constant as possible, a pre-column was employed. This also serves to keep back interfering ions possibly present in the samples.

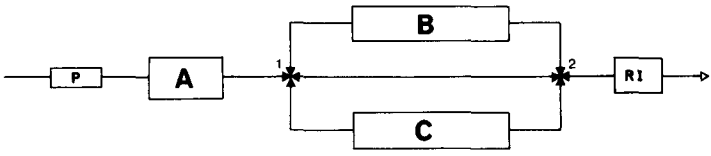

Fig. 2. Coupled column switching system with a pre-column (P), and the columns A–C, valves (1, 2) and, refractive index detection system (RI).

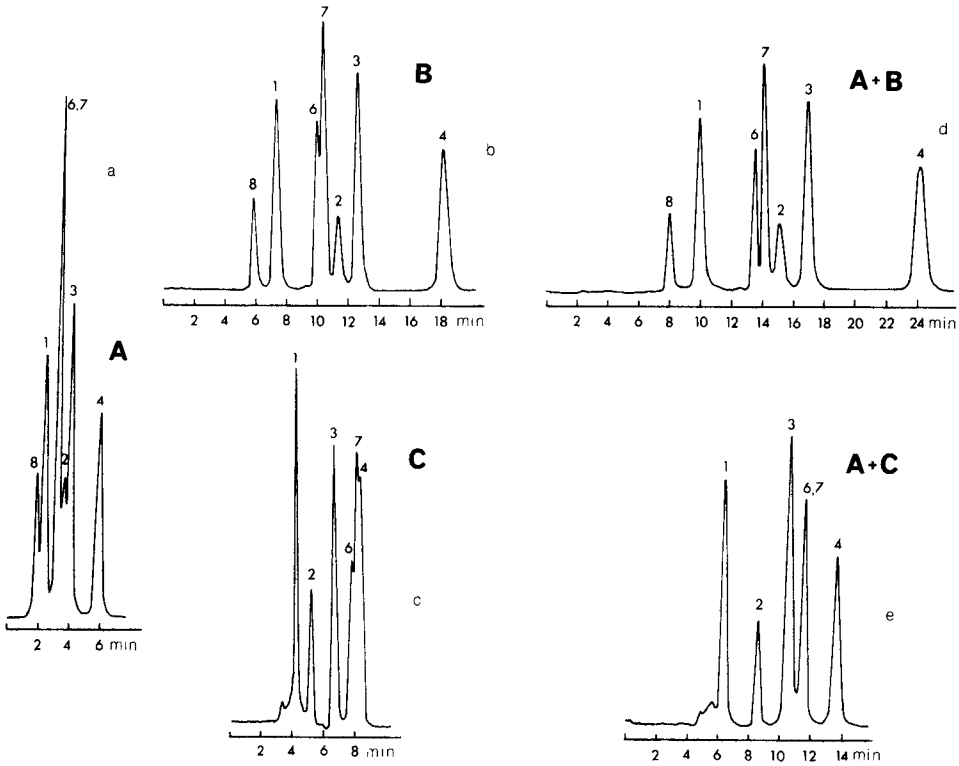

Fig. 3. Separation of a reference mixture on single and coupled columns. Stationary phases: a, A; b, B; c, C; d, coupled A and B; e, coupled A and C. Column temperature: 60°C. Mobile phase: water; flow-rate 0.8 ml/min. Detection: refractive index. For peak identification see Table I.

Fig. 3a shows a separation of reference compounds on a cation-exchange resin (lead form) with approximately 750 theoretical plates (based on glucose), and Fig. 3b that on a similar resin (B) but with approximately 7000 plates. At a column temperature of 60°C and a flow-rate of 0.8 ml/min, glycerol can be determined together with the carbohydrates saccharose, glucose and fructose; poorly resolved are methanol and ethanol in addition to DEG. An increase in column temperature to 80°C impairs resolution of the methanol/ethanol peaks and also changes the retention order with respect to fructose and ethanol/methanol. The use of an ion-exchanger resin (calcium form) (C) did not yield satisfactory resolution of methanol, ethanol and DEG (see Fig. 3c).

