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The determination of fluorochlorocarbons in air and body fluids

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A gas chromatographic method for the separation and determination of fluorochlorocarbons (FCC's) is described. Porapak Q is used as the solid phase. The column temperature is 150°. The compounds are detected by means of a tritium foil electron capture detector. The retention times under the conditions used are 1, 2, 4 and 7·5 min for the FCC's 11, 114, 12 and 113 respectively. Concentrations in air are determined directly, the concentrations in body fluids are determined by means of head space analysis. The detection limits for the various FCC's vary between 0·5 and 35 pg. The method is applied in determining FCC concentrations in air and in rat blood *in vivo*.

Since Taylor & Harris (1970) claimed that fluorochlorocarbons (FFC) are toxic to the heart, only few procedures for the determination of these compounds in biological material have been published. Dollery, Draffan & others (1970) and Paterson, Sudlow & Walker (1971) determined FCC's in blood by gas-chromatography. They injected blood samples directly on the column. Recently Shargel & Koss (1972) described a low temperature procedure, which should prevent losses normally occurring when extraction is carried out at room temperature.

We have developed a gas chromatographic method, based upon the head space technique as used by Fink & Morikawa (1970) for volatile anaesthetics and by Van Stekelenburg & De Bruijn (1970) for acetone in blood.

MATERIALS AND METHODS

Materials

FCC 12, FCC 114 and a mixture of the FCC's 11, 12 and 114 (1:2:1 by weight) were kept under pressure in special containers. FCC 11 and FCC 13 were kept at atmospheric pressure in tin cans. Chemical formulae and important physical constants are listed in Table 1.

Table 1. *Some chemical and physical data of FCC's 11, 12, 113 and 114.*

	FCC			
	11	12	113	114
Chemical formula	CCl_3F	CCl_2F_2	$FCl_2C–CClF_2$	$F_2ClC–CClF_2$
Molecular weight	137·4	120·9	187·4	170·9
Boiling point at 1 at (°C)	+ 23.8	−29·8	+ 47·6	+ 3·8
Vapour pressure at 25°C (at)	1·05	6·43	0·43	2·11
Fluid density at 25°C (g cm^{-3})	1·476	1·311	1·565	1·456
Solubility in water at 25°C and at 1 at (w%)	0·11	0·028	0·017	0·013

Animal experiments

A rat under urethane was kept in a Perspex inhalation chamber, breathing clean air or a mixture of air and FCC's flowing through the chamber. The gas mixture was stirred by an electric fan. Before the exposure an extracorporeal shunt was installed from carotid artery to jugular vein. The blood was pumped through the shunt by a roller pump. From this shunt samples were taken by a syringe and injected into the serum bottles for the head space analysis. Full details of the method are described by Wibowo, De Jong & others (1973).

Apparatus

A Varian Aerograph 1440 with a tritium electron capture detector is used. Samples are injected with Hamilton gas syringes of 100 μl. Samples, standards and dilutions are kept in rubber-capped bottles with accurately determined volumes of about 37, 75, 135 and 315 ml.

Sample handling

Air. Concentrations of FCC's in air are determined directly or after dilution of the air sample in a known volume of nitrogen.

Biological fluids. Calibrated bottles are flushed with nitrogen. Liquid samples (0·5 or 1 ml) or standards are injected through the rubber cap after removal of an approximately corresponding volume of gas from the bottle. The contents are then mixed and left to equilibrate for 2 h at room temperature (20°) in the dark or in diffuse daylight. Head space samples are then taken for injection into the gas-chromatograph.

Standards

A calibrated bottle is flushed with nitrogen at atmospheric pressure and room temperature (20°). It is then closed and weighed: let the weight be x g. Through an injection needle an arbitrary quantity of liquid FCC is filled into the bottle (in the case of FCC 12 and FCC 114 under pressure). After evaporation of the FCC and equilibration of the pressure through the needle the latter is removed and the bottle is weighed again: let the weight now be y g. The bottle now contains:

$$(y - x)\,\frac{M_{FCC}}{M_{FCC} - M_{N2}}\ \text{g of FCC}$$

(M is the molecular weight of the compound indicated by the index). The FCC concentration is calculated from weight and volume. From this "preliminary standard", serial dilutions are made to be used as working standards.

