[Chem. Pharm. Bull. 23(6)1363—1367(1975)] UDC 547.32.08 : 543.544

Determination of Perfluorochemicals in Organs and Body Fluids by Gas Chromatography[1)]

Kouichi Yamanouchi, Ryoichiro Murashima, and Kazumasa Yokoyama

Central Research Laboratory, The Green Cross Corporation[2)]

(Received October 7, 1974)

A gas chromatographic method for the quantitative determination of perfluorochemicals (FCs) contained in organs and body fluids was presented. Following destruction of FC emulsion by addition of ethanol, FCs were extracted with 1,1,2-trichlorotrifluoroethane and applied to a gas chromatograph equipped with a hydrogen flame ionization detector. A column (2 m×4 mm i.d.) of 20% Silicone OV-17 on Chromosorb W AW (60—80 mesh) was used at 60°. The retention time of each FC peak was found between 0.5 and 1.0 min. Complete separation of FC from the solvent was achieved and any undesirable, interfering peaks were not detected on the gas chromatogram. The determination of their contents was carried out by calculation of peak height ratio of them to benzotrifluoride added as the internal standard.

Recovery of four kinds of FCs by this method ranged from 94.7 to 102.6% when known amount of respective FCs in emulsified form was added to the organs or blood with the standard deviation of ±3.3%.

The property of perfluorochemicals (FCs) characterized by high oxygen solubility has led us to attempt to use them in an emulsified form as a substitute for erythrocytes. In the course of our studies on the metabolism of FCs in animals, attention has been directed to find a simple and accurate method for the quantitative analysis of the substances in organs and body fluids.

The oxygen flask combustion method has generally been used for the quantitative determination of fluorinated compounds.[3)]

This method, however, was not applicable to determination of the FCs, because-CF_3 group in them could not be completely decomposed by this method due to their stability and volatility,[4)] resulting only unsatisfactory quantitative analysis.

Recently, Holaday, *et al.* have described a gas chromatographic method for the simple quantitative analysis of perfluorobutyltetrahydrofurane.[5)]

This report refers to the gas chromatographic method for determining the FCs in organs and body fluids.

Matrials and Methods

1. Materials——Six FCs were used in this study. The chemical formulae and important physical constants are listed in Table I.

Another reagents used were benzotrifluoride (BTF) as an internal standard for gas chromatography and 1,1,2-trichlorotrifluoroethane (FC-113) as an extracting solvent, which were used after refining by distillation.

2. Preparation of FC Emulsions——The following procedure was performed to prepare the FC emulsions. The mixture of 430 g of an FC and 1000 ml of 4% Pluronic F-68 (Asahi Denka Co.) solution was dispersed

1) A part of this work was presented at the 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, Apr. 1972.
2) Location: *Midorijuji, 3-5-44, Miyakojima-nakadori, Miyakojima-ku, Osaka, 534, Japan.*
3) W. Selig, *Analyst*, **93**, 118 (1968); S. Ohta, *Bunseki Kagaku*, **18**, 1014 (1969); T.S. Light and R.F. Mannion, *Anal. Chem.*, **41**, 107 (1969).
4) B.Z. Senkowski, E.G. Wollish, and E.G.E. Shafer, *Anal. Chem.*, **31**, 1574 (1959).
5) D.A. Holaday and J.H. Modell, *Federation Proc.*, **29**, 684 (1970).

Table I. Some Chemical and Physical Constants of Perfluorochemicals

	Compounds	Molecular formula and weight	Boiling point	Density at 20° (g/ml)	Vapor press. at 37° (mmYg)
FC-43	perfluorotributylamine	$C_{12}F_{27}N$: 671.13	176—177°	1.87	1.14
FMD	perfluoro1-methyldecalin	$C_{11}F_{20}$: 512.12	160—161°	1.95	4.8
FDEA	perfluoro-N,N-diethyl-cyclohexylamine	$C_{10}F_{21}N$: 533.11	148—149°	1.89	8.7
FDC	perfluorodecalin	$C_{10}F_{18}$: 462.11	142—143°	1.93	12.7
FTC	perfluoro-2-isopentylpyran	$C_{10}F_{20}O$: 528.12	143—144°	1.83	9.9
FBA	perfluoro-N,N-dibutyl-methylamine	$C_9F_{21}N$: 521.07	134—136°	1.80	16.0

The abbreviations of the compounds were named by the authors.

with a homomixer at 2000 rpm for 10 min. The resultant coarse dispersion was further emulsified by passing it twelve times through a Manton-Gaulin homogenizer under the 2nd stage pressure of 50 kg/cm² and total pressure of 160 kg/cm². After dissolving sodium chloride to 0.9% (w/v), the emulsion was filtered with a milipore membrane of 0.45 μ pore size.