In order to separate ethanol and methanol on the resins in the lead form on the one hand, and, on the other, to separate DEG from methanol and ethanol on the resin in the calcium form, coupled systems were investigated. In Fig. 3d two ion-exchange columns (lead forms) (A and B) have been coupled in series, whereas in Fig. 3e column A is coupled with a column C (calcium form).

As has already been demonstrated[29], the non-additivity relationship calculated by Snyder and co-workers[30] also holds true in the present case for the coupled columns in the lead and calcium forms. Fig. 4 shows the dependence of the retention

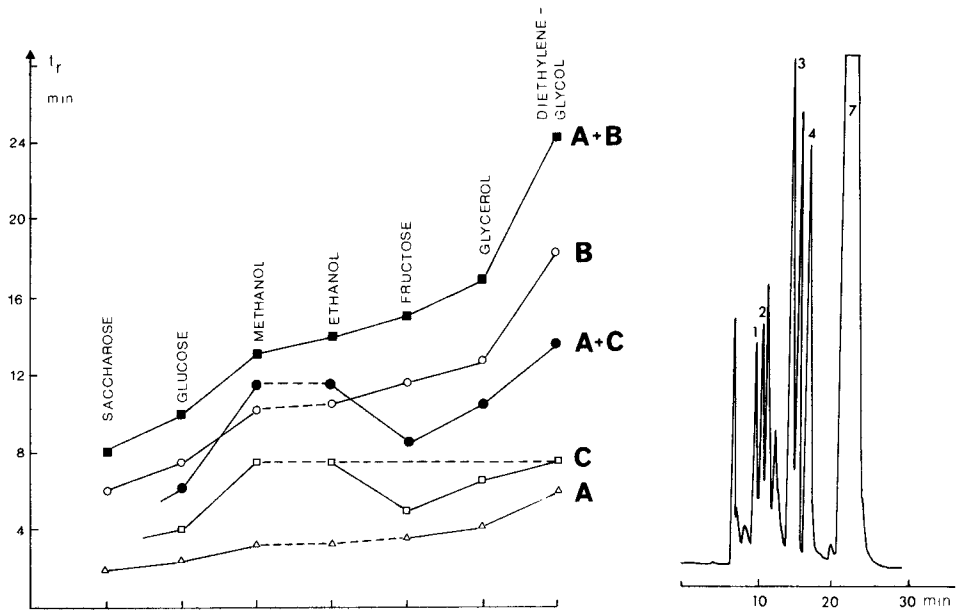

Fig. 4. Dependence of the retention time, t_R (min), on the use of single columns A, B, C and coupled columns A and B, A and C for the investigated substances. ——, Resolution, R_s, >1; – – – –, R_s <1. Chromatographic conditions as in Fig. 3.

Fig. 5. Separation of an Austrian red wine sample. Conditions as in Fig. 1. For peak identification see Table I.

time, t_R, of the substances studied on the use of individual ion-exchange resins and coupled systems.

The coupled system of columns A and C is excellently suited for quantitative analysis of DEG. In 15 min, up to 20 ppm DEG can be quantified. If all of the investigated substances are to be separated, this can be achieved within 26 min on this system, with only water as the mobile phase. In this case, the resolution, R_s, for all pairs of substances exceeds unity; thus, quantitative analysis is readily achieved.

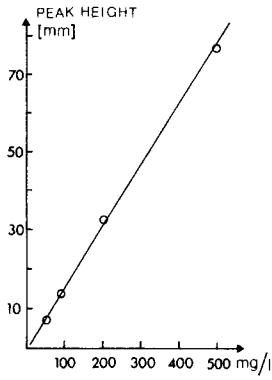

Fig. 6. Calibration curve for diethylene glycol. Conditions as in Fig. 1.

