CHROM. 8753

USE OF POLYOLS AS STATIONARY PHASES FOR THE GAS CHROMATOGRAPHIC SEPARATION OF VOLATILE COMPOUNDS FROM EXCESS AMOUNTS OF ETHANOL

H. VERACHTERT, D. VAN OEVELEN and J. BEVERS

Laboratory of Industrial Microbiology and Biochemistry, University of Leuven, Kardinaal Mercierlaan 92, Heverlee-Louvain 3030 (Belgium)

(First received July 7th, 1975; revised manuscript received September 15th, 1975)

SUMMARY

Erythritol, ribitol, arabitol, xylitol, mannitol, dulcitol and sorbitol were studied as stationary phases for the separation of volatile compounds ("volatiles") normally present in alcoholic beverages. Excellent separations of most of the volatiles from each other, and especially from the excess amounts of ethanol that are generally present, were obtained with 2- and 5-m glass columns filled with Chromosorb P coated with a polyol. A method is presented that permits the analysis of beer volatiles, from acetaldehyde up to phenethyl alcohol, in about 90 min. This method includes the direct injection of beer samples into the columns. After several months, no perceptible changes in column performance were observed; indicating that a rapid and cheap method for the analysis of beer volatiles is now available. Wines and spirits have been analyzed by the same method.

INTRODUCTION

The gas chromatographic analysis of volatile compounds ("volatiles") in alcoholic liquids or beverages is generally hindered by the presence of excess amounts of ethanol. This resulted in the need for pre-treatment of the samples, which included extraction with diethyl ether–pentane mixtures[1,2], carbon disulphide[3], methylene chloride[4] and many other solvents. Gas stripping[5], steam distillation[6] and distillation have also been used.

These procedures may reduce the relative concentration of ethanol, but mostly they result in losses of other volatiles also. Head-space techniques avoid the preliminary treatments but they require precise control of several parameters and they are therefore not simple. Moreover, certain compounds are still masked by ethanol. A better solution would be to find columns that would permit the separation of the major volatiles in the presence of excess amounts of ethanol. If such a procedure could be combined with the direct injection of samples of beer and other alcoholic liquids, maximum simplicity and accuracy would be obtained. Such an approach has been tested by Bertrand[7] and Cordonnier[8], and we have attempted to improve such

methods. Our experience with polyols[9,10] has resulted in the development of polyol-coated columns, on which beer samples may be injected directly and which separate the major beer volatiles from each other and from excess amounts of ethanol.

MATERIALS AND METHODS

All gas chromatographic separations were performed with a Carlo Erba Model GB apparatus equipped with two flame ionization detectors and thermal programming facilities. The column packing material was Chromosorb P (60–80 mesh) coated with 10% of a polyol. Glass columns with an I.D. of 4 mm and a length of 2 m (U-form) or 5 m (coiled form) were used. The vaporizer temperature was 180° and the detector temperature 190°. The oven temperature varied in different experiments and is given in each instance later. Nitrogen was used as the carrier gas at a flow-rate of 50 ml/min. The hydrogen flow-rate was 37 ml/min and the air flow-rate was 300 ml/min.

The columns were prepared as follows. A 4-g amount of a polyol was dissolved in 150 ml of water and 40 g of Chromosorb P were added. The mixture was slowly evaporated at 60° on a rotary evaporator and the residue was dried overnight at 80°. After filling the glass columns, they were further equilibrated in the gas chromatograph for 24 h at 90° under a flow of nitrogen. The polyols tested were *meso*-erythritol (Merck, Darmstadt, G.F.R.), D-arabitol, ribitol, xylitol, D-mannitol (Union Chimique Belge, Drogenbos, Belgium), D-sorbitol, dulcitol and *meso*-inositol. Glycerol was used as a reference polyol, as it has been used by several workers.

