AR226-3397

23/1

1

000017

AR226-3397

E. I. du Pont de Nemours and Company, Inc.
Polymer Products Department

Code No. B 465.5000
Original 3 Apr 81
Page 1 of 22

BLOOD

Determination of Perfluorooctanoic Acid

Gas Chromatographic Method

I. Scope and Applications

This is a method for the determination of perfluorooctanoic acid (C_8) and its salts in blood. Approximately 1 g of sample is required for measurement of concentrations down to 0.01 µg/g.

The method has been evaluated for analysis of human and rat whole blood, but preliminary experiments indicate that it could also be used for serum. A similar procedure can be used for the analysis of aqueous solutions (as from air impingers), and a modified method used for other solutions and solid samples.

II. Principle (See Note 1.)

The water is removed from sample aliquots by freeze-drying (lyophilization) to permit derivatization. Addition of methanolic HCl to the dried residue, along with perfluorodecanoic acid (C_{10}) internal standard, converts the acids, which are not amenable to GC analysis, to more volatile methyl esters. These are extracted from the reaction mixture into hexane solution for GC analysis. An electron capture detector (ECD) is used because of its sensitivity and selectivity, with either a packed or a capillary column.

$$C_7F_{15}COO^-M^+ + CH_3OH \xrightarrow{3\% \text{ HCl}} C_7F_{15}COOCH_3 + MOH$$

$M^+ = NH_4^+, Na^+, H^+$

III. Interferences

As in any GC analysis, compounds with the same retention time as the compound of interest will not be distinguished from it. These can include impurities present in the reagents or other components in the samples themselves.

Interfering peaks from solvents, derivatizing reagent, etc., are not generally observed in this laboratory with the reagents specified below (See Note 2), but each new batch should be checked and a blank included in every analysis. A small peak at the C_8 ester position is found in reagent blanks containing perfluorodecanoic acid internal standard, probably from C_8 present as an impurity in that material. This will give an intercept slightly greater than zero in the calibration plot, but will be a significant contribution to the C_8 peak area only at the lowest concentrations (about 1/3 of the total for 0.01 µg/g C_8, when about 1 µg/g C_{10} is added).

A very small peak at that position has also been found in some "blank blood" samples, as shown in the example in Figure 2a. Whether this represents a trace of C_8 (about 0.001 - 0.005 µg/g) or some other component of the blood would be difficult to determine at this low concentration and with the limited number of samples examined to date.

IV. Sensitivity, Precision, and Accuracy

The method has been used over the concentration range 0.01-150 µg/g C_8. Above 10-20 µg/g, however, interferences with the C_{10} internal standard are generally encountered so that some modification must be made for analyses at higher concentrations (See Note 15). Due to the relatively narrow linear range of the ECD, the calibration curve must always cover the region of interest for quantification.

From the data available at this time, precision is estimated to be ± 10% relative. Recovery of spikes is quantitative within this uncertainty when calculated relative to the C_{10} internal standard, although absolute recovery is affected by the presence of dried blood solids (See Note 3).

Accuracy will also be affected by the choice of C_8 standard (particularly when the packed column analysis is used), which should correspond to the fluorosurfactant composition of the samples as nearly as possible (See Note 4).

V. Apparatus

Instruments and equipment used in this laboratory are specified here; equivalent apparatus can be substituted.

1. Gas Chromatograph and Supplies - For packed column analysis. (See Appendix 1 for capillary column analysis.)

   Hewlett-Packard 5830 GC with EP 18803 Electron Capture Detector, equipped for on-column injection with glass-packed columns

   10 ft x 2 mm id glass columns (HP configuration 5 with ECD adapter), packed with 10% OV-210 on 70/80 mesh Chromosorb W.AW.DMCS. and conditioned at 200°C

   Hamilton 701N 10 µL syringe

2. Lyophilizer

Labconco No. 75352 bench-top freeze dryer (12-port, dry ice cooled)

Labconco No. 75406 (150 mL) or No. 75408 (300 mL) Fast-Freeze flasks, with No. 75476 stainless steel adapters

Vacuum pump with auxiliary cold trap

McLeod gage or electronic vacuum gage reading in the 5-0.005 torr range

3. Thermostated Reaction Block

Pierce No. 18800 Reacti-Therm Heating Module, with Reacti-Block to hold 2-dram vials and thermometer (Block C, No. 18804, has 12 holes, but they must be enlarged to about 19 mm to accommodate the 2-dram vials. Block B, No. 18802, can be used as is, but will hold only 9 vials and provides poorer thermal contact.) Lab-Line Multi-Blok Heaters can be used with the same blocks, and will hold 2-6 in a single unit.

4. Vials and Septum Caps

Wheaton No. 224884 or Pierce No. 13028 2-dram (about 7 mL) screw cap septum vials (borosilicate glass)

Pierce No. 12713 TEFLON-silicone septa and No. 13216 open-top screw caps

5. Pipets and Dispensers

Gilson P200 Pipetman variable volume micropipet for quantitative delivery of 20-200 µL aliquots

1, 2, and 5 mL volumetric pipets for measuring sample and reagent aliquots. (Brinkman Dispensette bottle-top dispensers are a great convenience for repetitive delivery of solvents.)

6. Disposable hypodermic syringes, 1-3 mL, if blood samples are to be taken from closed "Vacutainers".

7. Volumetric flasks, 10, 25, and 50 mL

8. Branson B-220 ultrasonic cleaner (See Note 5.)

9. Analytical balance

10. Common laboratory equipment (including a refrigerator for storage of samples)

VI. Reagents

1. C_8 standards: Perfluoro-n-octanoic Acid (PCR Research Chemicals), FC-143 ammonium perfluorooctanoate (3M), or other material in use in the area to be monitored (See Note 4.)

2. Perfluorodecanoic acid (PCR Research Chemicals)

3. Methanol (Fisher HPLC (A-452) or Fisher Certified ACS (A-412)) (See Note 2.)

4. Derivatization reagent, 3% HCl in methanol, prepared from Applied Science No. 18053 Instant Methanolic HCl Kit, but substituting Fisher HPLC or ACS methanol for the Lipopure methanol provided (See Note 2). The reagent should be stored in the refrigerator, and can be kept for about 1 month.

