

AR 226- 3400

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3701 Welsh Road
Willow Grove, PA
19090

National Medical Services

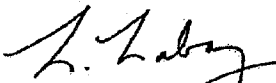
November 9, 2001

Mary Kaiser, Ph.D.
DuPont Central Research & Development
[REDACTED]
Wilmington, DE 19880-0269
[REDACTED]

Dear Dr. Kaiser:

We have completed work on the development and validation of a new procedure for the analysis of PFOA in blood. Please find enclosed an updated Test Information Summary Sheet and a summary of our findings. As always, we look forward to and welcome discussion concerning the particulars of this analysis. Specifically we greatly anticipate your comments concerning the proposed methodology change.

Sincerely,


Laura Kabay
Group Leader

Company Sanitized. Does not contain TSCA CBI

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PFOA Method Change Proposal
November 4, 2001

Background:

In an attempt to better certain aspects of the method currently in place to test for the presence and amount of perfluorooctanoic acid (PFOA) a new sample preparation procedure was developed and validated.

In brief, an internal standard (perfluorodecanoic acid) is added to the sample and the acids derivatized to their methyl esters utilizing methanolic HCl. All derivatives are then extracted into hexane prior to analysis by GC-ECD. The main advantages of using this new procedure in conjunction with the results of the validation for it are outlined and discussed below.

Reporting Limit:

The reporting limit for this analysis has historically been 10ppb and in this regard it is of importance that this value be consistently achievable. However, during the course of analysis we observed interferences that did not allow us to reproducibly detect this critical 10ppb point. In essence, this point was "buried" in the baseline and as a consequence the reporting limit had to be increased to a greater value. Investigation revealed that this problem was in fact due to one of the reagents that used during the sample preparation part of the procedure. Because purchasing fresh material did not alleviate this problem, it was determined that a new method be developed.

- By using the proposed method the interferences that caused the problem detailed above are no longer present. A reporting limit of 10ppb is consistently obtained (See Figure 1).

Patient Results:

To test the validity of the method 33 patient samples, along with the appropriate quality controls, were analyzed using both types of sample preparation procedures and their results compared.

- With the exception of four patient samples, all results are comparable with values not differing by more than $\pm 30\%$.
- The patient samples where this criterion did not hold true were samples where the described interferences were present.

Overall Conclusions:

1. A reporting limit of 10 ppb is consistently obtained.
2. Interferences that were observed and, in some samples, affected accuracy of results are no longer present.
3. Patient results are comparable when analyzed using both methods.
4. The greater ease of sample preparation allows us to handle a higher volume of samples at one time.

NAME OF TEST:

PERFLUOROOCTANOIC ACID

NMS # 3425

SYNONYMS:

PFOA, FC-143 Component, Perfluorocaprylic acid

ANALYTE CLASSIFICATION:

Fluorinated Organic Acid

METHOD PRINCIPLE:

This test is a quantitative analysis of perfluorooctanoic acid in whole blood and serum specimens. This is achieved by adding an internal standard (perfluorodecanoic acid). The acids are then derivatized to their methyl esters with methanolic HCl, which are then extracted into hexane.

Analysis is by gas chromatography with an electron capture detector on a DB-1701 capillary column.

ALTERNATIVE METHODS:

None available

SPECIMEN REQUIREMENTS:

Specimen	Requested Volume	Aliquot Volume	ONS Volume
Blood	4.0 mL	1.0 mL	0.5 mL
Serum	4.0 mL	1.0 mL	0.5 mL
For Tissue & Other Specimen Types, Call Lab.			

SPECIAL HANDLING:

Store refrigerated.

REPORTING LIMIT:

10 ng/mL

TAT:

Set-up on a by-arrangement basis.

PHARMACOTOXICOLOGIC DATA:

Little toxicological data is available on perfluorooctanoic acid. It is known to have a very long elimination half-life.

LIMITATIONS OF METHOD:

1. Quantitative results cannot exceed the highest working standard or be less than the lowest working standard for that run.
2. There are no known interferences for this analysis.

REFERENCES:

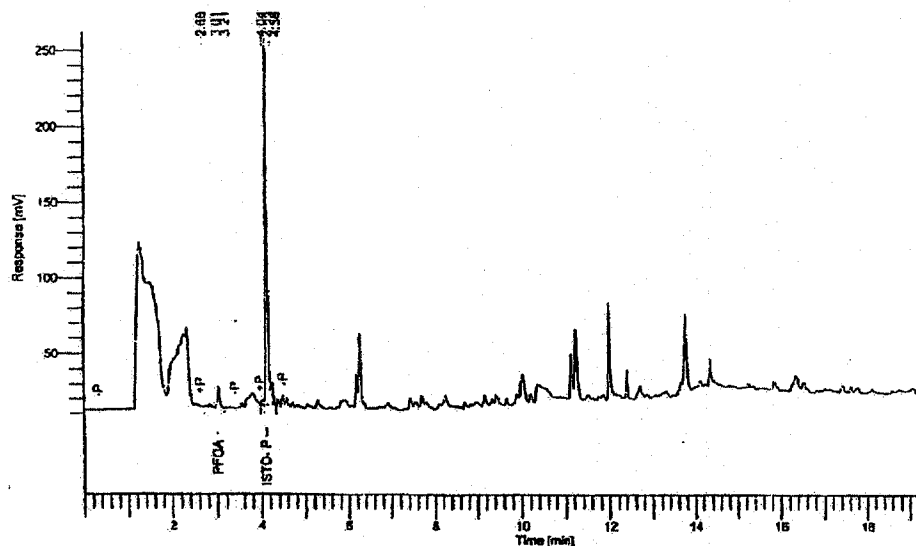
1. Belisle, J. and Hagen, D.F., A Method for Determination of Perfluorooctanoic Acid in Blood and Other Biological Samples, Analytical Biochemistry, 101, 369-376 (1980).
2. Ubel, F.A., Sorenson, S.D., and Roach, D.E., Health Status of Plant Workers Exposed to Fluorochemicals - A Preliminary Report, American Industrial Hygiene Association Journal, 41, p. 584 (1980).

Figure 1: Low point (10ppb) on calibration curve using liquid extraction procedure.

P:

Software Version : 6.1.2.0.1.D19	Date : 4/18/01 2:48:53 PM
Operator : lamadrid	Sample Name : Low Curve: 10 ng/mL
Sample Number : 053	Study : Homemade MeOH:HCl
AutoSampler : NCNE	Rack/Vial : 00
Instrument Name : E03	Channel : A
Interface Serial # : 7230910097	A/D mV Range : 1000
Delay Time : 0.00 min	End Time : 21.00 min
Sampling Rate : 7.1420 pts/s	
Volume Injected : 1.00000 ul	Area Reject : 100.000000
Sample Amount : 1.0000	Dilution Factor : 1.00
Internal Standard Amount : 1.0000	
Data Acquisition Time : 4/12/01 11:42:30 AM	Cycle : 38

Raw Data File : \Pe42001\MainLab\Env\EO-3\Data\041101\PFOA\pfoa-041101hgf-038.raw <Modified>
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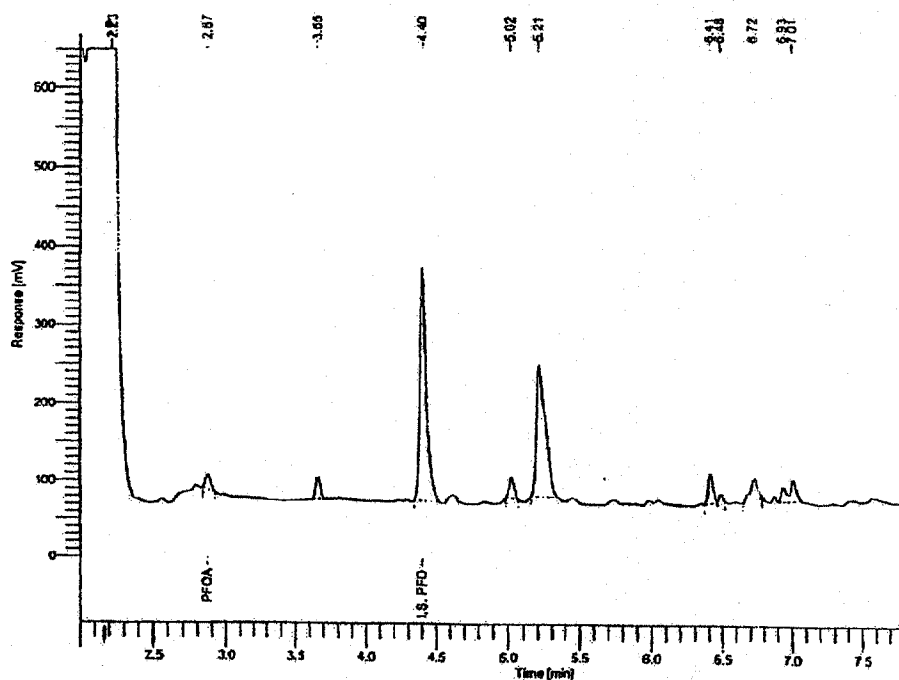
PFOA by GC-ECD (E03)

Peak #	Time [min]	Rel. RT	Area [uV-s]	Height [uV]	Area/Height [s]	ISTD Resp Ratio	Blank Column	Component Name	Concentration ng/mL
3	3.008	0.733	48421	14846	3.262	0.0627		PFOA	9
7	4.105	1.000	636461	236754	2.698	1.0000		ISTD-PFDA	9
			684882	251600		1.0627			