RESULTS

Isothermal analysis of volatiles on 2-m polyol-coated Chromosorb P columns

Different compounds were chromatographed on 2-m polyol-coated Chromosorb P columns at 60° in order to study the separation of the compounds from each other and from excess amounts of ethanol. Analyses were performed on 5-μl amounts of solutions containing 25 ppm each of the different volatiles mentioned in Table I and sometimes 5% (w/v) of ethanol. The results are given in Table I.

No separations were obtained on the inositol column. Some interesting differences were found in the retention times on the different columns. A better separation from ethanol of the compounds that elute after ethanol (isopropanol, n-propanol, etc.) was obtained with the erythritol and arabitol columns, and especially with the mannitol and dulcitol columns. Even in the presence of 5% of ethanol, the two C-3 alcohols were exceptionally well separated from ethanol on the mannitol and dulcitol columns. (Fig. 1). Unfortunately, these two columns did not separate ethyl acetate from ethanol or D-amyl alcohol* from isoamyl acetate. The glycerol, ribitol, xylitol and sorbitol columns, on the contrary, gave good separations from ethanol of the compounds that elute before ethanol (ethyl acetate, etc.), but they were not suitable for the separation of the compounds that elute after ethanol. Thus the arabitol and erythritol columns seem to offer the best possibilities for the separation of the different compounds from each other and from excess of ethanol. Modifications in the experimental procedure are necessary, however, in order to achieve the separation of ethyl acetate and the two C-3 alcohols from these excess amounts

---

* 2-Methyl-1-butanol.

TABLE I

RETENTION DATA OF VOLATILES ON POLYOL-COATED CHROMOSORB P

Columns were prepared as described in the text. Analyses were performed on 2-m columns at an oven temperature of 60°.

| Compound	Retention times on different columns (sec)								
	Glycerol	Erythritol	Arabitol	Xylitol	Ribitol	Mannitol	Dulcitol	Sorbitol	Meso-Inositol
Acetaldehyde	45	32	30	30	27	30	41	27	—
Methyl acetate	45	48	42	38	37	70	78	—	—
Diacetyl	71	52	48	46	39	70	80	—	—
Methanol	155	60	76	92	96	70	78	—	—
Ethyl acetate	50	73	70	56	54	95	96	50	—
Ethanol	155	84	94	97	99	102	96	99	—
Isopropanol	146	125	133	115	113	222	414	—	—
n-Propanol	192	146	168	132	129	276	520	126	—
sec.-Butanol	192	232	252	185	182	522	960	—	—
Isobutanol	228	269	282	210	196	606	1000	196	—
n-Butanol	306	353	370	270	255	846	1142	255	—
Isoamyl acetate	167	426	420	292	269	1536	3060	297	—
D-Amyl alcohol	394	600	618	436	404	1536	3060	420	—
Isoamyl alcohol	462	738	756	533	481	2056	3960	511	—
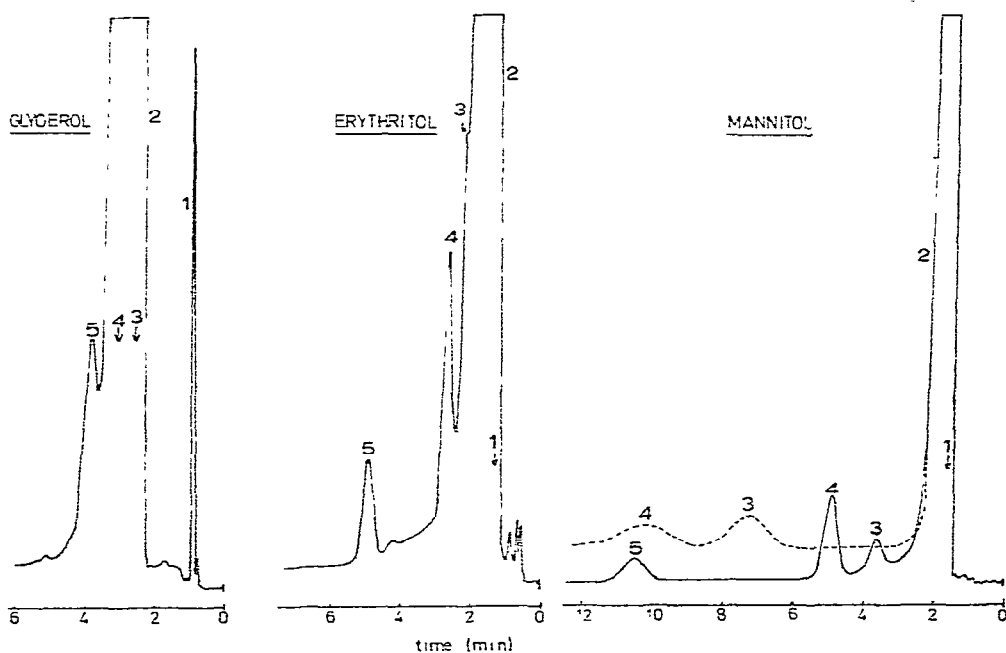
Fig. 1. Analysis of a distillate of a Belgian beer, showing the separation of ethyl acetate, isopropanol and n-propanol from excess amounts of ethanol (5% w/v) on glycerol, erythritol and mannitol columns at 60°. The broken line indicates the separations obtained on the dulcitol column. 1 = ethyl acetate; 2 = ethanol; 3 = isopropanol; 4 = propanol; 5 = isobutanol.