3. Extraction of FC from the Organs and Body Fluids——The animals used were Wistar strain male rats weighing 140 to 180 g. The rats given 1 g of FC per body weight in the emulsified form from tail vein, were sacrificed at 24 hr after injection and the blood and organs were removed. One hundred mg of the excised organs was homogenized in 3 ml of water in a glass homogenizer; The homogenate was transferred into a test tube and 5 ml of ethanol was added, while 1 ml of blood was transferred into a test tube without such pretreatment, and 3 ml of ethanol was added. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. By the addition of ethanol, emulsion was destroyed.

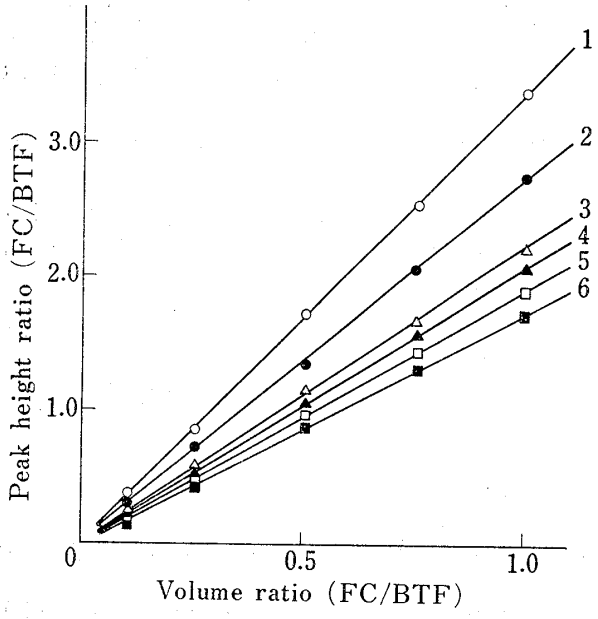

Fig. 1. Calibration Curves of Six Kinds of Perfluorochemical

1: —○—○— FC-43 2: —●—●— FTC 3: —△—△— FDC
4: —▲—▲— FBA 5: —□—□— FDEA 6: —■—■— FMD
FC: perfluorochemical BTF: benzotrifluoride

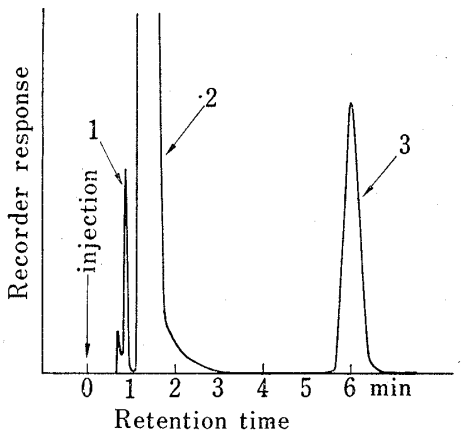

Fig. 2. Typical Gas Chromatogram of Extracted FDC from Rat Liver

1: FDC
2: FC-113 (solvent)
3: BTF (internal standard)
condition: 20% Silicone OV-17, 2 m×4 mm, 60°

The FC was then settled down together with the tissue fragments by centrifugation for 10 min at 3000 rpm. After the centrifugation, about 5 ml of the upper layer was discarded carefully. From 2 to 5 ml of FC-113 was accurately added to the residual layer in the tube. The tube was tightly closed with glass stopper, shaken vigorously and allowed to stand for 5 min in an ice-bath. After centrifugation, the lower FC-113 layer was washed twice with about 5 ml of water in order to remove ethanol from FC-113 completely. After drying FC-113 layer with anhydrous sodium sulfate, 1 ml aliquot was transferred to another test tube and 1 or 2 ml of 1.0% (v/v) BTF solution in FC-113 was accurately added as the internal standard.

