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COMBINED HEADSPACE AND EXTRACTION TECHNIQUE FOR PROFILE ANALYSIS BY CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

A method is described whereby a transevaporator is used for sampling 60–100 μl of aqueous sample. Volatiles are stripped from the sample either by a stream of helium and collected on a porous polymer, Tenax, or by 0.8 ml of 2-chloropropane and collected on glass beads. The volatiles are thermally desorbed into a precolumn which is connected to a capillary gas chromatographic column for analysis. The technique is shown to be reproducible and suitable for determining chromatographic profiles for a wide variety of sample types.

INTRODUCTION

Profile analysis has been the subject of considerable investigation, with various degrees of success, in disease diagnosis[1–5], in the study of metabolic disorders[6–18], in food quality evaluation[18–24], in air pollution studies[25] and in the characterization of automobile exhaust gases[21], marine sediments[26], engine oils[27] and marine oil spills[28–30]. Capillary columns are generally used for the analyses due to their superior resolving power.

The object of profile analysis is to correlate a characteristic "fingerprint" pattern in a chromatogram with the properties of a sample or, in biomedical studies, with the presence or absence of disease in a patient or animal from which the sample is taken. The components of interest are generally present in low concentration with the result that special techniques are necessary to overcome the problems associated with the small sample capacity of a capillary column and the need to introduce the sample as a concentrated plug. Two techniques that have been widely used for the concentration of volatile substances from dilute aqueous solution are solvent extraction[8,12–18,21,22,26–30] and headspace analysis using a trapping tube[1–5,20,24,25,31–35]. The techniques are complementary for samples that contain organics covering a wide volatility range. A shortcoming of the extraction technique is that some early peaks are masked by solvent whereas poor representation of late peaks occurs with the headspace technique. The extraction may be performed using a micro-technique but the headspace method generally yields unsatisfactory results where only small volumes of sample are available. Zlatkis and Kim[36] have recently described an improved method of micro-extraction using a transevaporator. An aqueous sample is placed on

a suitable adsorbent packed in a small tube, the organics of interest are extracted with a suitable solvent and transferred to a collection tube packed with glass wool and excess solvent is evaporated. The volatiles are then transferred to a capillary column via thermal desorption into a precolumn. The extraction procedure takes less than 30 min and may be used with as little as 25 μl of sample. The transevaporator extraction is about three times more efficient than a direct extraction. Purified 2-chloropropane was found to be a satisfactory extracting solvent and Porasil E was found to be the best adsorbent out of six possible candidates.

We report below on the use of the transevaporator in a "micro-headspace" technique, on an improved technique of using the transevaporator in the extractive mode, on the precision of the method and on several applications in connection with profile analysis.

EXPERIMENTAL

Adsorbents

Porasil E (80–100 mesh) and glass beads (80–100 mesh) were obtained from Analabs (North Haven, Conn., U.S.A.). Tenax GC (60–80 mesh) was obtained from Applied Science (State College, Pa., U.S.A.). Glass wool (Pyrex brand) was obtained from Corning (Corning, N.Y., U.S.A.).

All of the above were washed with distilled ether and conditioned at 280° in a stream of dry helium for 12 h.

Reagents

2-Chloropropane (Eastman-Kodak, Rochester, N.Y., U.S.A.) and *n*-pentane (J. T. Baker, Phillipsburg, N.J., U.S.A.) were distilled from phosphorus pentoxide (J. T. Baker). Diethyl ether anhydrous (Mallinckrodt, St. Louis, Mo., U.S.A.) was distilled from lithium aluminum hydride (Alfa Products, Danvers, Mass., U.S.A.) and water was distilled from potassium permanganate (Fisher Scientific, Pittsburgh, Pa., U.S.A.).

Apparatus

The transevaporator used is shown in Fig. 1 and is essentially of the same design as described by Zlatkis and Kim[36]. Differences are that the dimensions of the Porasil E tube are 70 mm × 40 mm O.D. × 2 mm I.D. and that a water condenser is included.