5. Hexane, Phillips Spectrograde or Applied Science Lipopure (See Note 2.)

6. Water, distilled or deionized

7. "Blank" Blood, to spike for standards. Heparinized "Vacutainer" blood collection tubes (B-D No. 6480) have generally been used for this, as well as for samples. Vacutainers containing liquid EDTA anticoagulant can also be used.

   NOTE: All blood samples must be stored under refrigeration.

8. Perfluorodecanoic acid (C_{10}) standard solution in methanol (See Note 6.)

   Prepare a stock solution and sequential dilutions as follows:

Solution	Preparation	$[C_{10}]$, µg/mL	[F] µg/mL
I	Weigh (to 0.1 mg) about 25 mg perfluorodecanoic acid into a vial. Add 10 mL methanol and shake to dissolve. (Solvent volume can be adjusted as needed to correspond to the actual sample wt.)	2500	1755
II	Dilute 2 mL I to 50 mL in methanol.	100	70.2
III	Dilute 2 mL II to 10 mL in methanol	20.0	14.0

9. Perfluorooctanoic acid (C_8) standard solution in water (See Note 6.)

Perfluorooctanoic acid is only sparingly soluble in water, and dissolves very slowly. At least 1-2 hr shaking is required at this concentration (about 2 mg/mL), and it is best to prepare the stock solution one day in advance of the dilutions. Acid concentration can be checked by titration.

Concentrations will vary, but solutions are needed from which at least four spiked blood standards can be prepared to cover the range of interest. For the example shown in the chromatograms and calculations below (0.01-0.9 µg/mL), a stock solution and sequential dilutions would be prepared as follows:

Solution	Preparation	[C_8], µg/mL	[F], µg/mL
I	Weigh (to 0.1 mg) about 45 mg perfluorooctanoic acid into a vial. Add 20 mL distilled water and shake to dissolve. (Solvent volume can be adjusted as needed.)	2200 (for 44 mg sample)	1514
II	Dilute 3 mL I to 50 mL in H_2O.	132	90.8
III	Dilute 5 mL II to 25 mL in H_2O.	26.4	18.2
IV	Dilute 2 mL II to 50 mL in H_2O.	5.28	3.63
V	Dilute 3 mL IV to 25 mL in H_2O.	0.634	0.436

VII. Operating Conditions

VIII. Safety and Health Precautions

Human blood should be considered potentially infectious, and the Laboratory Guidelines (See Reference 1) for handling, storage, and disposal of samples should be followed.

The 3M Company has found in preliminary studies that C_8 caused birth defects when fed to rats in a laboratory experiment. Female employees of childbearing capability should not engage in any portion of this procedure which offers significant chance for exposure. Although no health problems are known for workers exposed to C_8, it has been found in blood samples and may be very slowly eliminated from the body (See Reference 2). Standard laboratory safety practices for handling toxic, embryotoxic, etc., materials and for corrosives should be used in working with these perfluorinated acids and their solutions.

VIII. Safety and Health Precautions (Cont'd)

The electron capture detector contains a radioactive source (^{63}Ni), and the manufacturer's instructions for safe operation must be observed.

Care should be taken to avoid contact with the low-temperature baths used for freeze-drying.

IX. Procedure

A. Preparation of Blood Samples and Spiked Standards for Analysis (See Note 7.)

All blood samples should be well shaken before taking aliquots to insure a uniform distribution of cells and plasma.

1. Spiked standards

For each standard to be prepared, and for one or more blanks, pipet a 1 mL aliquot of "blank" blood into a 2-dram vial. Spike each with C_8 standard solution to give the range of concentrations required, as shown in the following example:

Spike	$[C_8]$, µg/mL blood	[F], µg/mL blood
33 µL 26.4 µg/mL C_8 in water	0.871	0.600
110 µL 5.28 µg/mL C_8 in water	0.581	0.400
69 "	0.364	0.251
41 "	0.216	0.149
110 µL 0.634 µg/mL C_8 in water	0.0697	0.0480
16 "	0.010	0.0070
Blank (no spike)	0	0

Mix gently, keeping the solution in the bottom of the vial, and sonicate for about 2 min for additional mixing (See Note 5). Store in the refrigerator until needed (See Note 8).

2. Samples

If the sample volume is sufficient, prepare in duplicate.

For each sample, pipet a 1 mL aliquot into a tared 2-dram vial and weigh to 0.1 mg. (Aliquots can also be taken with a disposable syringe, if it is felt preferable not to open the containers).

B. Freeze-Drying

1. A schematic diagram of the freeze-drying apparatus is shown in Figure 1. Each of the 12 ports has a separate valve so that flasks can be attached or removed while the system is under vacuum. The apparatus should be pumped down to an acceptable vacuum (See Note 9) and the cold traps filled before the drying flasks are attached.

2. Freeze the samples before placing them under vacuum to prevent bumping. To reduce the chance of contamination or sample loss, cover each vial with a light filter beforehand; a small piece of "Kimwipe" held in place with a rubber band works well. Freeze the solution in each (See Note 10) and keep the vials at dry ice temperature until all of them are ready.

3. Place up to four vials in each drying flask and attach to the drying chamber. The pressure should drop below 0.5 torr again within a few minutes if no leaks are present, and the samples should remain frozen as sublimation takes place. Allow them to dry for about 4 hr (See Note 11).

C. Derivatization

1. To the dried residue in each vial, add 2 mL methanolic HCl and 50 µL C_{10} standard solution (about 20 µg/mL in methanol). Prepare a reagent blank similarly, using an empty vial. Close with a septum screw cap and shake well. Sonicate for about 2 min (See Note 5) and shake again.

2. Thermostat for 1 hr at 65°C, then allow the vials to cool to room temperature and stand overnight (See Note 12).

3. Add 2 mL each of hexane and distilled water and shake for about 2 min.

4. After the phases separate, the upper hexane layer can be sampled directly for GC analysis (See Note 13). The solutions are stable in this form for at least several days, although hexane will gradually evaporate after the septum has been pierced.

