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A system for the quantitative determination of hydrocarbons in human breath

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Abstract: A method for the analysis of hydrocarbons in exhaled human breath samples has been developed and its quantitative performance optimized and exhaustively validated. The method involves preconcentration on a solid adsorbant at 0°C and desorption at 250°C to a packed column gas chromatograph. Calibrations for ethane and pentane are reproducible and linear over the concentration ranges found in human breath samples. The technique is now available for study of conditions, such as cystic fibrosis, in which an oxidative stress component in tissue injury is suspected.

Keywords: *Breath hydrocarbons; ethane; pentane; oxidative stress; gas chromatography.*

Introduction

In the past two decades there has been increased interest in the determination of breath hydrocarbons in clinical toxicology and occupational medicine. The focus has been on measurement of ethane and pentane because these hydrocarbons are final products of the oxidative breakdown of unsaturated lipids [1]. The major advantages of this approach are that it is non-invasive and can be applied to the study of a broad spectrum of clinical conditions. The method depends upon the ability of these gases to cross the alveolar–capillary membrane and partition between the bloodstream and aveolar air, thus allowing the concentration in breath to be related to the concentration in blood. It has been used to detect lipid peroxidation in man [2], the enhancing effect of an intravenous infusion of lipid [3] and hence, as an indirect index of vitamin E status [4]. Also it has been used to assess essential pulmonary damage, through lipid peroxidation, of exercise and exposure to ozone [5].

Despite these potential applications, there are major methodological problems because of the low concentrations of ethane and pentane in the sample matrix and the difficulty in quantitatively delivering the sample to a gas chromatograph. These difficulties have necessitated a preconcentration "trapping" step to improve the sensitivity and precision of the determination. For example, cold trapping utilizes solid absorbants cooled to liquid nitrogen, or solid carbon dioxide temperatures [3, 6, 7]. Ambient temperature trapping systems also have been explored [8]. Unfortunately, descriptions of these methods provide little detail on their performance and the inherent technical difficulties have precluded clinical application. This paper describes an accurate and reproducible higher temperature trapping system.

Experimental

Apparatus and procedure

The design of the apparatus is shown schematically in Fig. 1. The trap is made from 6 cm of 1/4″ o.d., 4.5 mm i.d. stainless steel tube with 1/4″ to 1/16″ Swagelok fittings. The first 3 cm (in the direction of adsorption) is packed with Tenax T.A. 60–80 mesh (Alltech Associates, Lancashire, UK) followed by 3 cm activated charcoal 80–100 mesh.

During the adsorption phase, while the six-port valve is in the 'load' position, the trap is placed in a water–ice mixture at 0°C. In order to desorb the trapped sample the trap is enclosed, as shown in Fig. 2, using a device fabricated from 1/16″ mild steel with an aluminium liner. After closing around the trap the nozzle 'A' is attached to a laboratory heat gun (RS Components, Northamptonshire, UK) which allows a temperature of 250°C to be

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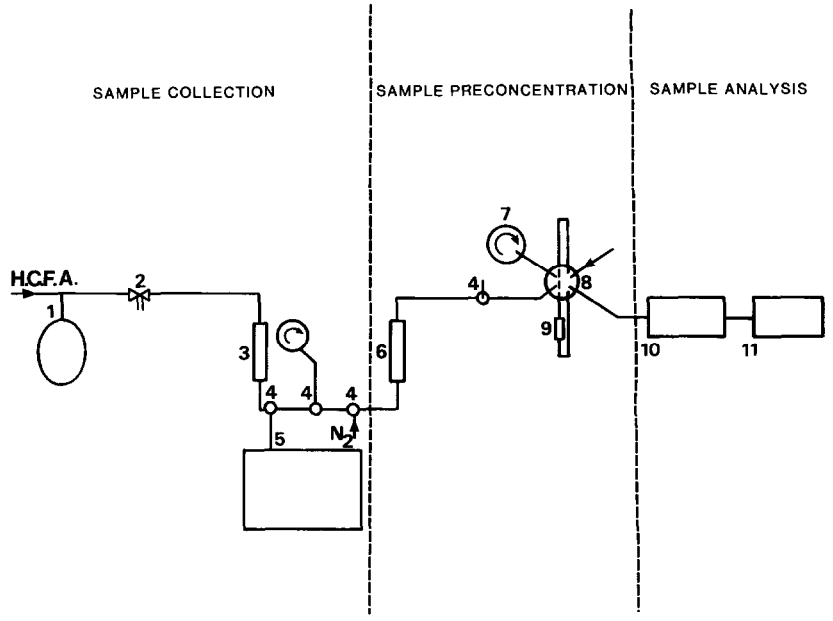