Sampling procedure

The apparatus used is shown in Fig. 1. The sampling procedure is performed in two steps, the first being collection of low molecular weight volatiles on a Tenax tube, the second being the solvent extraction of volatiles coupled with collection on glass beads. In the first step the sample (25–200 μl) is introduced to the inner tube which contains 0.3 ml of Porasil E, a strongly hydrophilic porous silica adsorbent. The upper tube contains 1.8 ml of Tenax, a 2,6-diphenyl-*p*-phenylene oxide porous polymer, which is an excellent trapping material for headspace analysis. A condenser is included in the apparatus to prevent excess water vapor from reaching the Tenax trap. A stream of helium gas (16 ml/min) is passed through the apparatus for 5–10 min

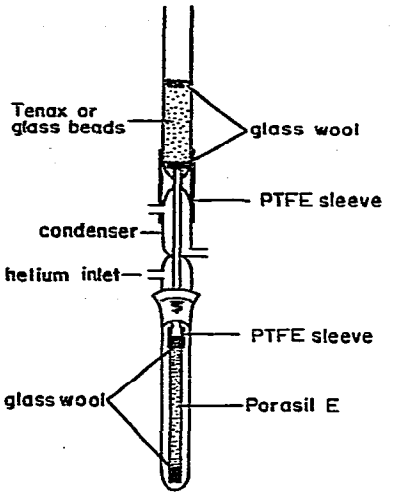


Fig. 1. Sampling apparatus.

to transfer the volatile compounds from the Porasil tube to the Tenax tube which is then removed from the apparatus. Helium (16 ml/min) is passed through the tube for about 3 min to remove traces of water which would block the precolumn during desorption. There is no apparent change in the resultant profile due to this procedure. The Tenax tube is removed from the apparatus and placed in a desorber at 280° for 10 min. A stream of helium (7 ml/min) desorbs the volatiles into a precolumn, cooled in liquid nitrogen, which is then connected to an analytical capillary column for chromatography.

In the second step the apparatus is converted to the form described by Zlatkis and Kim[36] by removing the condenser. The Tenax tube is exchanged for a tube of identical dimensions containing glass beads. 0.8 ml of 2-chloropropane is introduced into the tube and is swept through the Porasil tube into the upper tube by applying gas pressure. Soluble organics are extracted and transferred to the glass beads while most of the water and high molecular weight material is retained by the Porasil E. The Porasil E tube is removed, the apparatus is reassembled and the outer tube is placed in an air bath maintained at 50° to heat incoming helium which is passed through the apparatus at a rate of 16 ml/min for 10 min to remove excess 2-chloropropane and traces of water. As in the case with the Tenax tube there is no apparent change in the resultant profile due to this procedure. The organics are transferred from the glass bead trap to the capillary column by the same technique as is used for the Tenax tube.

Transference of sample from trapping tube to analytical column

The trapping tube was placed in a desorption chamber maintained at 280°, connected to a stainless-steel precolumn (30 cm × 1 mm I.D., coated with SF-96) and cooled in liquid nitrogen. The volatiles were transferred to the precolumn by passing helium through the system at 7 ml/min for 10 min. The precolumn was then connected to the analytical capillary column, the first 30 cm of which was cooled in liquid nitrogen. The sample was transferred from the precolumn to the analytical column

by heating the former to 180° with an air heating gun for 1 min while passing helium through the system at a rate of 1.5 ml/min.

Gas chromatography

A Hewlett-Packard 5830 A gas chromatograph (Hewlett-Packard, Avondale, Pa., U.S.A.) equipped with a flame ionization detector and connected to a Hewlett-Packard 18850 A gas chromatograph terminal was used. A stainless-steel capillary column (100 m × 0.25 mm I.D.) was coated with Witconol LA-23 (Witco, Houston, Texas, U.S.A.) by the dynamic coating method. The chromatograph was operated isothermally at 50° for 10 min, programmed at 1.5°/min to 160° and maintained at this temperature for a further 80 min.