D. GC Analysis - Packed Column. See Appendix I for capillary column analysis.

1. GC Conditions - Instrument and column as shown in V, Apparatus.

Temperatures:	Injection port	200°C
	Detector	325°C
	Column oven	100°C, isothermal

Carrier gas: 90% argon/10% methane (or nitrogen if recommended for the instrument) flow about 30 mL/min

Injection Volume: 2 µL

Sensitivity: Attenuation as needed to keep peak height measurable, 2^5-2^{13} on HP 5830.

Run time: 11-25 min, depending on sensitivity (See Note 14.)

After installing the column and establishing the conditions above, check the ECD base frequency and noise level according to the manufacturer's instructions, to insure that they are stable and within acceptable limits.

2. Run each solution twice (or run duplicates once each), bracketing samples with standards of similar concentration in the second series of injections.

   Representative chromatograms are shown in Figure 2 for blanks, standards and samples. Measure either peak height or peak area.

   As noted above, the calibration curve must cover the concentration range of the samples. If some samples are found to fall above the range of prepared standards, the hexane solution can be diluted with that from a blank (e.g., 100 µL + 400 µL for 5X dilution) to reduce the C_8 concentration without changing that of the internal standard. This type of dilution can also be used if peaks interfering with the internal standard are observed at high C_8 concentrations in either sample or standard preparations (See Note 15).

X. Calculations (See Note 16.)

1. Normalized C_8 Peak Values

A tabulation of peak heights for analysis of a series of standards (prepared as above) and samples as shown in Figure 3a.

To correct for any variations in injection volume, reagent volumes, etc., the raw C_8 peak heights (or areas) are first normalized relative to the internal standard average:

$$C_8 \text{ (corrected)} = C_8 \times \frac{C_{10} \text{ (average)}}{C_{10}}$$

Calculation of the C_{10} average also provides a measure of the precision in sample preparation and GC analysis.

2. Calibration Plot

A calibration curve obtained by plotting corrected C_8 peak height vs concentration of the spiked standards is shown in Figure 3b.

In this case, straight lines can be fitted to sets of points at higher (0.61-0.15 µg/mL) and lower (0.15-0 µg/mL) concentrations, and the slopes and intercepts calculated by linear least squares analysis.

In some plots no really linear region may be found, and a smooth curve should be drawn through the data points.

3. Calculation of Sample Concentrations

Using the corrected C_8 peak heights (or areas) for the samples, corresponding values of µg F/sample can be calculated from the LLS equations (as in Figure 3) or can be read directly from the calibration curve.

Divide each value by the sample weight (in grams) to obtain g F/g blood, and average the results for duplicate runs or duplicate preparations. Report as µg F/g blood (See Note 6).

XI. Calibration (See Under X, Calculations.)

XII. Notes

1. Initial method development was based on a procedure for determination of perfluorooctanoic acid in serum or plasma recently published by 3M (See Reference 3). In that method, C_8 is extracted from acidified serum into hexane/ether, the solution concentrated by evaporation, and diazomethane added for derivatization. With the lyophilization procedure described here, sample handling, time, and material required are reduced, and the use of diazomethane is eliminated. However, concentration of C_8 from a large volume of sample as in an extraction, is not possible. A sample preparation procedure similar to that in the 3M method has been used for aqueous solutions in this laboratory and can be followed by the same methanolic HCl derivatization and GC analysis as lyophilization. The extraction has not been tested with whole blood in either method, however.

GC analysis was initially done with a packed column (as in the 3M method but with a different stationary phase). A capillary column has also been used recently for better resolution of the branched C_8 isomers, and modifications for that analysis are given in Appendix I.

2. The solvents specified in Section VI were found preferable to several tried for this analysis, but others may also be satisfactory. Even with the same reagents, the appearance of the blank may depend on the condition of the detector. With the ECD used here, contamination results in a significant decrease in selectivity and increased reagent interference.

3. Recovery was determined by spiking blood, water, and methanol solutions at equivalent concentrations, obtaining the response factor for C_8 relative to C_{10} in GC analysis of the water and methanol spikes, and using this factor to calculate the C_8 concentration in the blood spikes. Average recovery (C_8 found/added) was 100 ± 5% for 18 samples of human blood spiked with 0.014-46 µg F/mL (FC-143 or perfluoro-n-octanoic acid) and for 7 samples of rat blood spiked with 0.007-67 µg F/mL. Absolute C_8 and C_{10} peak heights were about 15-20% lower in the presence of dried solids from 1 g blood, however, so that a corresponding error would result from external standard calibration against aqueous solutions.

   The question of whether recovery of spikes provides an accurate measure of recovery of the same material from samples should also be considered, particularly with a complicated substrate such as blood. Agreement between the results of this analysis and those of Wickbold Torch analysis for organic fluorine in a series of test samples seems to indicate that the spikes are a reasonable model for samples.

4. The FC-143 samples examined to date all appear to be a mixture of about 70-75% straight-chain material with several branched isomers present at about 1-10% (determined by F-19 NMR, capillary GC, and GC/MS). In contrast, commercial perfluoro-n-octanoic acid (PCR) contains about 95% straight chain C_8 with about 4% $(CF_3)_2CF(CF_2)_4COOH$ and traces of the other isomers. As shown in the chromatograms in Figures 2c and 2d, in the packed column GC analysis, only one of these is really separated from the main peak (small peak at 3.7 min) while others are visible as a shoulder following it. Chromatograms of samples and standards obtained using the capillary column analysis are shown in Figure 4 (Appendix I); under those conditions, at least partial resolution of four isomers from the main peak can be obtained.

