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Experimental Station Laboratory

Determination of Perfluorooctanoic Acid in Water

Gas Chromatographic Method

I. Applicability

This method has been developed for the determination of ammonium perfluorooctanoate (C_8-APFC) and perfluorooctanoic acid in aqueous solution, at concentrations from 0.01-10 µg/mL. The procedure described here is for the analysis of aqueous solutions from air impingers. The method has also been used for determination of C_8 in blood samples and, depending on specific interferences, could be applied to other aqueous systems. A modification which eliminates the drying step can be used for appropriate solid samples (such as Nuclepore filters used in air sampling) and for methanol solutions.

II. Unusual Safety Considerations

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 child bearing capability should be restricted from 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 (Reference 3). Standard laboratory safety practices for handling toxic, emoroyotoxic, etc. materials and for corrosives should be used in working with these perfluorinated acids and their solutions.

The electron caputre 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 in working with the low temperature baths used for freeze-drying.

III. Principle

Freeze-drying (lyophilization) is used to remove the water from sample aliquots and permit derivatization. Addition of methanolic HCl to the dried residue, along with perfluorodecanoic acid (C_{10}) internal standard, converts the acids 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 for sensitivity and selectivity.

IV. Fundamental Equations

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

$$(M^+ = NH_4^+,\ Na^+,\ H^+)$$

(acid not amenable to GC analysis) ——————> (non-polar, volatile ester for GC)

V. 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 (Note 1), 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 however, 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 is a significant contribution to the C_8 peak area only at the lowest concentrations (ca 1/3 of the total for 0.01 ppm C_8, when ca 1 ppm C_{10} is added).

In the air impinger samples examined to date, no obvious interference or contribution from compounds other than C_8 has been observed. Preliminary analyses of FEP "in-situ" dispersing agent indicate that while one component of that mixture would co-elute with C_8, it should be accompanied by other nearby peaks.

VI. Sensitivity, Precision and Accuracy

The method has been used over the concentration range 0.01-10 µg/mL C_8. Adjustments in the volumes of sample or reagents could probably be made to extend this somewhat in either direction, if necessary. Due to the relatively narrow linear range of the ECD, however, the calibration curve must always cover the region of interest.

From the limited data available at this time, precision is estimated to be ± 10% relative, with quantitative recovery of spikes within this uncertainty. Accuracy will also be affected by the choice of C_8 standard, which should correspond to the fluorosurfactant composition of the samples (Note 2).

VII. Apparatus

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

1. Gas Chromatograph and Supplies

Hewlett-Packard 5830 GC with HP 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. 18802 Reacti-Therm Heating Module, with Reacti-Block to hold 2-dram vials and thermometer (Block C, No. 18803, has 12 holes but they must be enlarged to ca 19 mm to accomodate the 2-dram vials. Block B, No. 18802, can be used as is, but will hold only 9 vials and provides poorer thermal contact).

4. Vials and Septum Caps

Wheaton No. 224884 or Pierce No. 13028 2-dram (ca 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, micropipet for quantitative delivery of 50 µL aliquots

1 and 2 mL volumetric pipets for measuring sample and reagent aliquots. (Brinkman Dispensette Bottle-Top Dispensers are a great convenience for repetitive delivery of solvents.)

6. Analytical Balance

7. Common laboratory equipment

VIII. Reagents

1. C_8 standard, FC-143 ammonium perfluorooctanoate (3M) or material in use in the area to be monitored (Note 2)

2. Perfluorodecanoic acid, PCR Research Chemicals

3. Methanol, Fisher HPLC (A-452) or Fisher Certified ACS (A-412) (Note 1)

* Reg. U.S. Pat. & Tm. Off.

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 (Note 1).

   The reagent should be stored in the refrigerator, and can be kept for ca 1 month.

5. Hexane, Phillips Spectrograde or Applied Science Lipopure (Note 1)

6. Water, distilled or deionized

7. Sodium hydroxide, 0.05 M aqueous solution

IX. Procedure

A. Preparation of Standard Solutions

Only final solution concentrations are given here without detailed preparation procedures, since amounts needed, concentrations of interest, etc., will vary between laboratories. Preparations should be such that concentrations can be calculated to three significant figures.