RESULTS AND DISCUSSION

The modifications of the transevaporator technique described here, result in a better overall reproducibility and eliminates the problem of the precolumn blocking during desorption due to the presence of excess water vapor.

The major modification is that a "headspace" trapping is performed before the solvent extraction step. Minor modifications are the substitution of glass beads for glass wool, the removal of the Porasil E tube and the warming of the helium during the evaporation of excess 2-chloropropane when the transevaporator is used in the extraction mode. Glass beads have been found to be a more efficient trapping material than glass wool. No differences have been found between silanized and non-silanized beads. Heating the helium carrier reduces the evaporation time for 2-chloropropane from about 20 min to 7 min. *n*-Pentane was substituted for 2-chloropropane in a few runs. This resulted in a lower extraction of volatiles without significantly changing the shape of the profiles.

Due to the small volumes involved, the transevaporator technique is faster than a macro-headspace sampling using a Tenax trap or a micro-extraction technique. Thus a complete transevaporator run with collection on both a Tenax and a glass bead tube takes about 30 min which is about the same time as required for a normal headspace sampling of a large sample on Tenax.

For the technique of profile analysis to be useful, it is essential that replicate runs of the same sample yield near identical chromatograms. The reproducibility of the entire transevaporator–desorption–chromatography–integration technique was investigated by performing replicate runs of a 100-μl aqueous sample consisting of 1.3 ppb* of each of the following components: *n*-propanol, *n*-butanol, 2-heptanone, benzaldehyde and 2-decanone. The transevaporator was operated in the 2-chloropropane extraction–glass bead collection tube mode. Table I lists the standard deviation and relative standard deviation for both retention time and peak area. The standard deviation for retention time varies between 5 and 10 sec and appears to be independent of retention time. Thus retention time is an adequate parameter for defining the identity (in the context of profile analysis) of all but the earliest peaks.

In the case of peak areas, it is more meaningful to compare relative standard deviations, which, for the compounds considered, vary between 4.5% and 9.8% in a

* Throughout this article, the American billion (10^9) is meant.

TABLE I

STANDARD DEVIATIONS OF RETENTION TIME AND PEAK AREA

Sample	*Retention time*			*Peak area*		
	Mean value (min)	*Standard deviation*	*Relative standard deviation (%)*	*Mean value (counts)*	*Standard deviation*	*Relative standard deviation (%)*
n-Propanol	26.54	0.11	0.41	9401	916	9.7
n-Butanol	36.46	0.16	0.44	37320	3228	8.7
2-Heptanone	41.81	0.08	0.19	65868	5559	8.4
Benzaldehyde	61.56	0.10	0.16	83330	3741	4.5
2-Decanone	67.40	0.08	0.12	63810	6255	9.8

random manner. These values are acceptable for compounds in the sub-ppm range and may be considered surprisingly good, if the degree of sample manipulation is considered. Our prime interest is in the profile analysis of biological fluids where components in "normal" samples are likely to vary over a range of 20–50%. Thus changes in concentration of less than 100% are not considered significant and a