TABLE II

RETENTION TIMES OF VOLATILES ON A 2-m ERYTHRITOL COLUMN AT 80°

Compounds that elute before 2-pentanol are not listed as they are masked by excess amounts of ethanol. The column was a 2-m chromosorb P (60–80 mesh) column coated with 10% erythritol. Other details are given in the text.

Compound	Retention time (min)	Compound	Retention time (min)
2-Pentanol	2.36	Methyl caprylate	9.21
Methyl caproate	2.36	Ethyl lactate	9.21
D-Amyl alcohol	3.21	o-Cresol	12.40
Isoamyl alcohol	3.92	Dimethyl succinate	12.71
Ethyl caproate	5.43	Benzyl alcohol	18.31
Hexyl acetate	5.53	Ethyl caprylate	21.12
Acetophenone	5.57	Ethylene glycol	22.91
Furfuryl alcohol	7.56	Propylene glycol	25.75
Diethyl oxalate	7.91	Phenethyl alcohol	27.40
Ethyl acetoacetate	7.94	2,4-Dimethyl phenol	29.05
Hexanol	8.03	Methyl caprate	34.02
Ethyl benzoate	8.39		

of ethanol, and these modifications are described later. Nevertheless, when 2-m erythritol columns are operated at 80°, excellent separations can be obtained of many compounds that elute after isoamyl alcohol (Table II). A typical chromatogram is shown in Fig. 2.

Isothermal analysis of volatiles on 2-m mixed-polyol columns

Compared with the erythritol column, the mannitol or dulcitol columns, with ethanol as reference peak, induced a shift towards higher retention times of all other volatiles. Glycerol, ribitol and xylitol columns induced the opposite effect. Mixtures of polyols were then tested in two different ways. Chromosorb P was coated with mixtures of different polyols, such as mannitol + ribitol and mannitol + erythritol, and the different phases were tested in 2-m columns, operated at 60°. Alternatively, 2-m glass columns were filled to a certain length with Chromosorb P coated with one type of polyol, followed by Chromosorb P coated with another polyol. It was found that the effects normally induced by one type of polyol became additive when mixtures were used but the separations obtained with erythritol alone could not be improved, and this extensive series of experiments will not be described further here.

Isothermal analysis of volatiles on 5-m erythritol columns

The main problem with the erythritol columns was the separation of ethyl acetate from large amounts of ethanol. When the compounds listed in Table I were injected into a 5-m erythritol column operated at 60°, excellent separations were obtained, except again for ethyl acetate, which was not separated from large amounts of ethanol (5% w/v).