Distribution coefficients

The use of the head space technique presupposes knowledge of the gas/liquid distribution coefficients of the compounds to be analysed. They are calculated from the data obtained by the two-samples method adapted from Fink & Morikawa (1970).

From a calibrated bottle with a net volume of approximately 75 ml, filled with nitrogen, 4 ml gas is replaced by 3 ml water or blood and 1 ml vapour containing FCC. After mixing and equilibration—which takes much more time than equilibration in the reverse direction—the FCC concentration in the gas phase is determined (C_{g_1}).

After this, the bottle is opened, flushed briefly with a stream of nitrogen and closed again. After another equilibration, the FCC concentration in the gas phase is determined again (C_{g_2}). If the volumes of the liquid phase and the gas phase are V_v and V_g respectively, the expression for $k = \frac{C_g}{C_v}$ may be deduced easily:

$$k = \frac{V_v}{V_g}\left\{\frac{C_{g_1}}{C_{g_2}} - 1\right\}$$

(C_g = concentration in the gas phase, C_v = concentration in the liquid phase).

In the same way FCC concentrations in samples with unknown distribution coefficients may be determined by a two-step procedure.

Gas chromatography

Column: glass 150 cm, o.d. 0·3 cm, filled with Porapak Q (100–120 mesh). Detector: tritium foil electron capture. Injector temperature: 165°. Oven temperature: 150°. Detector temperature: 150°. Carrier gas: nitrogen purified by molecular sieve 13 X. Flow rate: 25 ml min^{-1}.

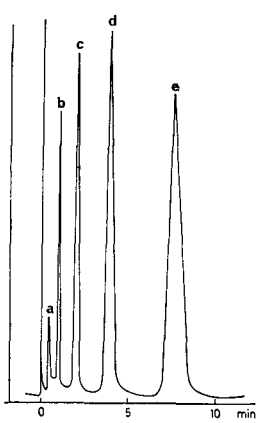

FIG. 1. Gas chromatogram of a mixture of fluorochlorocarbons; (a) oxygen contamination; (b) FCC 12, 0·33 ng; (c) FCC 114, 4·05 ng; (d) FCC 11, 0·06 ng; (e) FCC 113, 1·26 ng.

RESULTS

The separation of the four FCC's is shown in Fig. 1. The first peak is due to contamination with oxygen from the nitrogen used in flushing the bottles. Quantitation is performed by measuring peak height. The relation between quantity and peak height is linear in the measuring range used: attenuation 1×10^{-9} to 8×10^{-9}.

The detector sensitivity is dependent on the structure of the FCC's. This results in wide differences in detection limits. Owing to these differences, determinations in mixtures may have to be repeated at other attenuations to have all peaks in the optimal measuring range. The detection limits are given in Table 2, together with the corresponding concentration in the liquid sample when the gas:liquid volume ratio is 40 and the sample volume is 100 μl.

The retention times of the FCC's are clearly correlated with their boiling points, and do not exceed 10 min. The retention times and the distribution coefficients in plasma are listed in Table 2. The gas:liquid distribution coefficients in rat plasma

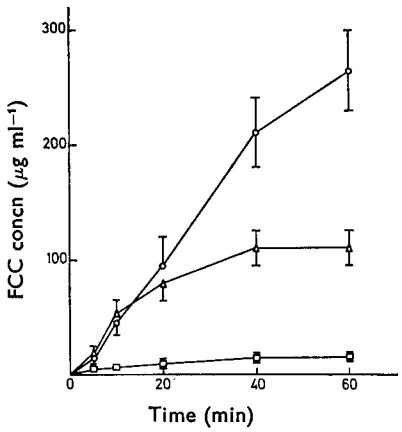

FIG. 2. Increase of FCC concentrations in rat blood during inhalation of a combination of FCC's 11 (○), 12(△) and 114 (□) (weight ratio 1:2:1, mean ± s.d., 6 rats).

are similar to those, found by Morgan, Black & others (1972), using human plasma. Distribution coefficients in blood are much lower than those found in plasma and increase markedly with degree of haemolysis.