Figure 1
Schematic representation of breath collection and analysis system. (1) Reservoir containing hydrocarbon-free air; (2) Ruben valve (Simonsen & Weel Ltd, Kent, UK); (3) waters canister; (4) three-way stopcocks (Vygon, France); (5) 20 l Tedlar sampling bag (S.K.C. Ltd, Dorset, UK); (6) flowmeter 0–250 ml min^{-1} (Platon); (7) rotary vacuum pump; (8) six-port valve, Valco 6UWP, port diameter 0.030″ (Phase Separations, Clwyd, UK). Arrow indicates carrier gas line in; (9) trap; (10) Varian 3300 gas chromatograph equipped with F.I.D. Column: 5 m × ¼″ o.d., 4.5 mm i.d. glass, packing: Porasil C (80–100 mesh); (11) Varian 4270 Integrator.

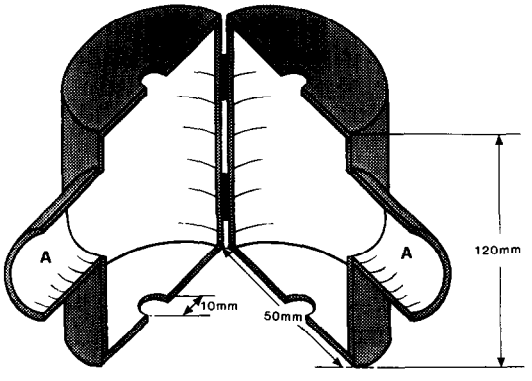

Figure 2
Heat enclosure for trap desorption. Inlet and exit lines are through the 10 mm holes. (A) is attached to heat gun nozzle.

reached in 10 s and afterwards maintained. Temperatures are measured by a Type K thermocouple attached to the bottom fitting of the trap.

Before use the apparatus, including the connecting tubing (translucent P.V.C. 12.20 mm o.d., 9.00 mm i.d.), 3-way stopcocks and gas sampling bag (Tedlar, S.K.C. Ltd, Dorset, UK) are all thoroughly flushed and cleaned with nitrogen, using the vacuum pump and nitrogen admission point located in the sample collection zone.

To prepare hydrocarbon standards for trapped and standard injections, calibrated gas mixtures (Phase Separations Ltd, Clywd, UK) are diluted appropriately with nitrogen, using gas-tight syringes and flasks of calibrated volume. Trapped injections are made by injecting the appropriate standard directly into the trap via a gas-tight syringe attached to the 3-way stopcock in the sample preconcentration area. This injection is followed by an equal volume of nitrogen. The trap is then desorbed in the usual way. Standard injections are made by admitting 5000 ml of hydrocarbon-free air (H.C.F.A., B.O.C. UK Ltd) into the pre-evacuated sampling bag followed by 5 ml of an appropriately diluted standard mixture introduced by syringe through a septum attached to the bag.

1000 ml of sample is pulled through the flow meter, three-way stopcock, six-port valve and onto the trap using vacuum pump 7 (see Fig. 1) at a flowrate of 40 ml min^{-1}. The six-port valve is in "load" position and the trap at 0°C. The trap is then desorbed in the usual way.

When used to sample human breath, the subject, equipped with an air-tight noseclip and disposable mouthpiece, breathes HCFA via an anaesthetic reservoir bag and Ruben valve for 10 min, to wash the lungs free of environmental contaminants. The expirate is vented out of the system. The open tubing is then attached to a Waters cannister containing soda lime and calcium sulphate. Expired air is collected in the Tedlar gas sampling bag for 2 min. As with standards, 1000 ml of breath is adsorbed onto the trap at 0°C at a flow-rate of 40 ml min^{-1}. The trap is desorbed in the usual way.