1. Perfluorodecanoic acid in methanol: ca 20 µg/mL

2. Ammonium perfluorooctanoate in water: At least four solutions should be prepared which cover the concentration range of interest. For the example in the chromatograms and calculations below (0.1-1.0 ppm), standard solutions were prepared containing 0.1, 0.25, 0.5, 0.75, and 1.0 µg/mL FC-143.

B. Preparation of Samples and Standards for Analysis (Note 3)

For each sample or standard solution, pipet a 1 mL aliquot into a 2-dram vial. Prepare a blank similarly, using distilled water. Add 50 µL 0.05 M NaOH to each and mix gently, keeping the solution in the bottom of the vial to avoid loss to the cap, sides, etc..

C. Freeze-Drying

1. A schematic diagram of the feeeze-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 (Note 4) and the cold traps filled before attaching the drying flasks.

2. To prevent bumping, the samples must be frozen before they are placed under vacuum. 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 (Note 5), 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 ca 4 hours, or until no ice is left in any of the vials.

D. Derivatization

1. To the dried residue in each vial, add 1 mL methanolic HCl derivatization reagent and 50 µL C_{10} standard solution (ca 20 µg/mL in methanol). Close with a septum screw cap and shake well.

2. Thermostat for 1 hour at 65°C.

   (At this point, the samples can be cooled to room temperature and left overnight to analyze the next day.)

3. Cool to room temperature, add 1 mL each hexane and distilled water, and shake for ca 2 minutes.

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

E. GC Analysis

1. GC Conditions - Instrument and column as above.

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 ca 30 mL/min	
Injection Volume:	2 µL	
Sensitivity:	attenuation as needed to keep peak height measurable, 2^{I} - 2^{II} on HP 5830.	
Run Time:	11-20 minutes, depending on sensitivity (Note 6)	

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. Each solution should be run twice (or duplicates run 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. Either peak height or peak area can be measured for quantitation.

X. Calculations (Note 7)

1. Normalized C_8 Peak Values

A tabulation of peak heights for analysis of a series of five standards and two samples (each prepared in duplicate) is shown in Figure 3.

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 standards is also shown in Figure 3.

For non-linear plots such as this, a smooth curve should be drawn through the data points. In some cases, a straight line can be fit through several points, and a linear least squares calculation made for the slope and intercept in that region.

3. Calculation of Sample Concentrations

Using the corrected C_8 peak heights (or areas) for the samples, corresponding concentrations can be read directly from the calibration plot as shown in Figure 3. Average the values from duplicates for each sample.

XI. Notes

1. The solvents specified in Section VIII 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.

2. The FC-143 samples examined to date appear to be a mixture of ca 70-75% straight chain material with several secondary components (including branched isomers) from 1-9%. (Determined by NMR and capillary GC with FID peak area). Under the GC conditions used here, this results in a group of poorly resolved peaks at the C_8 ester position, as seen in Figure 2. In contrast, commercial perfluoro-n-octanoic acid (PCR) and some other ammonium salts contain $\geq$95% straight chain C_8, and appear more nearly as a single peak in the analysis. The response factor determined relative to the C_{10} internal standard also differs for the two types of material, so it is important to choose the standard most similar to the samples to be analyzed. All of the aqueous samples examined thus far closely resemble FC-143 standards, but for chromatograms differing greatly in appearance it may be more appropriate to substitute a straight-chain standard.

3. At least for initial analyses using this procedure, duplicates should be prepared as a check on precision.

4. For this freeze-drying application pressures less than 0.5 Torr seem to be satisfactory, although 0.025-0.05 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.

5. 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 II, care must be taken to avoid contact with the cold bath.

6. As seen in the chromatograms in Figure 2, some small, broad peaks appear late in the run. (These appear to come from the C_{10} standard, and might be eliminated in purified material). When running at high sensitivity for low C_8 concentrations, these can interfere with the following run and must be allowed to elute before the next injection.

7. From the calibration plot in Figure 3, it can be seen that the ECD response is not linear with concentration over this range. Linear plots may be obtained over other ranges, but still with concentration-dependent response factors. The calculations described here thus make use of a calibration curve, where the internal standard is used to normalize raw peak values before plotting.

   Calculations could be done by a general internal standard procedure instead for an ECD which shows a more linear response and constant relative response factors. (A calibration plot of this sort has been observed with a Varian 3700 GC under similar conditions).