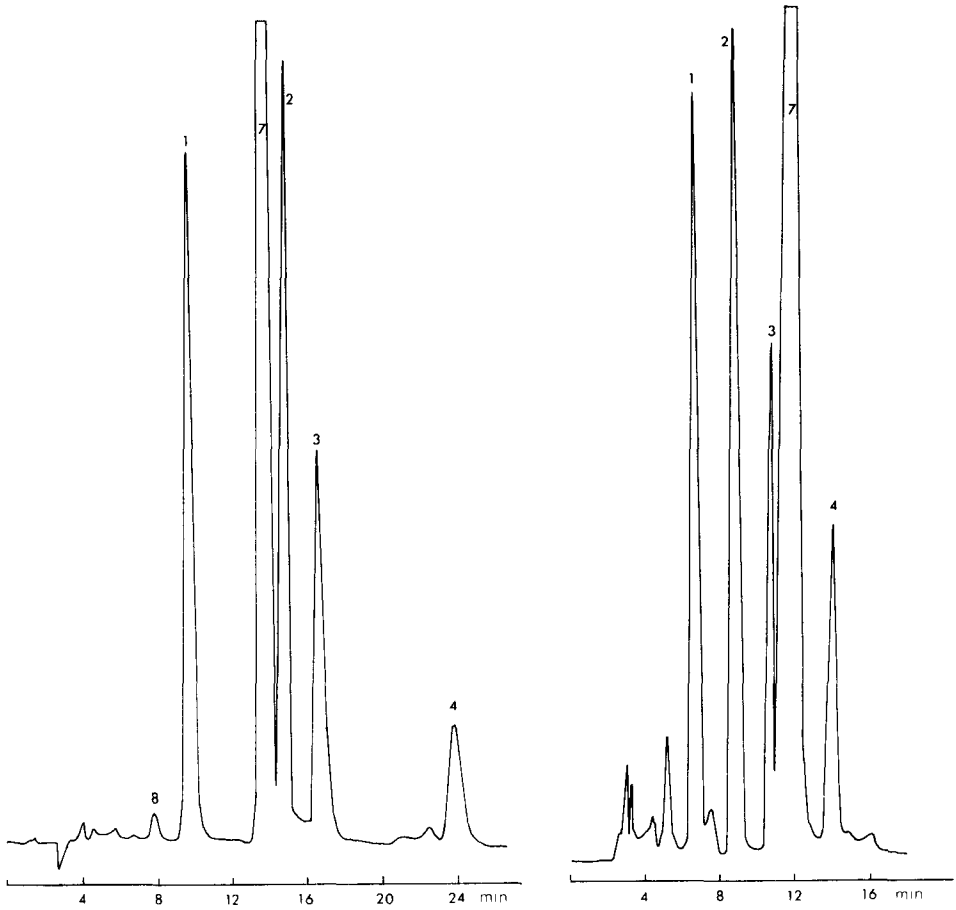

Fig. 7. Separation of an Austrian red wine sample. Stationary phase; coupled columns A and B. Conditions as in Fig. 3d. For peak identification see Table I.

Fig. 8. Separation of an Austrian white wine sample. Stationary phase: coupled columns A and C. Conditions as in Fig. 3e. For peak identification see Table I.

Applications

The selection of the differently loaded ion-exchange materials was directed towards quantitative detection of the illegal wine additive DEG as well as of carbohydrates and alcohols in wine and grape-juice soft drinks. Fig. 5 shows the separation of a red wine sample on a polystyrene–divinylbenzene ion-exchange resin (hydrogen form). In order to determine the detection limit for DEG, a calibration curve was constructed for this substance at low concentrations (see Fig. 6). As only water was used as the mobile phase, the sensitivity of refractive index detection is considerably higher than in the case of organic solvent mixtures. The detection limit for DEG is < 20 ppm, where the carbohydrates saccharose, glucose and fructose as well as the fermentation alcohols ethanol, methanol and glycerol can still be determined simultaneously.

Red and white wine samples can also be separated using coupled column sys-

tems as shown in Figs. 7 and 8. The detection limit for DEG in both cases was *ca.* 20 ppm.

CONCLUSION

Food sugars, fermentation alcohols and the illegal wine additive diethylene glycol were determined using differently loaded ion-exchange resins.

With water as the mobile phase and a refractive index detector, a detection limit for DEG of 20 ppm was achieved. An advantage of this system consists in its being based on an isocratic separation. Furthermore, the columns need not be regenerated. Thus the present system is exceptionally well suited for routine analysis.

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