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A Recirculating System for Concentrating Volatile Samples

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Key Words

Dynamic headspace sampling
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Human sweat

Summary

A recirculating system powered by a diaphragm pump which may be readily dismantled for cleaning, is described. The results of operating this system for dynamic headspace sampling in the closed or open mode to load volatiles onto a Tenax trap over a range of temperatures and loading volumes are shown. The system was designed to facilitate the analysis of volatiles in natural products and tested using an equimolar solution of 7 esters of boiling points ranging from 120 °C to 299 °C. The results showed that compounds in the boiling point range 120–200 °C could be loaded onto the trap at 30 °C by passing 1–2 l of carrier gas through. In order to trap compounds of boiling points approaching 300 °C, a temperature of 50–70 °C and a loading volume of 2.5–3 l was necessary. Using the apparatus, human sweat was analysed to show the degree of concentration possible with a natural sample and the variation in the chromatogram profiles of successive trapping from the same sample. Twelve chromatograms from the sweat of two pairs of identical twins were pattern matched to show the high degree of reproducibility possible using this apparatus to trap biological volatiles.

Introduction

The analysis of airborne volatiles is used extensively in environmental monitoring, the analysis of beverages, food and perfume and the study of chemical signals in biology. All these applications necessitate concentrating small amounts of compounds with a wide range of boiling points and the exercise is technically difficult.

The use of solid adsorbents for the isolation and concentration of the volatile components of biological samples has become well established over the past decade. Commonly used adsorbents are synthetic polymers such as Tenax [1], Chromosorb [2], Poropak [3] and carbon molecular sieves [4] which provide a range of chemical affinities and thermal characteristics. Traps packed with these adsorbents have been used for dynamic headspace sampling in closed loop recirculating systems and in open loading systems [5]. In the former mode, a small volume of air is recirculated over the sample and through the trap by a pump, so extracting and concentrating the volatiles onto the adsorbent. In the latter mode, a continuous stream of air or nitrogen is passed over the sample, through the trap and then discharged to the atmosphere. In each case, trapped volatiles are then introduced onto the gas chromatograph (GC) column, usually by thermal desorption. A somewhat different approach is described by Grob and Zurcher [6], in which volatiles are concentrated in a closed loop system onto a charcoal disc from which they are solvent extracted and injected onto the column for GC analysis [7].

The profiles of gas chromatograms obtained from headspace concentration depend upon the temperature and the capacity and affinity of the absorbent as well as the properties of the separation column. The highly volatile compounds may break through the trap and the low volatiles may never appear in the head space so that the results of analysis are unlikely to reflect the true concentration of the components in a complex mixture. Because of these constraints and small differences in technique, the results of headspace analyses are notoriously variable and difficult to compare from one sample replicate or one laboratory to another [8, 9]. A problem to be confronted, particularly by those studying chemical signalling, is that olfactory receptors are fine-tuned to respond to the biologically significant compounds which may be of lower volatility and present in much lower concentrations than other products [10]. It is therefore desirable that the analyst can eliminate some of the high volatiles in a controlled fashion. Care must be taken to avoid one sample being carried over to the next so all equipment must be

amenable to solvent washing.
Although each of these problems may be solved with existing commercial equipment, this is only possible through a considerable investment of time and money. For example, a typical recirculating head-space trapping system used in the food and flavours industry to analyse trace volatiles, has to run for many hours which is unacceptable for an animal secretion containing the microflora which modify the pheromones under investigation. During such a prolonged trapping period, the pheromones are likely to be degraded and the inevitable accumulation of dominant volatiles will make it difficult to find the trace volatiles unless appropriate selective techniques are employed.
We have designed a robust, inexpensive, recirculating system which is sufficiently versatile to allow whole biological samples to be analysed with minimal risk of degradation and can be readily dismantled and solvent cleaned. An advantage is that the pump generates sufficient pressure to rapidly load and concentrate volatiles into a large capacity Tenax trap minimising break through loss. We have demonstrated the system's versatility and chromatographic reproducibility by analysing a standard solution of esters of as large a range of volatility as one would find in natural products. Esters were chosen because they could be diluted in a single convenient solvent, gave well resolved peaks and are found commonly in fruit extracts [11] and some animal glandular secretions [9, 12, 13]. To show how the apparatus performs in practice, we have analysed human sweat and pattern matched 12 chromatograms from 2 pairs of identical twins.