XII. References

1. Research Notebooks E20029, E22306, E22424 (S. Stafford)

2. J. Belisle and D. F. Hagen, "A Method for Determination of Perfluorooctanoic Acid in Blood and other Biological Samples", Analytical Biochemistry 101, 369 (1980)

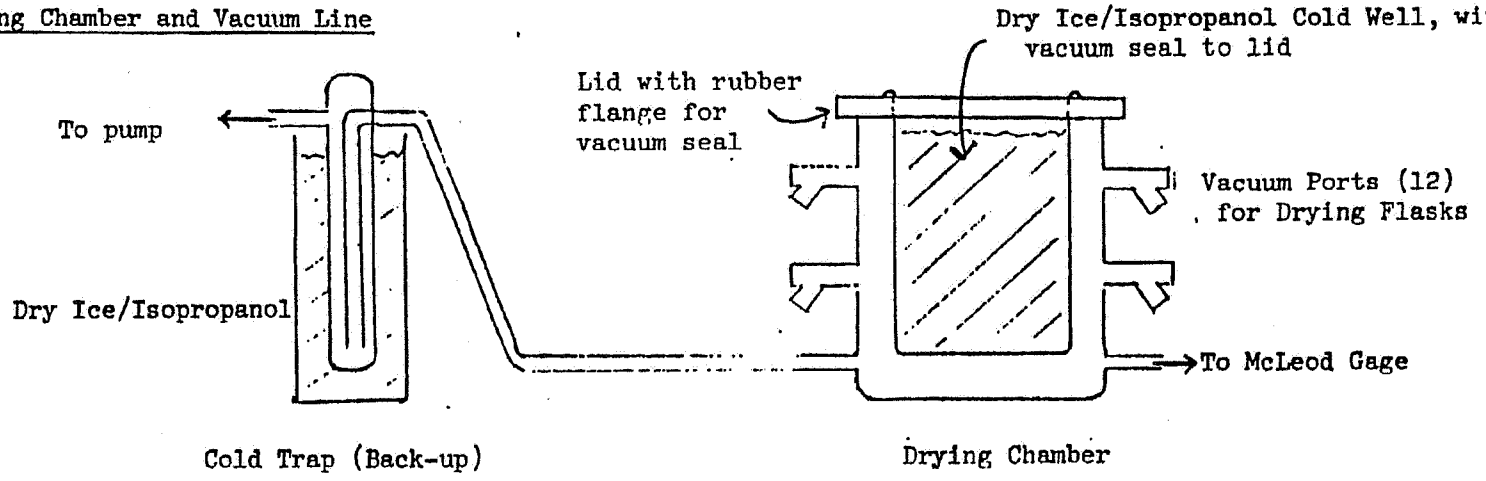
3. F. A. Ubel, S. D. Sorenson, & D. E. Roach, "Health Status of Plant Workers Exposed to Fluorochemicals - A Preliminary Report" American Industrial Hygiene Association Journal 41, 584 (1980)