   Since ECD response appears to be higher for the isomers than for the straight-chain ester (by a factor of 2 or more), the total area/concentration ratio also becomes higher as their percentage increases. In the packed column analysis, the response factor calculated for C_8 relative to C_{10} is about 30-40% higher for FC-143 standards than for perfluoro-n-octanoic acid standards, so that it becomes very important to use the one most similar to the samples to be analyzed. Although small variations in isomer distribution can be seen in human blood samples, all of those examined to date resemble perfluoro-n-octanoic acid much more than FC-143, and it has been an appropriate standard

XII. Notes (Cont'd)

4. (Cont'd)

for quantification within the uncertainty of the method. In a series of rat blood samples, however, considerable variation in distribution was observed. As seen from the chromatograms in Figure 4, neither standard would have been a good approximation to some samples, and analyses were done with the capillary column.

5. Gentle sonication (with the vials immersed about half-way in the water bath of a sonic cleaner) has been used to aid in mixing of spikes and in promoting contact of derivatizing reagent with the dried blood solids. Additional experiments are planned to determine whether this step is necessary or can be eliminated.

6. Since results that are to be compared with nonspecific analyses for total organic fluorine must be expressed as fluorine rather than as C_8, both concentrations are given for the standard solutions. Values for percent fluorine used in the conversion are: perfluorooctanoic acid, 68.82%, ammonium perfluorooctanoate, 66.10%, perfluorodecanoic acid, 70.22%.

7. An aliquot volume of 1 mL is generally used for both samples and standards, but it can be reduced to 0.5 mL when concentrations are expected to be relatively high.

Coagulation is generally a problem with rat blood samples kept for more than a week or two before analysis. When it is no longer a homogeneous liquid, the entire sample must be lyophilized, mixed well, and portions of the dried powder weighed out for analysis. The exact ratio of dry to liquid weight must be determined for each sample, but is about 0.2, so that 0.2 g powder will be comparable to 1 mL liquid. This procedure greatly increases the time required for sample handling and the probability for error in the weight, and it should be avoided if possible.

8. Spikes prepared for method development have been allowed to stand at least overnight before analysis, but a period of a few hours appears to give equivalent results.

9. For this freeze-drying application, pressures less than 0.5 torr seem to be satisfactory, although 0.025-0.05 torr may be achieved. Readings will also depend on the type of gage used, since the McLeod gage does not read the pressure of water vapor as electronic gages do.

10. A small dry ice/acetone bath or the center cold well of the drying chamber can be used for rapid freezing of the solutions in the bottom of the vials. As noted in Section VIII, care must be taken to avoid contact with the cold bath.

11. A 4-hr drying time has been adopted for preparation of blood samples by comparison with 1 mL water samples, where drying can be monitored by disappearance of the ice. Experiments with spiked blood samples indicate that longer drying times (8-15 hr) can also be used without loss of C_8.

12. Derivatization mixtures from aqueous samples can be partitioned with hexane/water immediately after cooling. In the presence of dried blood solids, however, additional time for equilibration of the C8 and C_{10} esters seems to be necessary. After standing overnight, peak areas for both C_8 and C_{10} are lower than in reaction mixtures prepared from aqueous or methanolic standards, but the relative response factors are equivalent (See Note 3). Preliminary experiments indicate that it might be possible to eliminate the overnight equilibration by spiking with aqueous C_{10} before lyophilization, rather than with methanolic C_{10} after.

13. Although a clear hexane layer is obtained after standing for about 1/4-1/2 hr, the solution has a relatively dark rust color and undoubtedly contains nonvolatile or high-boiling impurities. In the packed column analysis, these are injected onto the column head; and at the end of the day, the oven should be programmed to 180°C and held for several hours to reduce build-up. No deterioration of performance was observed in a column used for several weeks of analyses, but it may prove preferable to adopt flash vaporization with a glass injection port liner which can be changed daily, as in the capillary column analysis.

14. As seen in the chromatograms in Figure 2, some small, broad peaks can appear late in the run. These appear to come from the C_{10} standard and might be eliminated in purified material; others seem to be characteristic of older blood samples. At the high sensitivity used for low C_8 concentrations, these can interfere with the following run and must be allowed to elute before the next injection.

15. Several minor components are present in both the perfluoro-n-octanoic acid and FC-143 standards which elute near the C_{10} internal standard under these GC conditions and can interfere with quantification. Similar peaks are also often observed in samples at higher concentrations. With the C_{10} concentration generally added (about 1 µg/g), this seems to occur above about 20 ppm C_8 in the capillary column analysis and about 10 µg/g with the packed column. In some cases, an obvious shoulder can be observed on the C_{10} peak, varying in size with the C_8 concentration. In others, an unresolved peak may be indicated by an unusual change in C_{10} peak size, but is only seen if a sample is prepared without added internal standard. Depending on the type of analysis to be done, various techniques can be used for quantification under these conditions.

(a) Standards can be prepared only up to about 10 µg/g, and any samples found to be above this range diluted and run again, as described in IX, Procedure, Part D.

(b) If only relatively high concentrations are of interest, the C_{10} concentration can be increased and/or the sample size decreased so that the interference becomes negligible.

(c) With the increased resolution of the capillary column, components of the perfluoro-n-octanoic acid standard can be resolved from the C_{10} and the problem eliminated for standards. Samples must still be examined for interference, however.

(d) The calibration curve can be used for an external standard calculation, with uncorrected C_8 peak areas or heights. While good reproducibility is possible with care in measuring the injection and reagent volumes, it is obviously more critical here than in an internal standard calculation and must be checked regularly at each point.

16. From the calibration plot in Figure 3, it can be seen that the ECD response is not linear with concentration over the entire range. Linear plots may be obtained in some cases, but they still have concentration-dependent response factors. The calculations described here thus make use of a calibration curve, in which the internal standard is used to normalize raw peak values before plotting. For an ECD which shows a more linear response and constant relative response factors, calculations could be done by a general internal standard procedure instead. (A calibration plot of this sort has been observed with a Varian 3700 GC under similar conditions).

XIII. References

1. "Experimental Station Rules and Procedures for Human and Other Primate Blood and Blood Products", 3/80, Safety Office, Experimental Station.

2. F. A. Uoel, S. D. Sorenson, and D. E. Roach, "Health Status of Plant Workers Exposed to Fluorochemicals - A Preliminary Report" Am. Ind. Hyg. Assoc. J. 41, 584 (1980).