Experimental

Figure 1 illustrates the dynamic headspace loading system operating in closed mode. By removing the section of tubing A–B, it could be converted to operate in open mode. The tubing within the thermostatically controlled oven (Pye Series 104 GC), was 1/16th inch stainless steel and the rest was heavy duty PTFE (6 mm & 8 mm external and 4 mm & 6 mm internal diameter). The sample chamber was a 60 ml glass flask with a modified Quickfit (24/29) stopper and a glass to stainless steel seal at the base. The pump was of stainless steel with PTFE diaphragm and valves and a maximum output of 7 l per minute, generating a maximum pressure of 2 bars (Capex L2, supplied by Charles Austin Pumps Ltd., 100 Royston Rd., Byfleet, Weybridge, Surrey, UK). In the closed system with 1 trap in the circuit, the actual pump output was of the order of 500 ml/min., the precise flow depending on the resistance of each trap. It was found that a smaller capacity pump could not generate sufficient pressure to operate in the closed mode. The total volume of the system, without an in-line filter, was 120 ml. Although small amounts of solvent could be intermittently pumped through the system for cleaning, it was more efficient to dismantle the components and wash them

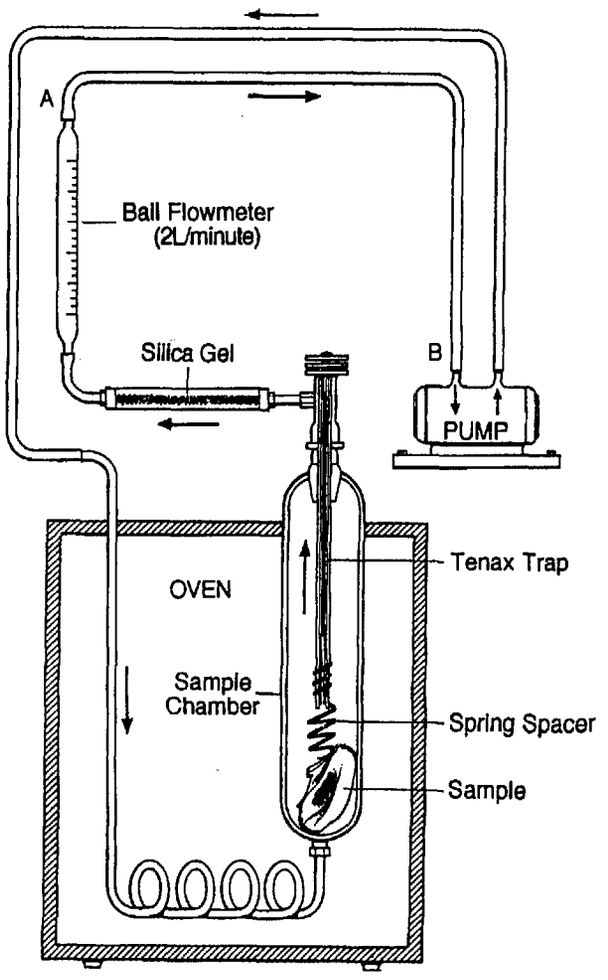

Figure 1
The headspace loading system connected to operate in the closed mode. If the section of tubing A–B was removed it would operate in the open mode.

separately. The pump is easily dismantled and the PTFE diaphragm overlay and valves may be washed with acetone. The reassembled system was blown dry by setting the loading oven temperature to 250 °C and pumping for 30 minutes in the open mode. When running samples contained in fabric etc. or in a small receptacle within the sample chamber, such heat cleaning alone proved sufficient to purge the system between successive samples unless there were very high boiling point volatiles present. An in-line silica gel filter cleaned the system very adequately recirculating in the closed mode for 10 minutes at a temperature of 150 °C and it prevented water condensing in the closed system with aqueous samples.

Each trap contained 86 mg of Tenax GC (60–80 mesh) and was desorbed at 320 C in an appropriately modified GC injector system (Concentrator/Headspace Analysis Injector up-graded System; S.G.E. UK Ltd., 1 Potters' Lane, Kiln Farm, Milton Keynes, MK 11 3LA,

UK) and the sample freeze focussed onto the proximal end of the column using ducted liquid CO_2. The capillary column used was non-polar polymethylsilicone BP1 equivalent, 25 m, 0.32 mm i.d., 0.5 µm film thickness. The carrier gas used was helium at a flow rate of 2 ml/min. and the temperature programme for the GC was 35–100 C @ 5°/min.; 100–300 C @ 12°/min. The distal end of the column was interfaced with a mass spectrometer (Finnigan MAT 5100).