Prepared by S. S. Stafford, 8/8/80
Revised by S. S. Stafford 4/3/81

Approved by L. J. Papa

FIGURE 1 - APPARATUS FOR FREEZE-DRYING SAMPLES

(a) Drying Chamber and Vacuum Line



(b) Vacuum Port, Drying Flask, and Samples

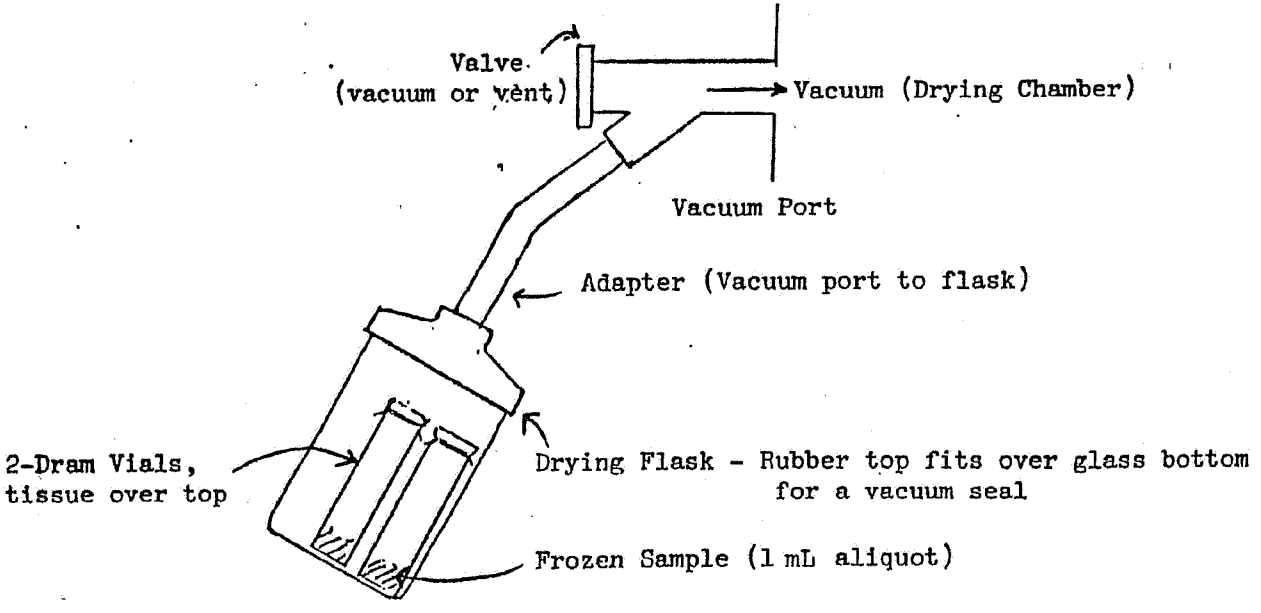
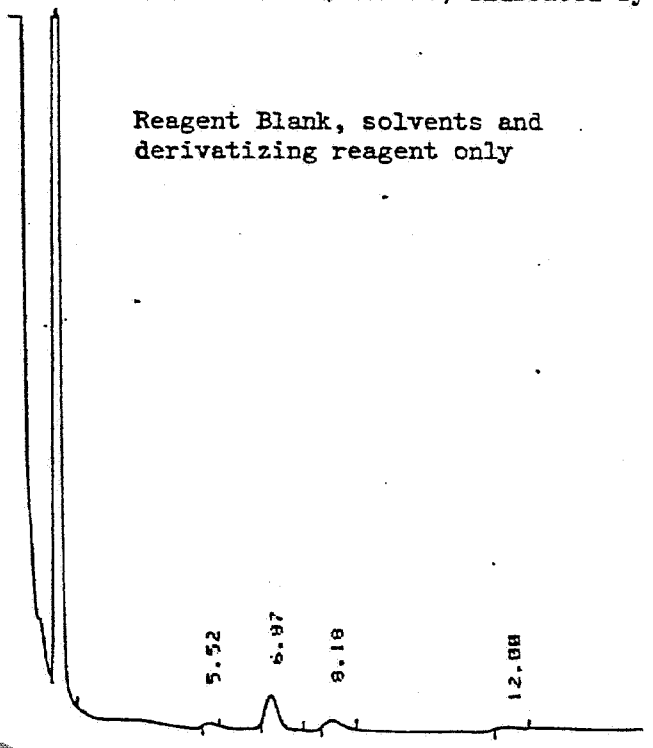


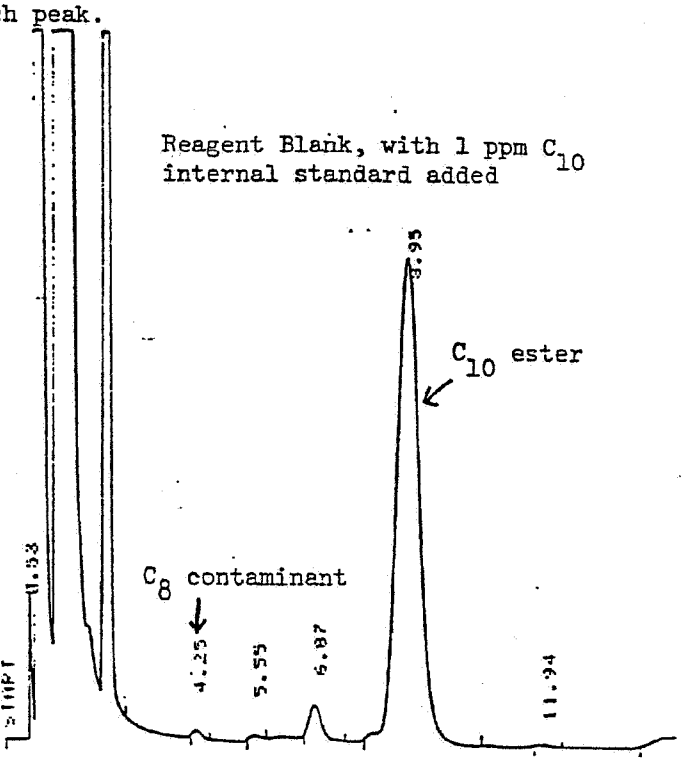
FIGURE 2 - CHROMATOGRAMS FOR C_8 DETERMINATION *