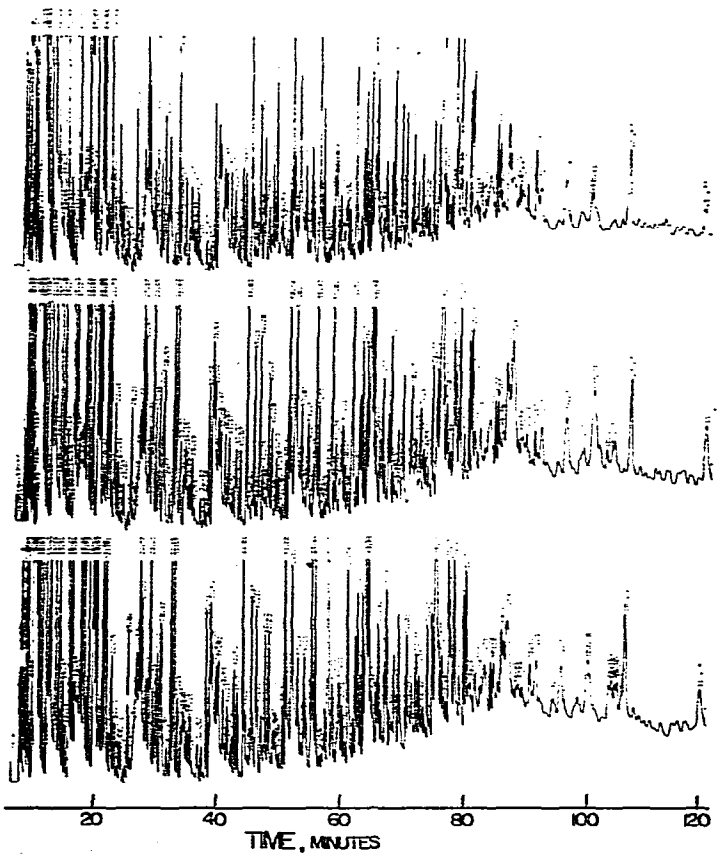
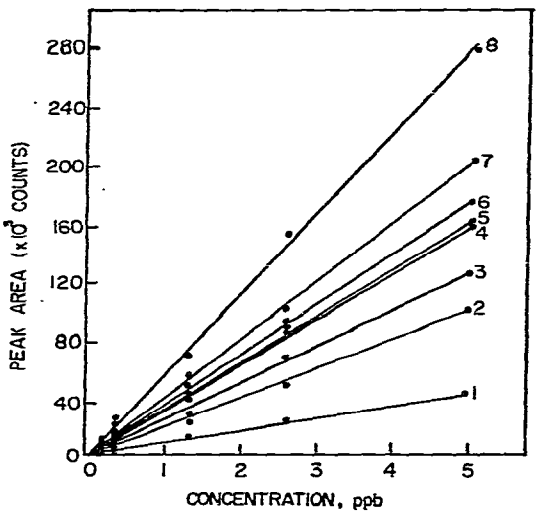


Fig. 2. Three replicate chromatograms of volatile compounds in 60 μl of normal serum. Trans-evaporator used in the glass bead mode.

relative standard deviation of 10% is more than adequate for the combined sampling and chromatographic technique. No increase in precision is obtained by considering either relative retention time or relative peak area. The reproducibility of the technique is further illustrated in Fig. 2 which shows three replicate runs of 60 μl of the same normal serum using the transevaporator in the 2-chloropropane–glass bead mode. The reproducibility is excellent, and with the exception of a few areas, the chromatograms are visually identical. The reproducibility is similar when the transevaporator is operated in the Tenax mode.

The linearity of overall response is good when the transevaporator is operated in the 2-chloropropane–glass bead mode as is shown in Fig. 3 for a variety of aldehydes, alcohols and ketones. However the actual recovery (comparing aqueous sample introduced into Porasil E tube to equivalent amount of dry sample directly introduced to glass bead tube) varies from about 20% to 80% for the five compounds illustrated in Fig. 4. The recovery varies slightly over a concentration range of 0.3–5 ppb but is remarkably consistent, considering that the actual weight of each component is in the nanogram range. Because of this consistency, the non-identical recovery for different compounds is of little consequence to profile analysis which is a comparative rather than an absolute technique.