The test mixture used was a 0.05 molar solution in dichloromethane (Fison's Distol grade solvent as supplied for pesticide residue analysis) of the esters shown in Table I.

Unless otherwise stated, 0.1 µl (5×10^{-9} moles) of this solution was placed in the sample container for each trap load and the peak numbers in the figures refer to the esters listed above. Each test was carried out 4–6 times to establish the typical result presented in the figure.

In order to test the performance of the apparatus with a small natural sample, a cotton strip held in the hand for 5 minutes was analysed. Larger sweat samples were collected on cotton squares which were pinned to the armpit part of T-shirts and worn for 8 hours by 2 pairs of identical twins (monozygosity proved by DNA profiling) [14]. Before collecting the samples, the cotton strip and squares had been in an oven at 120 C for 2 hours with a fan circulating the air, to render them chromatographically clean. The sweat analyses were carried out using a 12 m capillary column of medium polarity (BP10; OV1701) in a gas-chromatograph (CG) with a flame ionisation detector (FID). An ATARI ST 1040 stored the output from the FID on disc for the pattern matching [15]. The results of comparing the profile correlations of pairs of chromatograms were subjected to a Student's t-test.

Results and Discussion

Figure 2 shows a chromatogram obtained by injecting 0.1 ml of the test solution directly onto the column. The ratios of the peak areas are close to 1 which reflect the equimolar quantities in the test solution as accurately as would be expected. Peak 7 has the highest boiling point of any compound in the test solution and offers the greatest challenge to accurate head-space analysis, therefore, for the purposes of this report, a ratio of areas of 6 : 7 close to 1 is taken as the target to be achieved.

Table I.

Compound peak (no.)		Molecular Weight	Boiling Point °C
Ethyl butyrate	(1)	116	120
Amyl acetate	(2)	130	149
Butyl butyrate	(3)	144	164–165
Methyl benzoate	(4)	136	198–199
Ethyl benzoate	(5)	150	212
Ethyl caprylate	(6)	172	206–208
Phenyl benzoate	(7)	198	298–299

Figure 3a and 3b demonstrate how much extremes of sampling technique can affect the profile of the chromatogram. The open loading system was used at

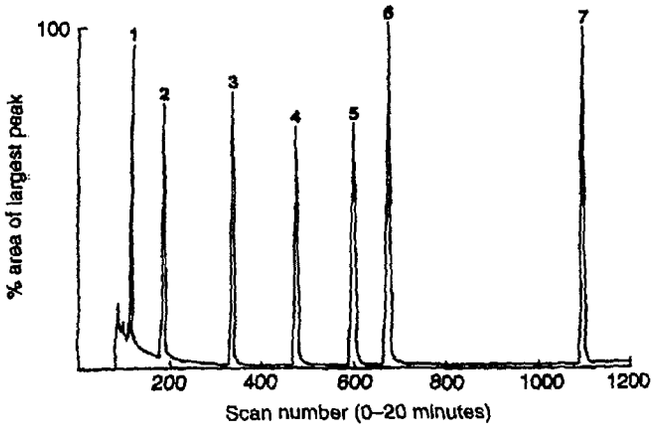

Figure 2

A chromatogram obtained by injecting 0.1 µl of the test solution directly onto the column. The peak numbers in this and all subsequent chromatograms refer to the compounds composing the test solution as designated in the "methods" section. The ratio of the peak areas are: (1) : (2) = 0.9 (2) : (3) = 0.9 (3) : (4) = 1.1 (4) : (5) = 0.8 (5) : (6) = 0.8 (6) : (7) = 1.0