3. J. Belisle and D. F. Hagen, "A Method for Determination of Perfluoro-octanoic Acid in Blood and other Biological Samples", Anal. Biochem. 101, 369 (1980).

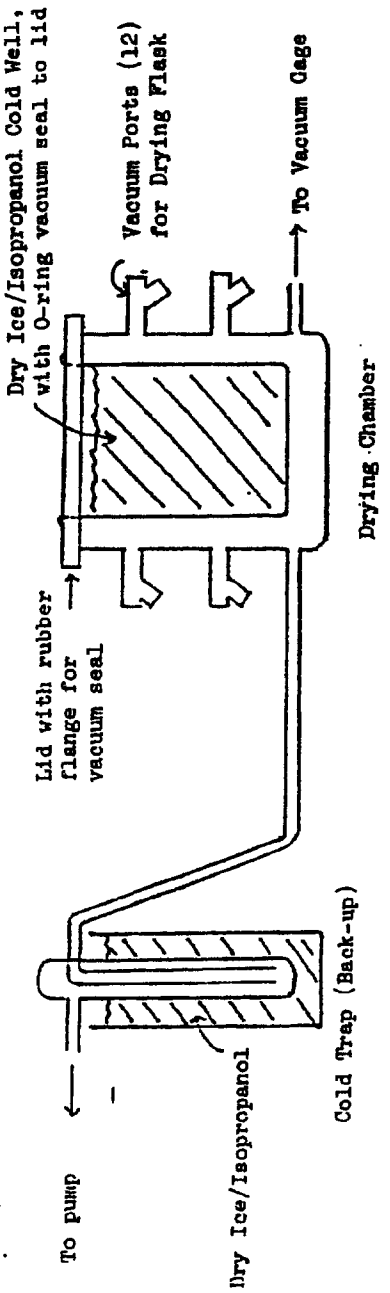
[4. [redacted]]

Origin: Experimental Station
Prepared by: S. S. Stafford Approved by: L. J. Papa
Approved by Laboratory Methods Committee, 9 Nov 81

ES-567

FIGURE 1 - APPARATUS FOR FREEZE-DRYING SAMPLES

(a) Drying Chamber and Vacuum Line



(b) Vacuum Port, Drying Flask, and Samples

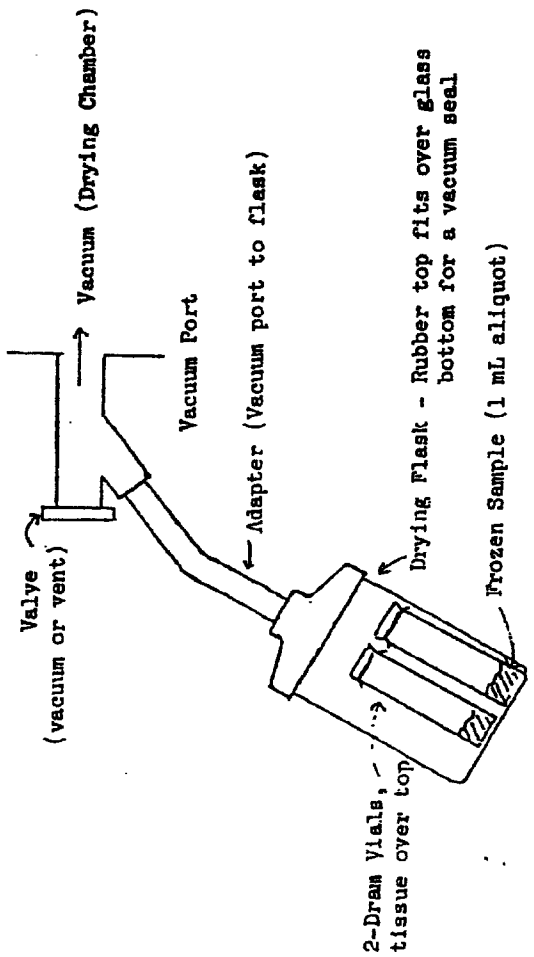
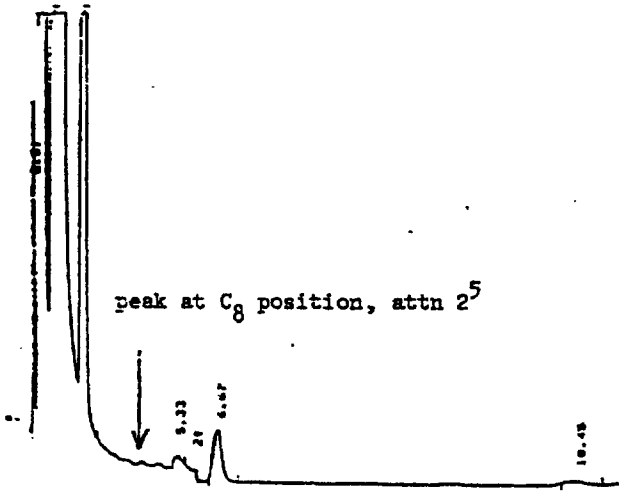


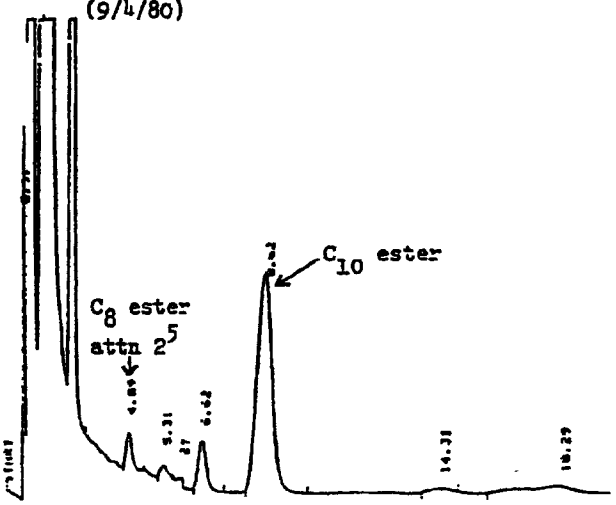
FIGURE 2 - CHROMATOGRAMS FOR C8 DETERMINATION, PACKED COLUMN ANALYSIS

GC conditions as on p. 8. Attenuation as noted for 0-6 min, 2^7 after 6 minutes. Chart speed 1 cm/min. Retention time (minutes) indicated by each peak.