Temperature-programmed analysis of volatiles on 5-m erythritol columns

Ethyl acetate could be separated from ethanol (5% w/v) only by temperature programming. The best conditions were injection of 5-μl samples into the column,
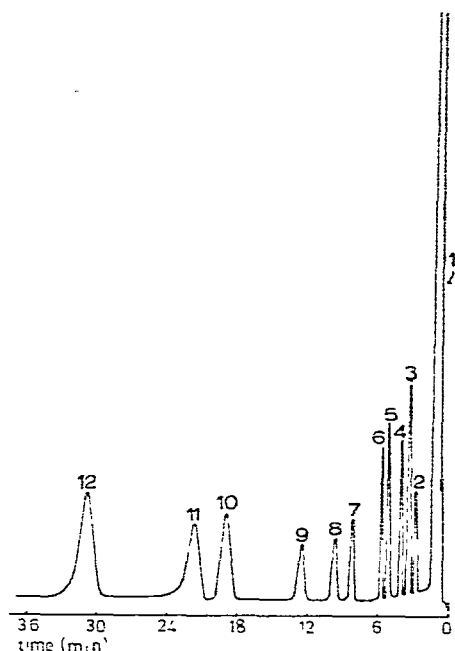
Fig. 2. Elution profile of several volatiles on a 2-m erythritol column operated at 80°. 1 = ethanol, etc.; 2 = 2-pentanol; 3 = D-amyl alcohol; 4 = isoamyl alcohol; 5 = ethyl hexoate; 6 = hexyl acetate; 7 = hexanol; 8 = ethyl lactate; 9 = dimethyl succinate; 10 = benzyl alcohol; 11 = ethyl caprylate; 12 = phenethyl alcohol.

the oven temperature being 50°, followed immediately by starting the temperature programming from 50° to 80° at the rate of 1°/min. Under these conditions, all of the volatiles listed in Tables II and III could be separated from each other and from large amounts of ethanol. A typical separation is shown in Fig. 3. Compounds that

TABLE III

RETENTION TIMES OF VOLATILES ON A 5-m ERYTHRITOL COLUMN

The column was a 5-m chromosorb P column coated with 10% erythritol and operated by temperature programming from 50° to 80° at the rate of 1°/min. Other details are given in the text.

| Compound	Retention time (min)	Compound	Retention time (min)
Acetaldehyde	1.94	Isobutanol	17.65
Ethyl formate	2.52	n-Butanol	20.96
Diacetyl	3.39	tert.-Amyl alcohol	21.70
Methanol	4.72	Isoamyl acetate	23.54
Ethyl acetate	5.15	Isobutyl acetate	26.14
Ethanol	6.25	D-Amyl alcohol	28.57
Ethyl propionate	7.72	Isoamyl alcohol	31.45
Propyl acetate	8.50	n-Amyl alcohol	32.26
Isopropanol	9.70	Ethyl caproate	35.90
n-Propanol	11.32	Hexyl acetate	37.95
Ethyl butyrate	14.20	Hexanol	47.56
Butyl acetate	15.92	Ethyl lactate	50.58
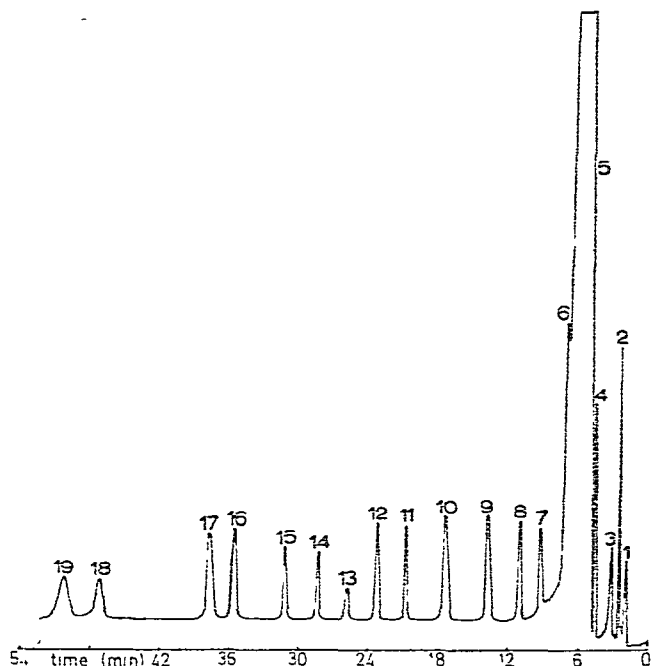
Fig. 3. Elution profile of several volatiles on a 5-m erythritol column operated by temperature programming from 50° to 80° at 1°/min. 1 = acetaldehyde; 2 = ethyl formate; 3 = diacetyl; 4 = ethyl acetate; 5 = ethanol; 6 = ethyl propionate; 7 = isopropanol; 8 = propanol; 9 = ethyl butyrate; 10 = isobutanol; 11 = butanol; 12 = isoamyl acetate; 13 = isobutyl acetate; 14 = D-amyl alcohol; 15 = isoamyl alcohol; 16 = ethyl hexoate; 17 = hexyl acetate; 18 = hexanol; 19 = ethyl lactate.