The gas sample bag is flushed and evacuated five times before and between use. The trap is replaced after 80 samples have passed through it, or recovery values have begun to fall. Before use, new traps are activated by heating at 330°C for 15 h in a stream of nitrogen. Restandardization is carried out each time the trap is replaced.

Chromatographic conditions

The oven temperature was 32°C for 6 min; 70°C at 6°C min^{-1}, held for 12 min; and 130°C at 15°C min^{-1}, held for 15 min. The carrier gas was nitrogen and the flowrate was 30 ml min^{-1}. The F.I.D. detector had attenuation of 8, a range of 11 and a temperature of 190°C. The injector temperature was 180°C.

Standardization

Recovery levels of ethane and pentane are determined using a combination of three types of injection:

(i) direct 25 μl on-column injection of known concentrations of the gases;
(ii) trapped injections, to validate the adsorption/desorption recovery from the trap by peak area comparison with direct injections;
(iii) standard injections, to validate the recovery from the complete system by peak area comparison with trapped injections.

Blank injections are made by sampling equal volumes of HCFA from the bag in the same manner as for standards.

Results

Recoveries from the adsorption/desorption cycle of the trap were validated by comparison of trapped injections of ethane (1.234×10^{-10} moles) and pentane (5.147×10^{-11} moles) in 8 ml nitrogen with direct injection of ethane (1.222×10^{-10} moles) and pentane (5.264×10^{-11} moles) in 20 μl. The recoveries were: ethane 98% ± SD 0.5%, $n = 6$ and pentane 105% ± SD 0.6%, $n = 6$.

The overall recoveries of the breath analysis system were obtained by comparison of standard injections of ethane (8.125×10^{-10} moles) and pentane (4.03×10^{-10} moles) in 1000 ml samples with trapped injections as above. These recoveries were: ethane 106% ± SD 7%, $n = 5$ and pentane 106% ± SD 6%, $n = 5$.

Calibration curves for mixtures of ethane (6.17×10^{-11}–9.75×10^{-10} moles) and pentane (2.57×10^{-11}–4.84×10^{-10} moles) were found to be linear for both trapped and standard injections (Table 1).

The ultimate sensitivity of the technique in its present form was considered in terms of limits of detection. These values, shown in

Table 1
Linearity for standard and trapped injections

Component	Injection format	Intercept	Slope	Correlation coefficient
Ethane	Trapped	-5.36×10^{2}	4.05×10^{13}	0.9956
Ethane	Standard	-6.47×10^{2}	4.45×10^{13}	0.9984
Pentane	Trapped	-8.46×10^{2}	1.47×10^{14}	0.9977
Pentane	Standard	1.45×10^{3}	1.46×10^{14}	0.9989

Table 2
Limits of detection for standard injections

Gas	Mean limit of detection (moles)	Range (moles)
Ethane	8.34×10^{-12}	5.7×10^{-12}–11×10^{-12}
Pentane	6.86×10^{-12}	3.8×10^{-12}–8.9×10^{-12}

Table 2, were regarded as being the amount of each component equivalent to the sum of the intercept of the calibration line and the standard deviation of the cumulative blank peak area.

Figures 3 and 4 show typical chromatograms from a standard and a control sample injection, respectively. The concentrations of the hydrocarbons were calculated using the linear regression of multi-level standard injections expressed in moles. Typical control values are shown in Table 3.

Discussion

It is clear from previous studies that a lack of standard methodology has hampered progress towards a reliable breath analysis system [9]. Problems have often been caused by build-up of contaminants in the apparatus and uncertain or unreliable quantitation. The present study seeks to eliminate chronic contamination by a variety of means, not least by meticulous attention to detail and the use of controlled and standardised operating procedures for the analysis system.