Then, 0.2 μl of this analytical sample was injected with Hamilton microsyringe into the gas chromatograph. The chromatography was repeated at least twice. The contents of FC were calculated from the standard curves shown in Fig. 1, which were related to peak height ratio and volume ratio of the FC to the internal standard (BTF).

4. Apparatus——A Shimadzu gas chromatograph GC-4B type equipped with a flame ionization detector was used in this study. A column used was a coiled glass tube, 2 m long and 4 mm i.d. packed with 20% silicone OV-17 on Chromosorb W AW (DMCS), 60—80 mesh (Nishio Kogyo Co., Ltd.). Here the related conditions were as follows: column oven, injection port and detector temperature were 60, 175 and 180°, respectively. Nitrogen gas flow rate was 40 ml per min.

An LKB gas chromatograph-mass spectrometer Type 9000 equipped with a 3 m long and 3 mm i.d. stainless steel column packed with 20% Silicone OV-17 on Chromosorb W AW (DMCS), 60—80 mesh, operated at 70 eV was used for gas chromatographic-mass spectrometric determination.

5. Quantification——The peak height ratios (R) of each FC to BTF were determined by gas chromatography. The average of R was plotted against the volume ratio of them to BTF. A linear relation between the peak height ratio and the volume ratio was observed.

Results and Discussion

The FCs used in this study were detected as the first peak on the chromatogram which were eluted within 0.5 to 1.0 min after injection and excellent separations from FC-113 peak were obtained. Many FCs used were able to analyze characteristically under the same condition. BTF used as an internal standard was detected at 6 min after injection, which was completely separated from the solvent FC-113 on the chromatogram.

Any undesirable peaks such as impurities of FC-113 or volatile substances extracted from organs with FC-113 were not detected on the chromatograms.

A typical gas chromatogram of FDC was shown in Fig. 2. The minor peak found before the main peak of FDC on the chromatogram was the isomer contained in FDC.

To identify the peaks of FDC on the chromatogram, the major peak was analyzed by gas chromatography-mass spectrometry. The mass spectra of the peak of the standard FDC and extracted one from rat liver are shown in Table II. As is evident from Table II, no difference between standard FDC and extracted one was observed. In both mass spectra, the most abundant ion was at m/e 69, corresponding to CF_3^+, and prominent peaks were also seen at m/e 131, 293 and 462, corresponding to $C_3F_5^+$, $C_7F_{11}^+$ and M^+, respectively. The

Table II. Mass Spectra of Standard FDC and Extracted One from Rat Liver

m/e	Abund.		m/e	Abund.	
	Standard[a]	Extracted[b]		Standard[a]	Extracted[b]
31	73	129	205	163	157
50	21	30	219	18	10
69	1000	1000	231	77	49
93	365	303	243	740	798
100	417	601	255	90	105
119	365	337	281	12	7
131	854	873	293	560	693
143	223	145	305	50	50
155	146	133	343	125	127
162	183	154	355	81	84
169	102	180	393	77	141
181	379	218	443	188	112
193	244	309	462	37	110

Numbers represented the relative intensity of mass peaks.
The mass spectra were obtained using an LKB 9000 gas chromatograph-mass spectrometer under the following conditions: column 20% Silicone OV-17 (60°), electron energy 70 eV, ion source temperature 250°, accelerating voltage 3.5 K.V.
a) FDC in FC-113
b) extracted FDC from the liver of rat given the FDC emulsion by our method

identity of these fragments was confirmed by the coincidence of a characteristic abundance pattern for FDC which was demonstrated by Middleditch.[6] The stndard curves of six FCs were shown in Fig. 1. The relations between peak height ratios and volume ratios of them to BTF were linear and extrapolated to zero.

For examining the reproducibility of this method, five determinations were made on the mixtures of each FC and BTF in three different volume ratio. The results are shown in Table III. In each case the coefficient of variation was less than 3%.