No significant adsorption of FCC's to glass or rubber caps has been observed.

Spiked blood samples prepared by equilibrating blood with the respective FCC's and distributing it over sample bottles were used to test the reproducibility of the method. From independent determinations the following results were obtained (mean ± s.d. in μg ml^{-1}): FCC 11: 2·29 ± 0·14 (n = 4); FCC 12: 12·05 ± 0·45 (n = 6); FCC 114: 1·46 ± 002 (n = 4). Our method has been applied in *in vivo* experiments with rats. Fig. 2 shows the time course of FCC concentrations in blood of rats exposed to a 1:2:1 (by weight) mixture of FCC's 11, 12 and 114. Fig. 3 shows the decline of the blood concentration of FCC 11 in two rats after the inhalation has been stopped.

DISCUSSION

Separation of very volatile materials is best made by gas-solid chromatography, as gas-liquid chromatography often does not permit rapid separation without tailing. Porapak Q is excellently suited to this purpose, as shown by Foris & Lehman (1969).

Table 2. *Some analytical data of FCC's 11, 12, 113 and 114.*

	FCC			
	11	12	113	114
Retention time (min)	4·0	1·0	7·5	2·0
Distribution coeff. Nitrogen: water*	0·53 ± 0·05	3·89 ± 0·28	0·96 ± 0·06	4·92 ± 0·38
Distribution coeff. nitrogen: plasma**	0·51 ± 0·03	8·35 ± 0·75	–	4·15 ± 0·31
Detection limit*** (pg)	0·5	3·3	10	35
Detection limit in sample**** (ng ml^{-1} blood)	0·21	1·35	4·20	14·0

* mean ± s.d., room temperature (20°C).

** rat plasma, mean ± s.d., room temperature (20°C).

*** sample 100 μl, attenuation 1 × 10^{-9}.

**** head space sample 100 μl, attenuation 1 × 10^{-9}, gas: liquid ratio 40.

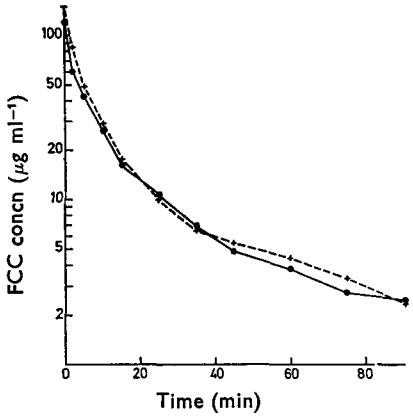

FIG. 3. Decrease of FCC 11 concentration in rat blood after stopping a 20-min inhalation (2 rats).

Under our conditions the FCC peaks were well separated and the retention times were conveniently brief. The separation characteristics of Porapak column material are adversely influenced by loading with water vapour. Therefore direct injection of blood samples has disadvantages, apart from column contamination.

The head space technique was chosen because sample handling is simple and cheap, and prevents losses. While greater precision in manipulation liquid and gas phases would be obtained if gas tight syringes were used (Fink & Morikawa, 1970), their cost precludes their use for large series of samples.

We found that total haemolysis may increase the gas : liquid distribution coefficient several fold in comparison with native blood. This fact seems disturbing, but our procedure with a gas : liquid volume ratio > 30 makes the method insensitive to variations in distribution coefficients, the more so if these have values $> 0{\cdot}5$.

As haemolysis, chemically, is not a very drastic change, other subtle influences might also affect distribution coefficients. Thus samples as small as possible should be taken and haemolysed in the bottle before equilibrating.

Another advantage of the head space method is the purity of the samples. A single column may be used for more than a year and hundreds of samples may be injected without loss of performance. For determinations of FCC's 11, 12 and 114 the head space technique is entirely satisfactory. The volatility of FCC 113, however, is not sufficient for easy application of this technique and here the method Jones, Molloy & Rosen (1972) used for methoxyflurane, may be more suitable.

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