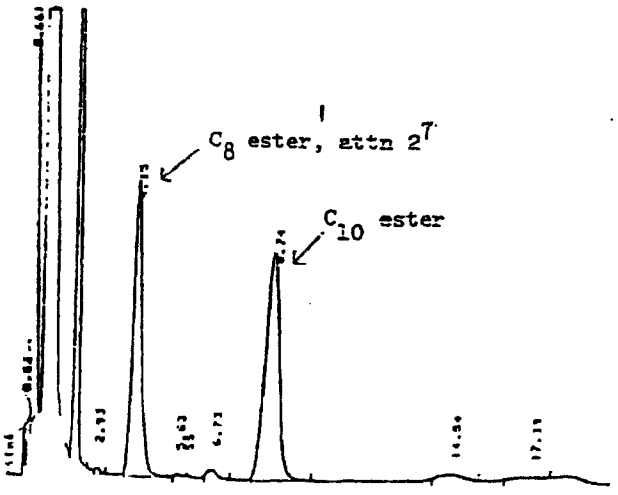
(a) Blank blood, no C_8 spike or C_{10} standard added (9/4/80)



(b) Standard, blood spiked with 0.01 µg/mL perfluoro-n-octanoic acid (0.007 µg F/mL) (9/4/80)



(c) Standard, blood spiked with 0.9 µg/mL perfluoro-n-octanoic acid (0.6 µg F/mL) (9/10/80)



(d) Sample E22514-28-1 (9/10/80)

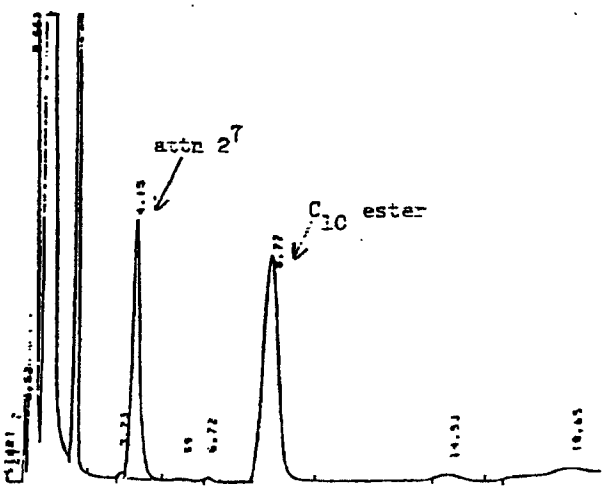


FIGURE 3 - CALCULATION OF CORRECTED PEAK HEIGHTS AND CALIBRATION PLOT

(a) Peak height values for runs of seven standard and five sample solutions (duplicate runs were made, and shown on the calibration plot, but only one is included in the table).

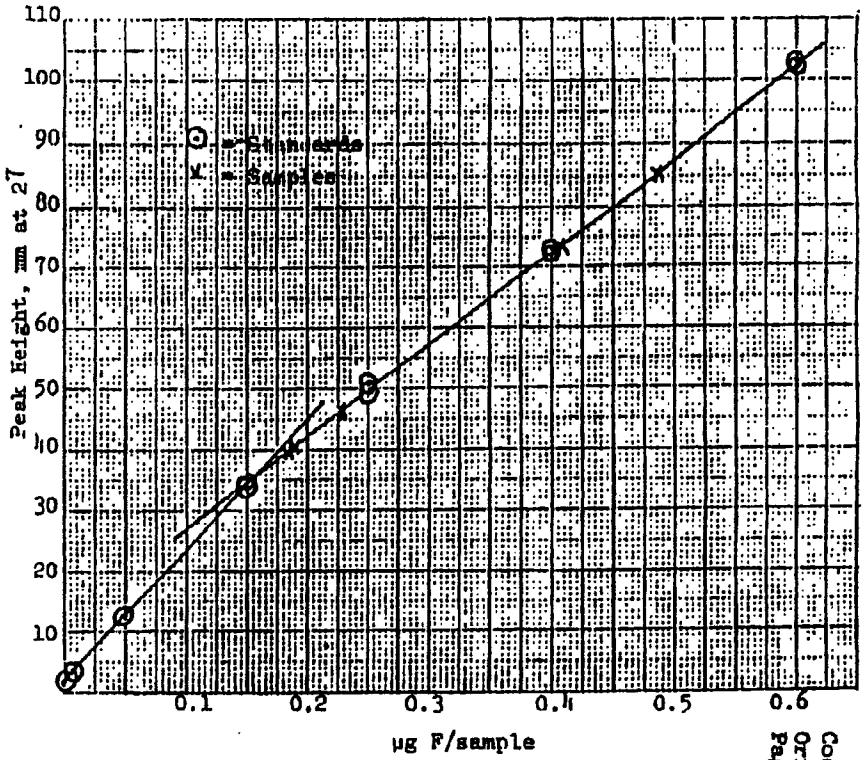
Solution	[C_8], µgF/sample	Run No.	Peak Height, mm at attn 2^7		
			Raw C_8	Raw C_{10}	Corrected $C_8 \times \frac{79}{C_{10}}$
	Added				
Standards	0.601	8	99	76	102
	0.399	6	71.1	78.1	71.9
	0.250	10	49.9	77.5	50.9
	0.149	12	34.1	79.9	33.7
	0.0480	23	12.6	78.2	12.7
	0.0070	4	3.80	81.7	3.67
	Blank + C_{10}	3	1.95	80	1.93
				Avg. (11 values) = 79 ± 3	
	Calculated*				
Samples	0.491	9	87.8	80.8	85.8
	0.409	7	72.5	78.5	73.5
	0.230	5	48.3	82	46.5
	0.191	11	36.5	70.8	40.7
	0.186	20	38.1	75.5	39.9

*Sample concentrations calculated from linear least squares fit,

0.6-0.15 ppm standards (8 points): H = 11.98 + 150.3 [F]