elute after isoamyl alcohol, such as phenethyl alcohol, involved very long retention times and gave flat peaks. This difficulty was overcome by adopting the method described below.

Two-step analysis of volatiles on 2- and 5-m erythritol columns

In order to reduce the long waiting times involved in the analysis of compounds such as phenethyl alcohol and to avoid the flat peaks obtained with these compounds, the following method was adopted. The double-column gas chromatograph was fitted with two columns of different lengths. A 2-m erythritol column was joined to one detector and a 5-m column was joined to the other. In the first step, a sample to be analyzed was injected into the 5-m column, the temperature of the oven being 50° and the detector of this column being connected to the recorder. The analysis was started by programming the temperature from 50° to 80° as described above. When isoamyl alcohol had left the column (after about 35 min), the analysis was stopped and the recorder was disconnected from the 5-m column detector. The recorder was then connected to the 2-m column detector. The oven temperature was kept constant at 80° and a second sample was injected into the 2-m column. The compounds that elute after isoamyl alcohol were then analyzed on this 2-m column at 80°. About 10 min after phenethyl alcohol had left this 2-m column, the analysis was stopped. It was found, by testing about 30 different types of beer, that no compounds with higher retention times were present.

Under these circumstances, relatively sharp peaks were obtained for all compounds. During the analysis on the 2-m column (about 40 min) the compounds that elute after isoamyl alcohol have time to leave the 5-m column, making this column ready for a new sample injection. Therefore, the temperature was reduced to 50° and a completely new analysis was started after rearranging the connections between the 5-m column detector and the recorder.