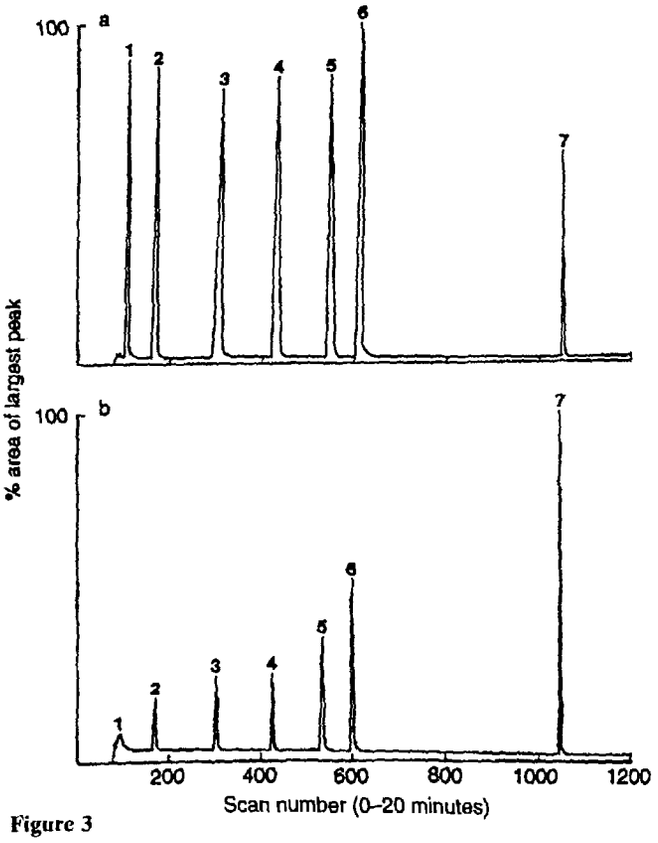

Figure 3

Chromatograms demonstrating how extremes of sampling technique can affect the peak profile of chromatograms.

a) The open mode system was used at 70 °C to load a trap with 250 mls of air passed over 0.1 µl of test solution just introduced into the sample chamber.

b) Another trap was loaded with 750 mls of air passed over the same sample immediately after loading the trap for 3a.

17 °C. Figure 3a shows the result of loading 250 ml of air passed over the sample and onto the trap immediately after putting 0.1 µl of test solution into the sample container and Figure 3b shows the results of subsequently loading a trap with a further 750 ml of air from the same sample. In Figure 3a, a relatively small amount of the high boiling point peak 7 was trapped while in Figure 3b, the lower boiling point compounds have been dissipated and peak 7 is higher than the others. The loss of the low boiling point peaks might have been due to break through because the capacity of the trap had been exceeded. To test this possibility, two traps were joined in series so that any sample breaking through the first trap would be caught in the second. No break through occurred until about 80 times (8 µl) the normal test dose was used. Only a small amount of peaks 1 and 2 appeared in the second trap but the large amount of sample in the first trap over loaded the column causing poor peak resolution.

In all the test analyses, the loading system was operating in the open mode and was dismantled and cleaned between each run.

The effect of different loading temperatures and loading volumes (LV) was investigated by measuring the ratio(R) of peak areas 6 : 7 under four different conditions: a. 30 °C LV = 1500 ml R = 12.4 : 1; b. 50 °C LV = 1200 ml R = 3.4: 1, c. 50 °C LV = 2500 ml R = 1.7 : 1 and d. 70 °C LV = 1250 ml R = 2 : 1. These results show that a higher loading temperature can compensate for a lower loading volume, when analysing high boiling point compounds.

Since it may be contra-indicated to load some samples at a temperature as high as 70 °C, Figure 4 shows the relationship between the loading volume and the area ratio of peaks 6 : 7 at a temperature of 30 °C. The ratio is reduced with increased loading volume but even 2500 ml only achieves a ratio of 5 : 1. The points all lie close to the line indicting a linear relationship between loading volume and peak area ratio and also confirming the good reproducibility of the loading system.

In order to achieve, conveniently, an accurate assessment of high boiling point compounds, it is best to use a higher loading temperature. At 70 °C it was found that a. LV = 125 ml R = 3.2 : 1; b. LV = 250 ml R = 2.3 : 1 and c. LV = 250 ml R = 1 : 1. So even at 70 °C, high loading volumes are required to obtain profiles which accurately represent the concentration of the high boiling point components in the sample.

When water was added to the test mix to simulate an aqueous sample and the system used in the closed mode, condensation occurred in the flow meter unless a silica gel trap was used (see Figure 1). The in line silica gel column cannot be used if any sample break through is likely to occur when the closed mode is being used to achieve sample/trap equilibrium.