0.15-0 ppm standards (5 points) : H = 2.13 + 214.4 [F]

(b) Calibration plot, corrected C_8 peak height vs. concentration (expressed as fluorine)



APPENDIX I

Modifications for Capillary GC Analysis

As discussed in the method, a capillary column can be substituted for the original packed column in analyses where separation of the C_8 isomers is necessary for quantification, or where determination of the isomer distribution is of interest. Chromatograms obtained with the column and conditions currently in use are shown in Figure 4. A wide-bore WCOT is used to permit large injections (1-3 µL, as with the packed column) for detection of low concentrations although the efficiency is probably less than that of a lower capacity column. The major isomer peaks are generally resolved well enough for quantification, however, or at least to indicate whether a significant peak is present (as in Figures 4c and d). Comparison of these chromatograms with those in Figure 2 shows that the analysis time is presently longer than with the packed column; this may be reduced by further column modifications.

Preparation of samples and standards is the same for both analyses. Modifications needed in apparatus, GC conditions, and calculations for the capillary analysis are as follows:

1. Apparatus (V)

   HP5830 GC with HP18803 Electron Capture Detector, equipped for splitless injection with glass capillary columns. A flame ionization detector should also be available on this or another capillary instrument.

   Glass injection port liners (inserts) for use with the capillary system in splitless mode.

   Silanized glass wool for packing injection port liners.

   Glass WCOT column, 50 m x 0.5 mm, OV-210 (high load). (The column currently in use is a custom-coated column from Alltech Associates. It can also be specially ordered from Quadrex or Chrompack, but is not presently available as a stock column.)

2. GC Analysis (IX, Procedure, Part D)

   Instrument and column as above; straighten the column ends, recoat them with FFAP in CH_2Cl_2, and install the column according to the manufacturer's instructions.

   Before inserting the glass injection port liner, pack it loosely with about 1 cm silanized glass wool. This should be positioned just below the point of injection, to prevent nonvolatile sample components from reaching the column.

GC Conditions:

Temperatures:	Injection port	200°C
	Detector	325°C
	Column oven programs	(a) 15 min 55°C, 8°/min to 130°C, 5 min 130°C
		(b) 10 min 60°C, 2°/min to 76°C then 6°/min to 120°C, 6 min 120°C

Carrier and make-up gas: 90% argon/10% methane
column pressure 3 psig, about 2.7 mL/min flow at 100°C
make-up about 26 mL/min (total flow about 30 mL/min)
splitter about 100 mL/min

Injection volume: 2 µL, splitless injection with 60 sec hold before opening splitter vent

Sensitivity: Attenuation as needed to keep peak height measurable 2^5-2^{13} on HP5830

Run time: About 30 min (depending on program) plus cool-down.

Temperature program and column flow rate will vary with the condition of the column, instrument configuration, carrier gas, etc., and must be chosen and modified as needed to maintain adequate resolution. Column oven program (a) was used for the chromatograms in Figure 4; program (b) and similar ones have also been used.

After installing the column and establishing the conditions above, check the ECD base frequency and noise level according to the manufacturer's instructions to insure that they are stable and within acceptable limits. Base frequency should also be checked before each day's operation.

Maintenance of a clean system is especially important for temperature-programmed ECD analyses at high sensitivity. A clean injection port liner and new septum (low-bleed) should be installed at the end of each day, and the column oven temperature raised to 130°C (or the maximum program temperature) overnight and when the instrument is not in use.

3. Calculations (X)

The calculations are done in two parts, first determining the concentration of the main component, then adding a percentage correction for the secondary isomers.

(a) Concentration of perfluoro-n-octanoate

Calculation of normalized C_8 peak values, preparation of the calibration curve, and use of it and the sample weights to calculate µg/g for each

sample are done as in the original method. Since only the area of the main peak is used, however, the concentrations of the standards must first be corrected to correspond to it rather than to the total C_8 concentration. For the perfluoro-n-octanoic acid standard used in this laboratory, the concentration of straight-chain acid is taken to be 95% of the total (See Note 4 above).

For some samples, such as the one shown in Figure 4d, the isomer peak just ahead of the main peak may not be large enough to be resolved but still makes a significant contribution to the total. Peak height rather than area can be used to minimize the error in quantification of the main peak.

(b) Correction for secondary components

(1) Relative response factors

As discussed in Note 4 above, ECD response for the branched isomers is higher than that for the straight-chain ester, so that relative response factors must be determined in order to calculate their concentrations. Since FID response appears to be more nearly equal for all of the isomers, the factors can be estimated by comparing peak area percent values for standards analyzed on the same column by the two different detectors.

Differences in carrier gas, reagent interference, and relative peak sizes make it difficult to reproduce the chromatography exactly, but conditions should be chosen to make the analyses as similar as possible. Calculate average area percent values by both detectors for the groups of five C_8 isomer peaks, then use the ratios to determine ECD response factors relative to the main peak, as in the following example for an FC-143 standard:

	Peak Number, 14.95-17.22 min in Figure 4b				
	#1	#2	#3 (main peak)	#4	#5
ECD area percent (average of several runs)	7.38	11.94	51.76	10.02	18.91
FID area percent (average of several runs)	3.55	4.38	72.88	8.45	10.73
$F_i' = \left(\frac{A_i}{A_{main}}\right)_{ECD} \times \left(\frac{A_{main}}{A_i}\right)_{FID}$	2.93	3.84	1.00	1.67	2.48

For perfluoro-n-octanoic acid standards (Figure 4a) the only measurable isomer peak is #5. The factor calculated for it generally differs somewhat from that for FC-143 standards, as might be expected from the incomplete resolution and different contributions from neighboring peaks. For samples ranging in type between the two standards, the average factor is usually taken.