Two-step analysis of beer volatiles by direct injections of beer

The two-step method described above permitted the separation of the volatiles listed in Tables II and III. Nevertheless, about 90 min are required for a gas chromatographic analysis. Therefore, it was decided to examine the possibility of the direct injection of beer samples, which would reduce the total analysis time of a beer sample to about 90 min. After the application of this method during more than 8 months, it was concluded that direct injections of beer samples were possible. During this period, 30 types of beer were injected into the 2- and 5-m erythritol columns. The only preliminary treatment was the filtration of a few millilitres of beer through a filter-paper in order to decarbonate the beers. After 3–4 months, the first 1 cm of the column fillings was discarded and replaced with new material. After 8 months, the performance of the columns decreased and new columns had to be made. It is not certain, however, that this decrease in performance resulted from the direct injections. Moreover, even if the columns had to be replaced more frequently, it is not a major problem as they are very easy and cheap to make. Figs. 4 and 5 show typical analyses of a Belgian beer. The
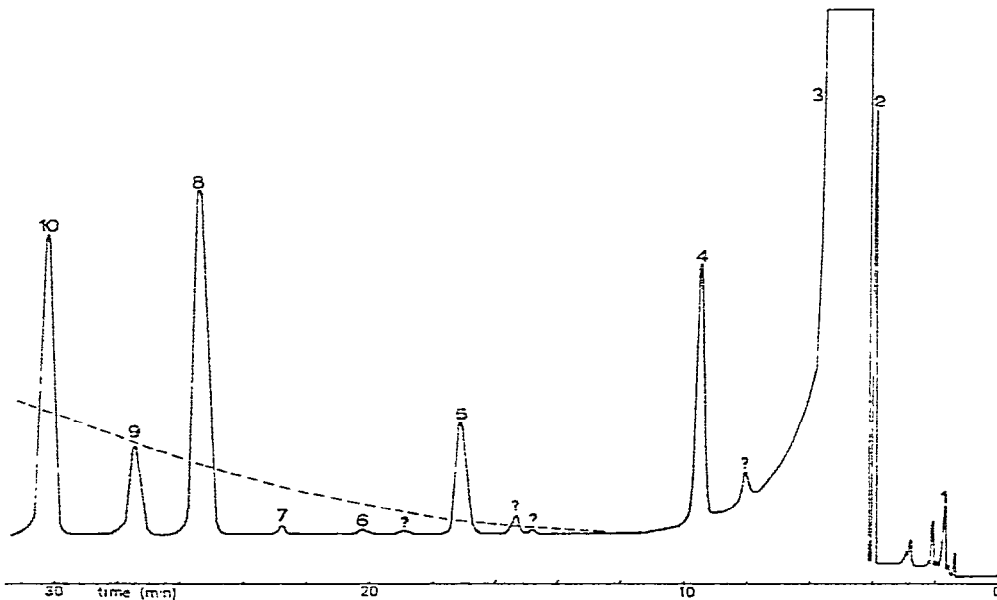

Fig. 4. Analysis of a Belgian beer by the two-step method; elution profile on the 5-m erythritol column. Amounts directly injected were 5 μl. The attenuation factors were 10 × 16. The broken line indicates the increase of the base-line during temperature programming. 1 = acetaldehyde; 2 = ethyl acetate; 3 = ethanol; 4 = propanol; 5 = isobutanol; 6 = butanol; 7 = isoamyl acetate: 8 = 2-pentanol; 9 = D-amyl alcohol; 10 = isoamyl alcohol.
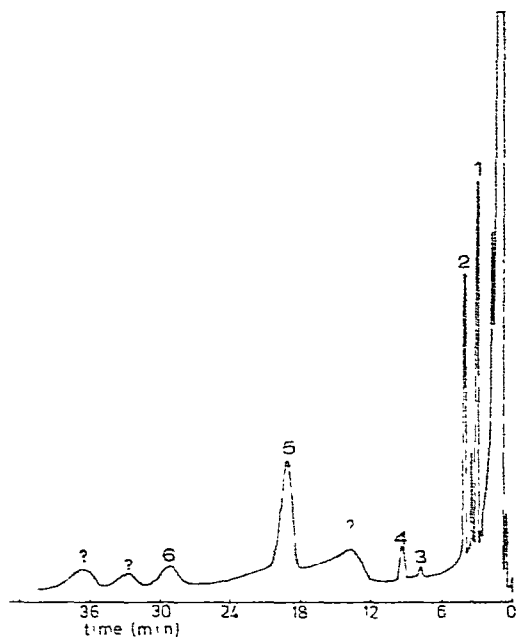
Fig. 5. Analysis of a Belgian beer by the two- step method; elution profile on the 2-m erythritol column. Attenuation 10 × 32. 1 = 2-pentanol; 2 = isoamyl alcohol; 3 = hexanol; 4 = ethyl lactate; 5 = benzyl alcohol; 6 = phenethyl alcohol.
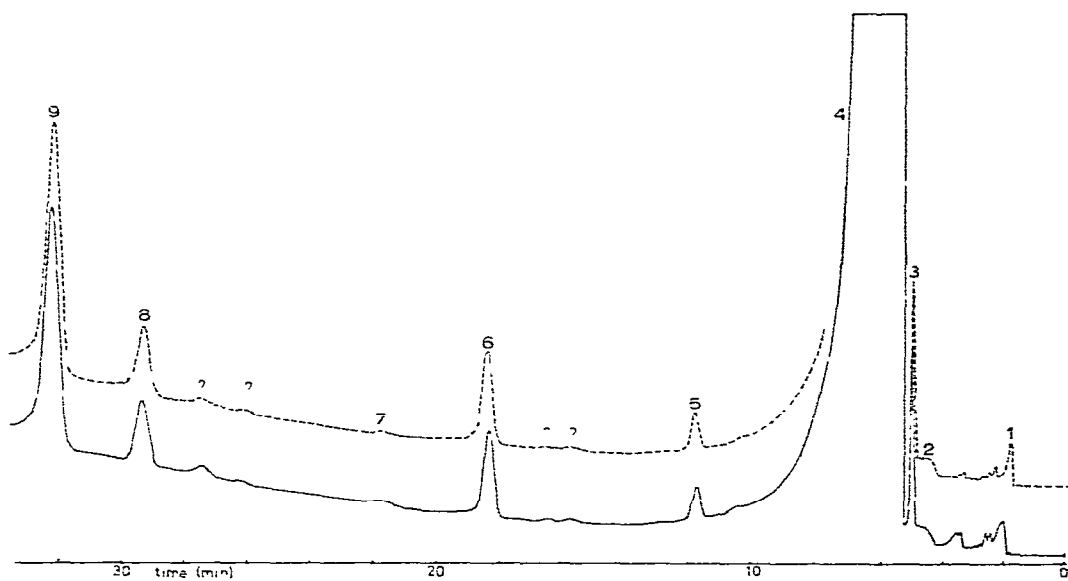