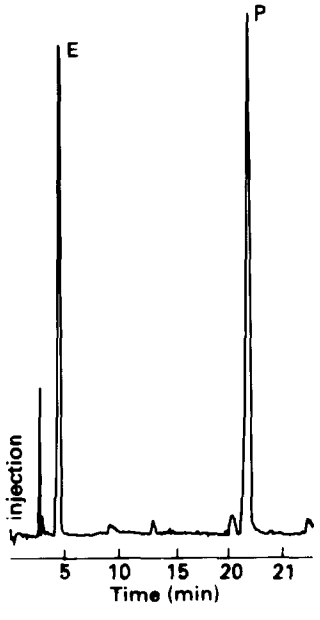

Figure 3
1000 ml standard injection of ethane (E) and pentane (P). Chromatographic conditions as described in the text.

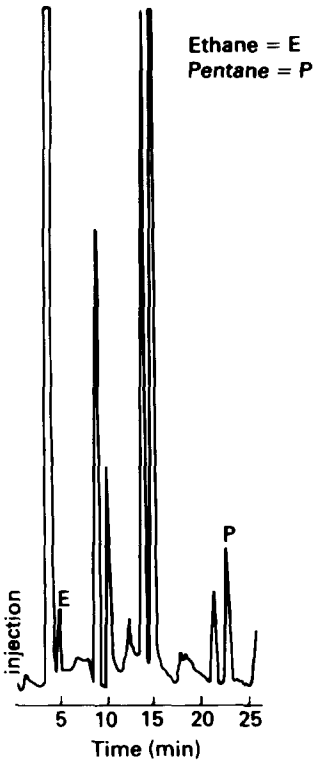

Figure 4
Breath chromatograph from a control (E = ethane, P = pentane). Chromatographic conditions as described in the text.

It has been found that medical grade compressed air (BS 4275) is entirely unsatisfactory for breath analysis studies due to the presence of hydrocarbons. On the other hand, HCFA contains less than 0.1 vpm total hydrocarbons and does not cause noticeable contamination.

In previous studies, lung wash-out periods, required to distinguish between endogenous hydrocarbon production and hydrocarbons

Table 3
Ethane and pentane production in five control subjects

Subject number	Weight (kg^{-1})	Hydrocarbon production (moles kg^{-1} min^{-1}) Ethane	Pentane
1 (M)	65	Below L.O.D.	0.96×10^{-9}
2 (F)	64	1.42×10^{-9}	1.06×10^{-9}
3 (F)	58	2.50×10^{-10}	2.48×10^{-9}
4 (F)	55	6.05×10^{-9}	1.81×10^{-9}
5 (F)	53	4.08×10^{-9}	3.13×10^{-9}

inspired from the atmosphere have varied between 0 [10] and 60–90 min [7]. The present work has shown that a 10 min wash-out is adequate.

In order to avoid leakage, adsorption of sample, memory effects and subsequent ghost peaking, the choice of material for the sample bag is important. Tedlar is ideal for short-term sampling and has the advantage of reasonable robustness.

Control of sample flowrate during adsorption is critical and it has been found that a flowrate of 40 ml min^{-1} is satisfactory for a trap of the dimensions described. As flowrates approach 100 ml min^{-1}, recovery values drop significantly.

Good quantitative performance is well demonstrated by the correlation observed between trapped and standard injections and between trapped and direct injections of standards. Taken together these correlations demonstrate the efficiency and reproducibility of sampling, over the range of hydrocarbon concentrations found in breath.

As the chromatographic peaks are sharp and well-defined (within the constraints of packed column technology), good resolution of the peaks emerging between ethane and pentane permits future identification and quantitation of these intermediate hydrocarbons (Fig. 4).

The technique for measuring hydrocarbons in breath is a dynamic non-invasive one. It combines immediacy with sensitivity and reliability and can be used clinically to study the pathological effects of exogenous and endogenous phenomena. It seems particularly applicable to detecting oxidative stress and monitoring its response to additional pro-oxidant forces and to antioxidant therapy. In the first instance it is hoped to test the method with patients suffering from the congenital disease cystic fibrosis since in this condition, in which the exocrine pancreas is a primary target as in acquired chronic pancreatitis [11], oxidative stress has been clearly documented.

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