(2) Percent correction for total concentration

For each sample run, use the peak areas and response factors to calculate the concentration of isomer peaks relative to the main peak as in the following example (peak numbering as in the factor calculation above):

Figure 4e; Sample 80-68609, Run #21, 12/30/80
Main Component Concentration 7.63 µg/g F

Peak No.	F_i'	Peak Area (integrator units)	% of main peak $= \left(\frac{A_i}{A_{main}}\right)\left(\frac{10^2}{F_i'}\right)$	
1	2.93	83 560	4.80	Total % correction = 24.1
2	3.84	70 460	3.09*	
3	1.00 (main peak)	59 4000	(100)	7.63 x 1.241 = 9.5 ppm F total
4	1.67	32 430	3.27**	
5	2.73	20 9300	12.91	

*Peak #2 is frequently visible as a shoulder, but not resolved sufficiently for quantitative measurement. Depending on the size, the correction may be considered insignificant or can be estimated by comparison with the other isomers.

**This value is probably too high, by comparison with peaks #1 and #3 in the chromatogram in Figure 4e. Since the correction is only a few percent, however, the effect on the total concentration will not be appreciable.

These calculations clearly involve many approximations, especially for samples unlike either of the standards in isomer distribution. Determination of the isomer concentrations is difficult in those cases, since the "response factors" for a group of poorly resolved peaks will be influenced by the relative sizes and contributions from each other as well as by detector response. For peaks #2 and #4 in particular, the individual values must be considered estimates only. Since the contribution of each isomer to the

total concentration is usually small, however, the overall uncertainty will probably still be less than if the composite peak were used and no correction is made for differences in composition. In most of the blood samples examined to date, the composition has appeared similar to that of perfluoro-n-octanoic acid (See Note 4), so that the isomer correction is small and calculated for the well-resolved peaks 1 and 4. For those samples, the packed column analysis should give equivalent results (within the uncertainty of the method), but the capillary analysis has the advantage of verifying that a significant error has not been introduced by undetected isomer peaks.

/30/80
conditions as on p.18
mperature program:
℃ 55°C, 8°/min → 130°C,
130°C

art speed 1 cm/min

tenuation 2^{10} for 20 min,
1 after

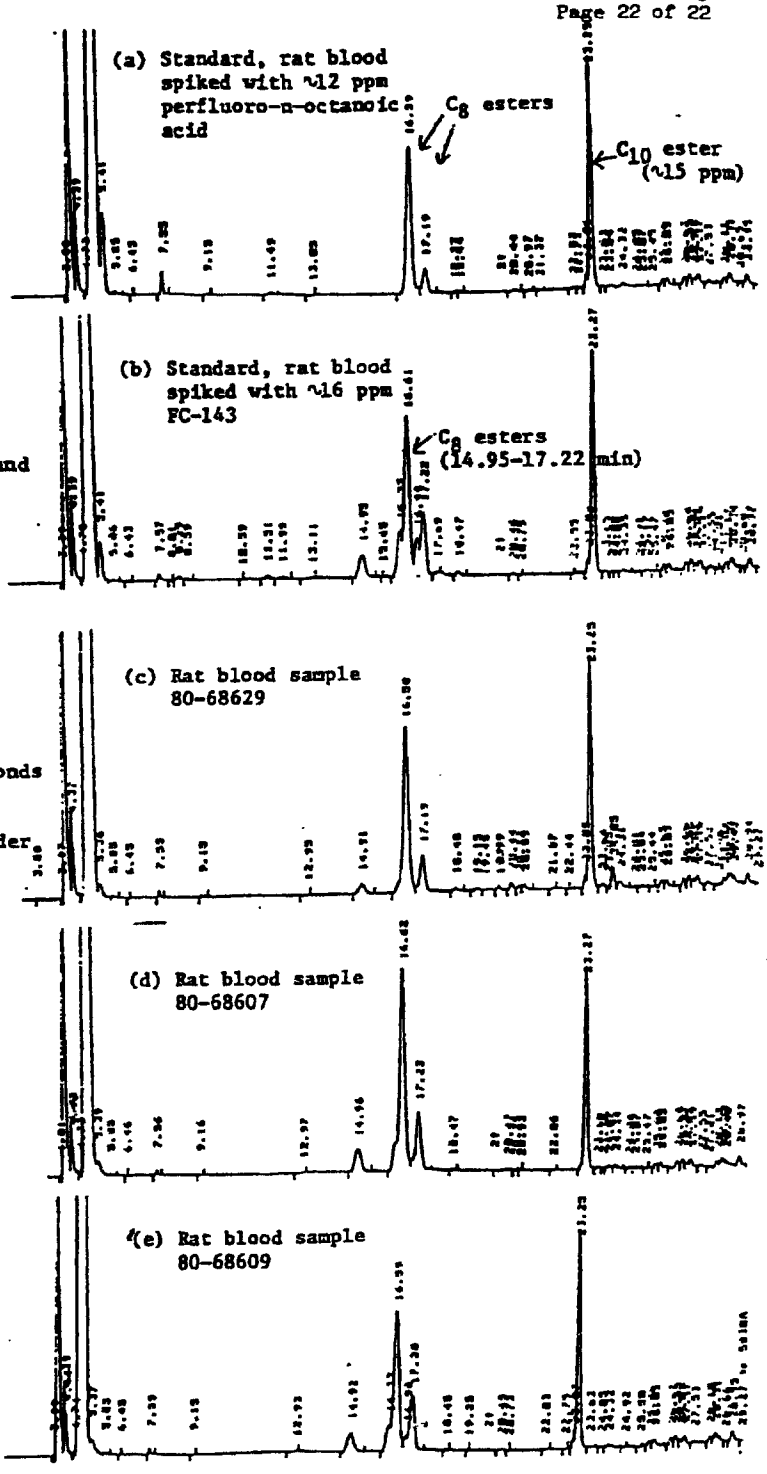
thyl perfluoro-n-octanoate
pears at 16.6 min, branched
omers at 14.9, 16.3, 17.0, and
.2 minutes.

r comparison with the
matograms in Figure 2
(ed column analysis):

ie peak at 14.9 min corresponds
) that at 3.7 min

ie peak at 16.3 min lies under
ie main peak

ie peaks at 17 and 17.2 min
ppear as a shoulder on the
ack of the main peak



Company Sanitized. Does not contain TSCA CBI

000039