GC conditions as on p. 5. Attenuation 2^7, chart speed 1 cm/min.
Retention time (minutes) indicated by each peak.

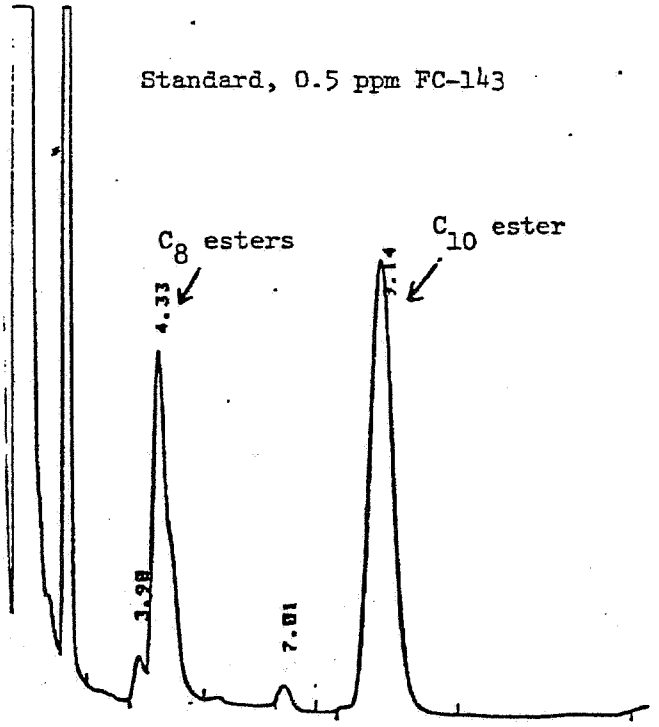
Reagent Blank, solvents and derivatizing reagent only



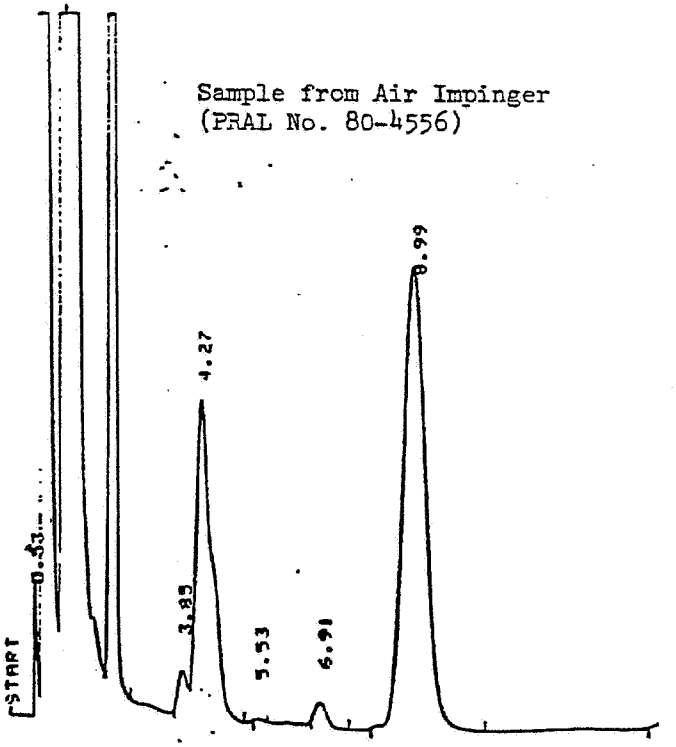
Reagent Blank, with 1 ppm C_{10} internal standard added