Figure 5 shows 3 chromatograms obtained from a sample of human skin volatiles on a strip of clean

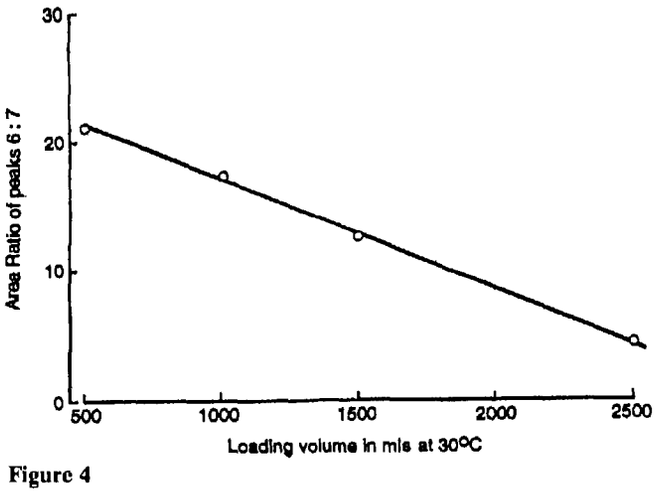

Figure 4

Graph summarising the data from 4 chromatograms to show the relationship between the loading volume and the area ratio of peaks 6 : 7 at a temperature of 30 °C using the system in the open mode.

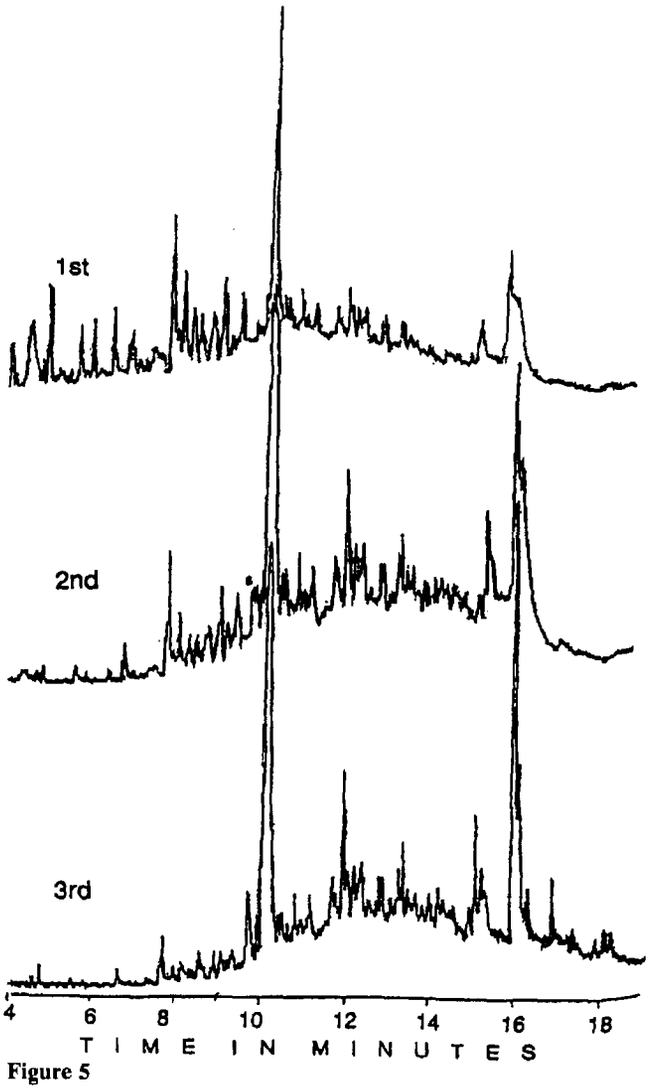

Figure 5

Chromatograms showing 3 successive loads from a single sample of skin volatiles from a cloth held in the hand for 5 minutes. The traps were loaded in the closed mode at 70 °C:- 1st.-2 min. (total recirculated volume = 860 ml); 2nd.-3 min. (1290 ml); 3rd.–5 min. (2150 ml). GC-FID attenuation = 10 × 64.

cotton fabric held in the hand for 5 minutes. It demonstrates that the volatiles in small biological samples can be concentrated to give easily detectable peaks with good replication. The difference between consecutive trappings in the closed recirculating mode are shown, 1st. = 2 min. (860 ml total volume), 2nd. = 3 min. (1290 ml) and 3rd. = 5 min. (2150 ml), with the loading oven set at 70 °C. The attenuation used on the GC was 10 × 64. It will be noted that the low volatiles dominate the chromatogram pattern in the 1st load and the high volatiles are more evident in the 3rd. The large peak on the right has a boiling point of about 160 °C and, although present in all three runs, it is better resolved and higher in the 3rd.