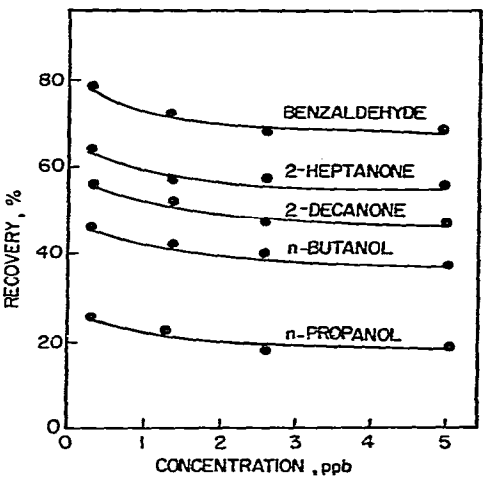


Fig. 3. Plot of peak area *vs.* concentration of various compounds dissolved in 100 μl of aqueous sample. 1 = *n*-Propanol; 2 = *n*-butanol; 3 = cyclohexanone; 4 = 2-undecanone; 5 = 2-decanone; 6 = 2-heptanone; 7 = 2-methyl-2-heptene-6-one; 8 = benzaldehyde.

Fig. 4. Plot showing percentage recovery *vs.* concentration for five compounds dissolved in 100 μl of aqueous sample.

The chromatogram obtained from a given sample when using the transevaporator sampling technique depends on a number of factors such as the nature and volume of the solvent used for extraction, the volume of adsorbents and the temperature and volume of gases flowing through the apparatus during the trapping procedure. Thus a standard set of conditions must be chosen for the complete pro-

cedure when comparing profiles. A blank should be run periodically to check for contamination of the system.

Application

The transevaporator technique has been applied to a large number of diverse samples in our laboratory. It is especially attractive for biological samples due to the small sample size requirements.

Chromatograms of normal serum volatiles (Fig. 2) collected using the transevaporator in the glass bead mode were referred to earlier in connection with reproducibility studies. Fig. 5 shows the corresponding chromatogram for a serum sample collected from a diabetic patient. About 200 peaks are present in each of the chromatograms. The heavy ends of the chromatograms are visually different. In addition there are several quantitative differences between the two profiles that are evident from inspection of an integrator print-out. Fig. 6 shows the chromatograms obtained from a diabetic serum using the transevaporator in the Tenax mode. In these profiles the relative size of the arrowed peaks is reversed in normal and diabetic serum.

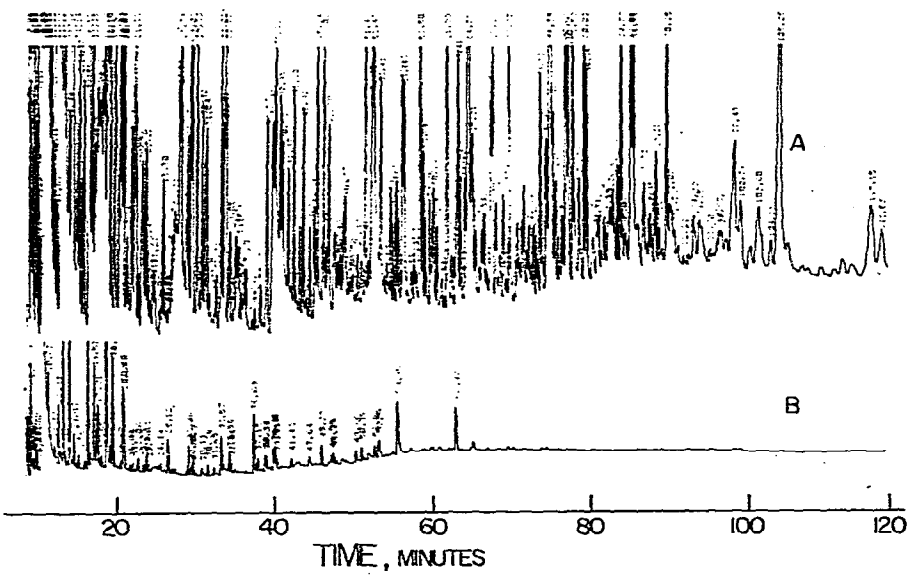


Fig. 5. (A) Chromatogram of volatile compounds in 60 μl serum collected from a diabetic patient. Transevaporator used in the glass bead mode. (B) Blank for system.