Table III. Reproducibility of Peak Height Ratio (R)

Compounds FC/BTF (volume ratio)		R Mean±S.D. (N=5)		C.V.
FC-43	0.5/1	1.73	0.0412	2.38
	1/1	3.40	0.0532	1.56
	1.5/1	5.02	0.0392	0.78
FMD	0.5/1	0.88	0.0248	2.28
	1/1	1.75	0.0151	0.86
	1.5/1	2.61	0.0412	1.58
FDEA	0.5/1	1.01	0.0235	2.33
	1/1	1.98	0.0193	0.98
	1.5/1	2.95	0.0327	1.11
FDC	0.5/1	1.13	0.0284	2.51
	1.1	2.28	0.0443	1.94
	1.5/1	3.35	0.0459	1.37
FTC	0.5/1	1.35	0.0284	2.10
	1/1	2.73	0.0278	1.02
	1.5/1	4.00	0.0588	1.47
FBA	0.5/1	1.04	0.0199	1.91
	1/1	2.11	0.0270	1.28
	1.5/1	3.19	0.0274	0.86

gas chromatographic condition: packing 20% Silicone OV-17, glass tube 2 m×4 mm, column temp. 60°, N_2 40 ml/min FC: perfluorochemical C.V.: coefficient of variation

Table IV. Recovery of Perfluorochemicals Added to Homogenates

Compounds	Organs	Added amount of respective compounds 0.76 mg Found	(Recovery %)	1.52 mg Found	(Recovery %)
FC-43	liver	0.74	(97.4)	1.53	(100.7)
	spleen	0.77	(101.3)	1.51	(99.3)
	blood	0.72	(94.7)	1.55	(102.0)
FMD	liver	0.78	(102.6)	1.45	(95.4)
	spleen	0.73	(96.1)	1.49	(98.0)
	blood	0.73	(96.1)	1.50	(98.7)
FDEA	liver	0.72	(94.7)	1.55	(102.0)
	spleen	0.77	(101.3)	1.51	(99.3)
	blood	0.78	(102.6)	1.55	(102.0)
FDC	liver	0.72	(94.7)	1.48	(97.4)
	spleen	0.73	(96.1)	1.44	(94.7)
	blood	0.72	(94.7)	1.47	(96.7)
	Mean±S.D.		(97.69±3.27)		(98.85±2.53)

FC (0.76 mg or 1.52 mg) in emulsified form was added to the homogenate of the rat organ (0.1 g) or to the heparinized blood (1 ml), and the mixture was treated as described in the experimental section. Values in table indicate the amount of FC in mg recovered from the homogenate and the blood. FC: perfluorochemical

6) B.S. Middleditch, *Anal. Chem.*, **41**, 2092 (1969).

The results indicated that this method was suitable to determine the FCs in organs and biological fluids.

The sensitivity for detection of FCs was little affected on their chemical structure. The minimal detectable concentration of them in biological materials was less than 40 μg/g tissue.

The recovery tests were carried out after addition of 0.76 and 1.52 mg of four kinds of them in emulsified form into liver, spleen and blood. The results of these determination were shown in Table IV and were found to be in a good agreement with added amount. The recovery from the blood and the organs was more than 94.7% and the standard deviation within 3.3%.

The method was applied to biological materials. Six FCs in liver, spleen and blood of rats given the 1 g per kg body weight of respective compounds were determined by this method. The results were shown in Table V.

Table V. Perfluorochemical Contents in Rat Liver, Spleen and Blood

Compounds	FC Contents[a] (N=5)		
	Liver (mg/g)	Spleen (mg/g)	Blood (mg/ml)
FC-43	9.37±1.03	25.41±1.15	5.09±1.37
FMD	8.55±2.01	22.17±1.04	3.35±0.51
FDEA	8.92±1.07	21.24±2.18	3.84±0.98
FDC	4.83±0.57	15.38±1.09	1·89±0.83
FTC	6.30±1.00	16.41±1.12	1.75±0.77
FBA	9.49±1.67	19.62±2.83	3.73±0.56

Wistar strain male rats (140—180 g) given 1 g of FC per kg body weight in the emulsified form from the tail vein were sacrificed at 24 hr after injection and FC contents in liver, spleen and blood were determined by gas chromatography.
a) Values in liver and spleen indicate FC mg/g tissue and those in blood indicate FC mg/ml blood.
FC: perfluorochemical

These results indicated that this method was applicable to the determination of FCs in the biological materials and could be carried out with satisfactory accuracy and reproducibility.