Standard, 0.5 ppm FC-143



Sample from Air Impinger (PRAL No. 80-4556)



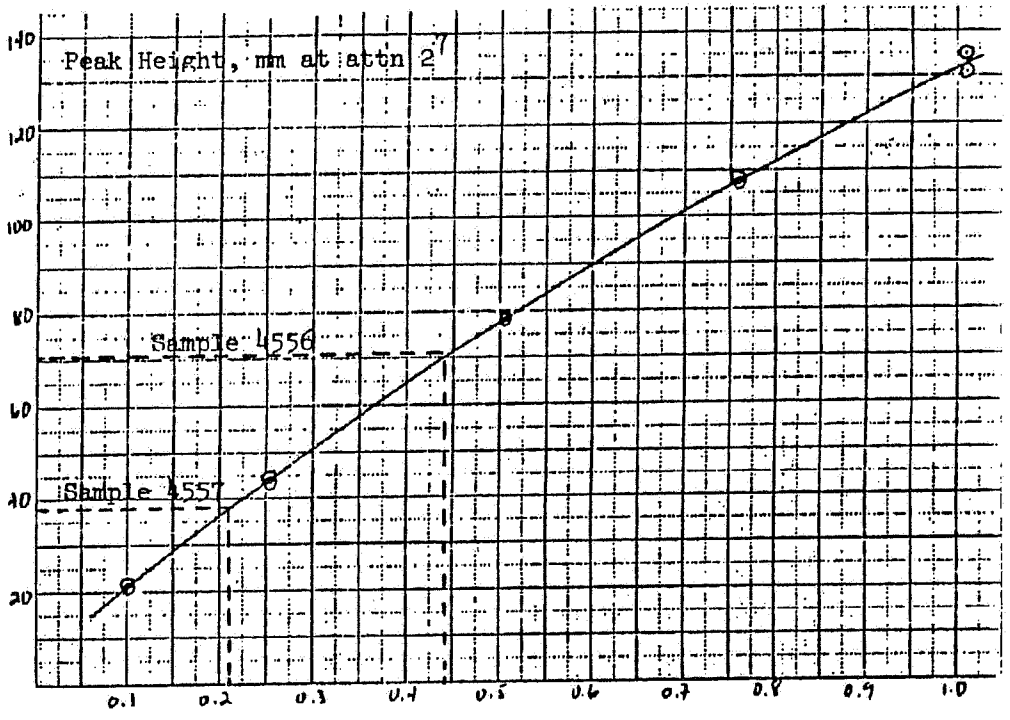
*The results shown here are for solutions prepared using 2 mL each derivatizing reagent, hexane, and water, rather than 1 mL as in the method. Relative peak heights will not be affected, but attenuation is 2x lower.

FIGURE 3 - CALCULATION OF CORRECTED PEAK HEIGHTS AND CALIBRATION PLOT *

(a) Peak Height values for runs of ten standard and four sample solutions (duplicate preparations at each concentration)

Solution	[FC-143], µg/mL Added	Run #	Peak Height, mm at 2 attn Raw Values C_8	Raw Values C_{10}	Corrected $C_8 \times \frac{102}{C_{10}}$
Std A-1	1.01	7	138	101	135
" A-2	"	20	131	102.2	131
" B-1	0.758	8	107.2	101.5	108
" B-2	"	19	106	101.5	107
" C-1	0.505	9	99	103	78.2
" C-2	"	16	77.5	100.8	78.4
" D-1	0.253	10	44	103.5	43.4
" D-2	"	13	44.2	101.2	44.5
" E-1	0.101	11	22	102.5	21.9
" E-2	"	12	21.5	106	20.7
** Sample	Calculated				
80-4556-1	0.440	17	70	102	70.0
80-4556-2	0.445	18	70	101	70.7
80-4557-1	0.210	14	38.5	103.5	37.9
80-4557-2	0.200	15	37.8	105	36.7
				ave = 102±2	

(b) Calibration Plot, Corrected C_8 peak height vs. concentration



** Calculated concentrations for duplicate sample preparations read from the curve

*The results shown here are for solutions prepared using 2 mL each derivatizing reagent, hexane, and water, rather than 1 mL as in the method. Relative peak heights will not be affected, but attenuation is 2x lower.