Fig. 6. Analysis of a Bordeaux wine by the two-step method; elution profile on the 5-m erythritol column. Amounts directly injected were 2 μl. Attenuation 10 × 16. The broken line represents the elution profile for 2 μl of a distillate diluted to its original volume. 1 = acetaldehyde; 2 = methanol; 3 = ethyl acetate; 4 = ethanol; 5 = propanol; 6 = isobutanol; 7 = butanol; 8 = D-amyl alcohol; 9 = isoamyl alcohol.
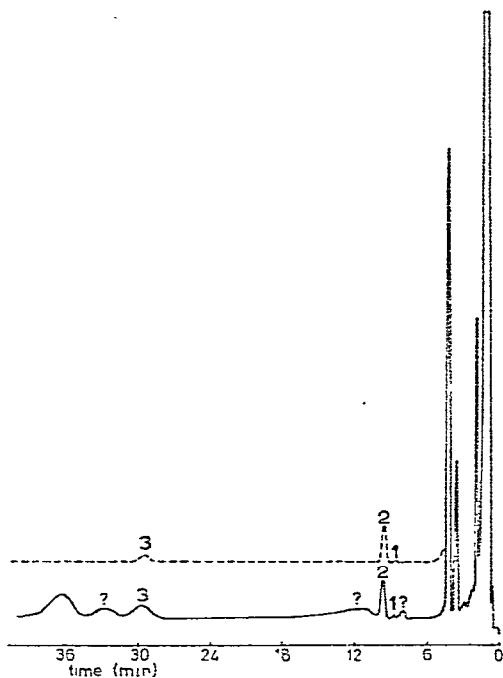
Fig. 7. Analysis of a Bordeaux wine by the two-step method; elution profile on the 2-m erythritol column. Attenuation 10 × 16. 1 = hexanol; 2 = ethyl lactate; 3 = phenethyl alcohol. Broken line, see Fig. 6.

method enabled the presence of ethyl lactate in some types of beer to be ascertained. For the quantitative analysis of volatiles, internal standards must be used. It was found that several compounds could be used: 2-pentanol for the 5-m column and 2-pentanol, acetophenone and benzyl alcohol for the 2-m column.