Figure 6 shows 4 chromatograms of a larger sample of underarm sweat from each of 2 pairs of identical twins. Each run is a second load, equivalent to the 2nd chromatogram in Figure 5, and was recorded at a GC attenuation of 1 × 128. It will be noted that the patterns of the twin pairs A_1 & A_2 and B_1 & B_2, are more similar to each other than to members of the other pair. Using the profile coefficient in the ATARI match programme, the higher the number the better the match is, with a perfect match scoring 1 and no match scoring 0. Matching chromatograms within a twin pair [A1/A2 & B1/B2] gave a very significantly ($p < 0.0001$) better match (0.95 ± 0.08 sd) than matching across the pairs [A/B] (0.08 ± 0.28 sd). Before we began to use this technique, chromatographic replication was too erratic to achieve convincing pattern matching [14].

This recirculating system fulfils the conditions for accurate quantification of headspace volatiles set out by Gordon [16]. It is possible to work with small samples so that virtually all the volatiles can be transferred to the trap in order to achieve an accurate picture of the chemical composition. We know that olfactory receptors are selective and do not respond in a linear dose-related fashion but the system described allows a dependable assessment of the volatile composition of a sample and this may then be related to physiological and behavioural data.

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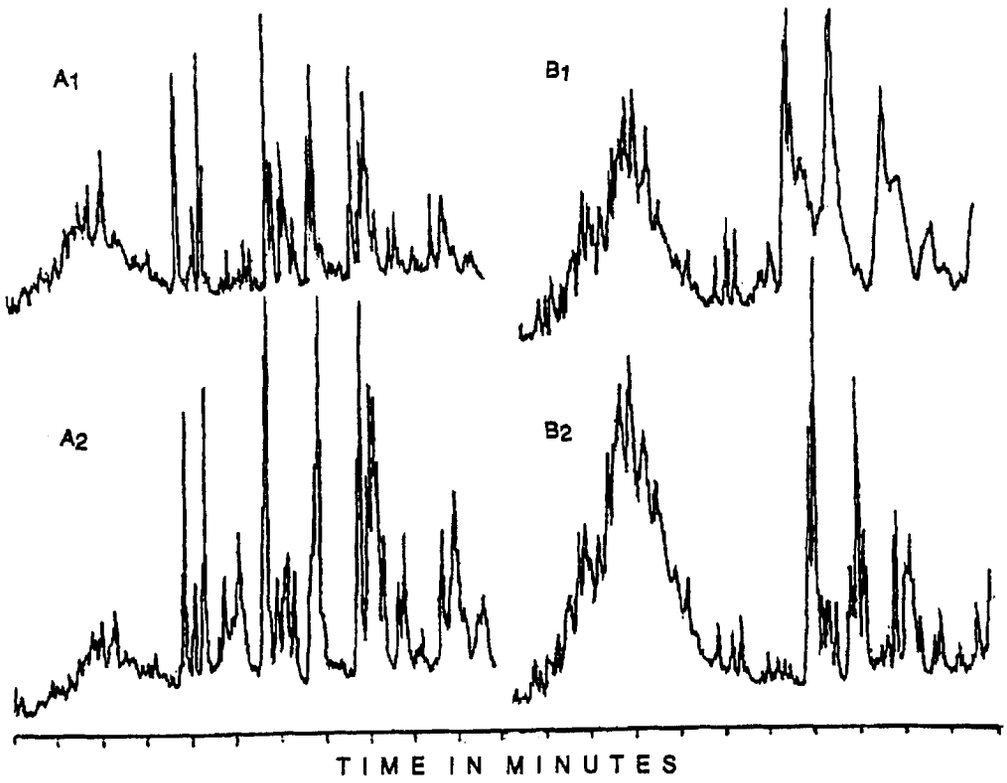

Figure 6

The middle section (70 °C–180 °C) of chromatograms of underarm sweat from each of 2 pairs (A & B) of identical twins. The second trap-load was used (1290 ml recirculated in the closed mode at 70 °C). GC-FID attenuation = 10 × 64.

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