Differences are also found between the profiles (not shown here) of the sera of influenza patients and the sera of controls. These differences are manifested by a greater overall concentration of volatiles in the infected sera and by ratios of peak heights that are characteristic of either the infected or non-infected sera.

The transevaporator technique is well suited to other body fluids apart from serum. Fig. 7 shows the profile of 200 μl of saliva using the transevaporator in the 2-chloropropane extraction mode. The late peaks are small but their size could be increased four-fold by increasing sensitivity without introducing noticeable noise.

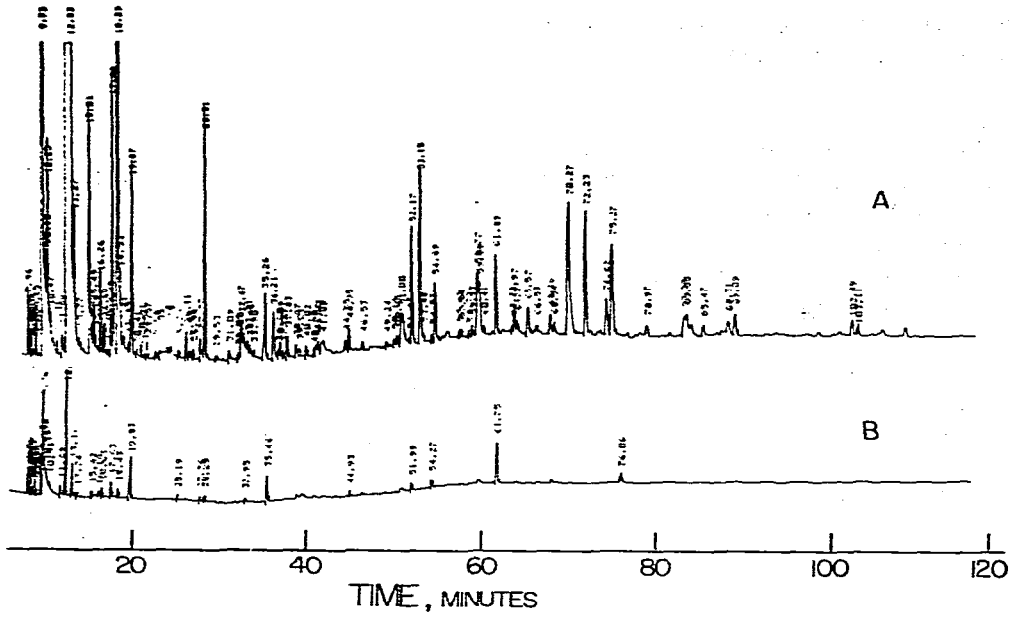


Fig. 6. (A) Chromatogram of volatile compounds in 60 μl serum collected from a diabetic patient. Transevaporator used in the Tenax mode. (B) Blank for system.

This profile, while not having as many volatiles as a serum profile, is of interest to biomedical studies due to the ease of obtaining samples. A profile (not shown here) was also determined for 50 μl of breast milk which, due to its viscosity, was first diluted with an equal volume of distilled water. The profile is complex and the concentration of volatiles is larger than in serum. It is estimated that an adequate profile may be obtained with as little as 5 μl of breast milk. The profile has virtually no resemblance to commercial cow milk which has a relatively simple profile. The transevaporator technique is not suitable for urine due to the low concentration of volatiles.