Analysis of other alcoholic liquids

Some preliminary experiments were carried out in order to determine whether other alcoholic liquids such as wines and fortified wines might be injected directly into the erythritol columns. Figs. 6 and 7 show the results for the injection of a Bordeaux wine and indicate that such a direct analysis is possible. Fig. 6 also shows the very irregular peaks given by methanol. In this instance, the shape of the peak helped in the identification of methanol. A clear example of peaks that appear only after direct injection is shown in Fig. 7. Fig. 7 also shows that phenethyl alcohol cannot be quantitated by using a preliminary distillation. Similar results have also been obtained with other wines and fortified wines. With spirits, excellent results were obtained provided that not more than 0.5 μl of the spirit was injected into the columns.

DISCUSSION AND CONCLUSION

The results indicate that polyol-coated Chromosorb P columns offer a useful alternative for the analysis of the volatiles that are most often found in alcoholic beverages. Their main advantages are the separation of the major volatiles from each

other and their separation from excess amounts of ethanol. According to the type of polyol used, different types of separations can be obtained, *e.g.*, the use of mannitol or dulcitol for the rapid and excellent separation of isopropanol and *n*-propanol from large amounts of ethanol.

An extensive study of the erythritol columns also showed that the direct injection of beer samples was possible, which allowed the analysis of all major beer volatiles to be carried out in about 90 min. Temperature programming had to be used in this analysis and, as no reference columns were used, this resulted in a slight increase in the base-line when the temperature was increased from 50° to 80°. However, this did not interfere with the quantitative measurement of the peak areas. Of all compounds to be analyzed, isoamyl acetate was the most difficult to quantitate, as only small amounts are usually present in beer and the retention time is too high to give very sharp peaks. Nevertheless, amounts down to 1 ppm could be determined. Methanol was difficult to determine as it formed very irregular peaks, emerging just before ethyl acetate. The irregular form of the peak sometimes helped in the identification of methanol, however, although it is not present in beer. Direct injections of beer samples always resulted in the appearance of peaks that were not detected after distillation of the samples. In only a few instances did one or two of these peaks mask the peaks of known volatiles. A typical example of peaks that are detectable only after direct injection is the two peaks with the longest retention time on the 2-m erythritol column at 80°, shown in Fig. 5. Direct injection thus permits the detection and analysis of more compounds. Moreover, it permits the quantitative analysis of compounds such as ethyl lactate and phenethyl alcohol, which are not recovered completely by distillation. Liquids other than beers, such as wines, aperitives and spirits, were also injected directly into the columns. In all instances, the separations were excellent although some unknown peaks sometimes partially masked one or two of the known volatiles. This was the case, for example, with some aperitives that contain a large amount of sugar. These results will be published later.

It is probable that the columns can also be used for the direct injection of blood serum for the determination of the ethanol content of blood.

REFERENCES

1 B. Drews, H. Specht and G. Bärwald, *Monatsschr. Brau.*, 17 (1964) 101.
2 J. Baraud and A. Maurice, *Ind. Aliment. Agr.*, 80 (1963) 3.
3 A. D. G. Powell and I. H. Brown, *J. Inst. Brew.*, 72 (1966) 261.
4 A. D. Webb and R. E. Kepner, *Amer. J. Enol. Vitic.*, 13 (1962) 1.
5 J. N. Boidron, S. P. Avakiantz and A. Bertrand. *Conn. Vignes Vins*, 3 (1969) 43.
6 B. Ahrenst-Larsen and H. L. Hansen, *Brauwissenschaft*, 16 (1963) 393.
7 A. Bertrand, *Conn. Vignes Vins*, 2 (1968) 179.
8 R. Cordonnier, *Bull. OIV*, 490 (1971) 1128.
9 L. Dooms, G. L. Hennebert and H. Verachtert, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 37 (1971) 107.
10 L. Dooms, D. Declerck and H. Verachtert. *J. Chromatogr.*, 42 (1969) 349.