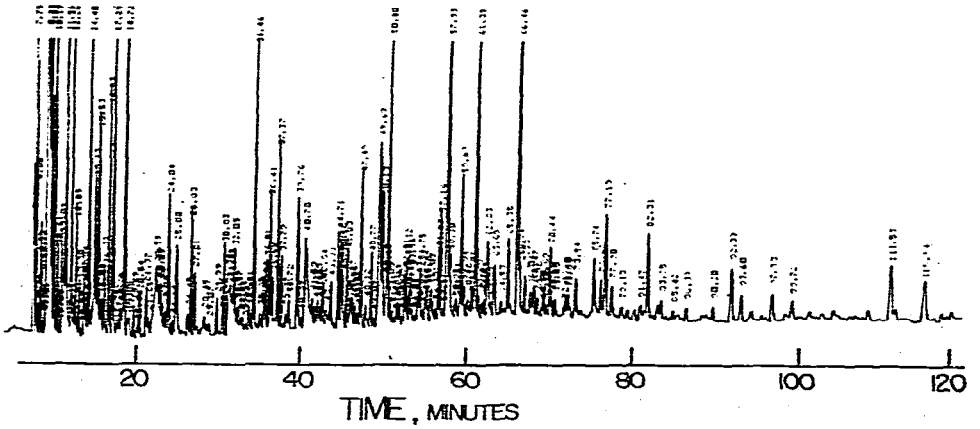


Fig. 7. Chromatogram of volatile compounds in 200 μl of saliva. Transevaporator used in the glass bead mode.

Figs. 8A and 8B show the different profiles obtained from samples of cognac using the transevaporator in the Tenax mode and also in the 2-chloropropane–glass bead mode. Other samples that have been run by this technique include wine, cola, pressed coffee oil and brewed coffee, all of which give complex profiles. Differences between Robusta and Arabica coffee were found for the latter two sample types.

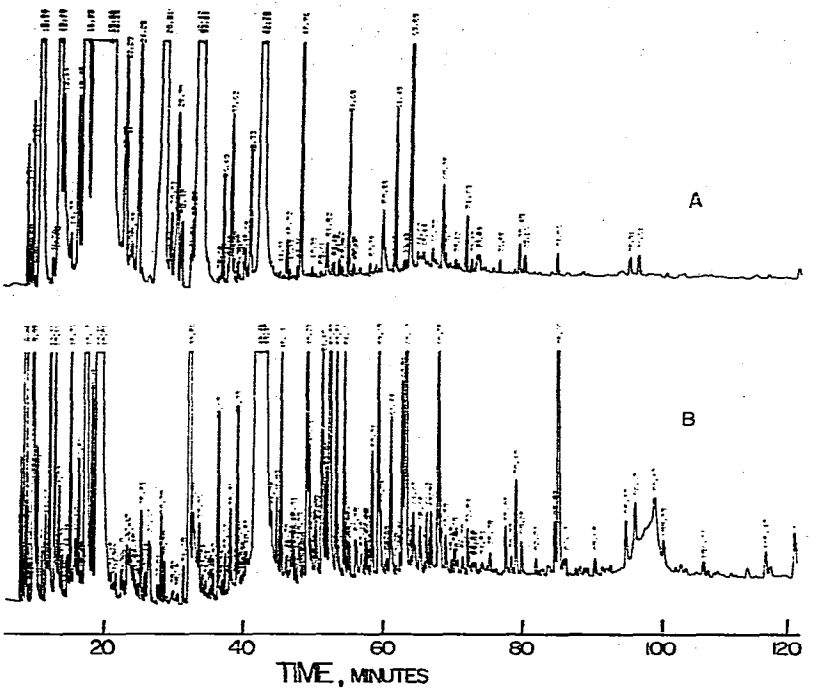


Fig. 8. Chromatogram of volatile compounds in 50 μl of cognac. A, transevaporator used in the Tenax mode; B, transevaporator used in the glass bead mode.

CONCLUSIONS

Complex profiles may be obtained from aqueous samples in the volume range from about 5 μl (for very concentrated samples) to about 200 μl by the transevaporator technique for a wide variety of biological and other sample types. Both a "headspace" and an extraction profile may be obtained from the same sample. These methods are complementary as each emphasizes different parts of the profile. The precision of the method is good considering the small sample and the trace quantities of volatiles present.

The technique may be used to show differences between normal and diabetic sera, between normal and influenza infected sera, and between Arabica and Robusta coffee for both pressed coffee oil and brewed coffee.

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