Figure 2: Freeze Drying Method (Low QC)

Software Version	: 6.1.2.0.1.D19	Date	: 4/15/01 4:15:44 PM
Operator	: hagarie	Sample Name	: Low QC
Sample Number	: 025	Study	: WL#311904
Auto Sampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: E03	Channel	: A
Interface Serial #	: 7230910097	A/D mV Range	: 10000
Delay Time	: 0.00 min	End Time	: 21.00 min
Sampling Rate	: 10.0000 pts/s	Area Reject	: 100.000000
Volume Injected	: 1.000000 mL	Dilution Factor	: 1.00
Sample Amount	: 1.0000	Cycle	: 25
Internal Standard Amount	: 1.0000		
Data Acquisition Time	: 4/7/01 6:23:55 PM		

Raw Data File : \tpe42001\MainLab\Env\EO-3\data\040701\PFOA-040701HGF-025.raw <Modified>
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PFOA by GC-ECD (E03)

Peak #	Time [min]	Rel. RT	Area [μV.s]	Height [μV]	Area/Height [s]	ISTD Resp Ratio	Blank Column	Component Name	Concentration ng/mL
2	2.871	0.652	45541	18614	2.447	0.0624		PFOA	34
4	4.401	1.000	909923	298336	3.050	1.0000		I.S. PFOA	34
			955464	316950		1.0624			

PFOA Method Comparison: Freeze Dried Method versus Liquid Extraction Method of Perfluorooctanoate methyl ester from Blood

sample number	Control Number	Freeze Drying				Liquid Extraction				% diff > +/- 30%
		PFOA Ht.	ISTD Ht.	ISTD Resp.	ng/mL	PFOA Ht.	ISTD Ht.	ISTD Resp.	ng/mL	
1	Low QC	17222	243073	0.071	48	22049	256135	0.086	49	2
2		8053	247169	0.033	13	20971	281763	0.074	38	98 **
3		21927	252004	0.087	63	27751	291816	0.095	57	10
4		12329	208416	0.059	37	24076	297100	0.081	44	17
5		8168	212227	0.038	18	15910	285704	0.056	22	20
6		21538	274340	0.079	55	21841	303446	0.072	36	42 **
7	Mid-QC	50612	183210	0.276	256	79640	243951	0.326	299	15
8	Low QC	17312	251466	0.069	46	24268	273951	0.089	53	14
9		76733	195603	0.392	410	130769	307048	0.426	420	2
10		71339	253949	0.281	2700	85973	281786	0.305	2800	4
11		71436	226788	0.315	310	101613	300800	0.338	310	0
12		60243	256633	0.235	2100	68749	293512	0.234	2000	5
13		116082	194232	0.598	810	207364	325202	0.638	730	10
14		34108	184427	0.185	160	60516	340561	0.178	140	13
15		39543	247914	0.160	538	28829	291402	0.099	627	15
16		40585	238555	0.170	140	46740	302389	0.155	120	15
17		56678	233943	0.242	220	68624	303009	0.226	190	15
18		45512	255324	0.178	1500	26280	266231	0.099	1249	18
19		63205	218966	0.289	2800	73665	258508	0.285	2543	10
20	Mid-QC	2178	216499	0.010	0	5060	310214	0.016	0	N/A
21		49680	220045	0.226	2000	55698	263645	0.211	1752	13
22		68453	223282	0.307	298	86938	295411	0.294	265	12
23		40573	249224	0.163	140	58153	331323	0.176	140	0
24		3309	236671	0.014	0	7399	321543	0.023	0	N/A
25		47659	248200	0.192	673	228640	323630	0.706	860	24
26		10975	239652	0.046	25	12966	303100	0.043	10	86 **
27		12113	249430	0.049	28	15902	314680	0.051	17	49 **
28		78299	229317	0.341	340	100095	302138	0.331	310	9
29		10037	167416	0.060	38	24353	304819	0.080	44	15
30		52963	191978	0.276	261	85457	274960	0.311	283	8
31			139762	0.000	0	7902	298280	0.026	0	N/A
32	Low QC	3518	198673	0.018	0	9273	281886	0.033	0	N/A
33			153525	0.000	0	10052	303660	0.033	0	N/A
34			149912	0.000	0	7813	326413	0.024	0	N/A
35		7358	168129	0.044	23	21948	343206	0.064	29	23
36		79791	264217	0.302	1169	55987	318673	0.176	1386	17
37		19411	137065	0.142	117	57208	329714	0.174	136	15
38	Mid QC	117280	263930	0.444	2438	90354	323824	0.279	2477	2
39		59401	272670	0.218	784	42979	336052	0.128	909	15

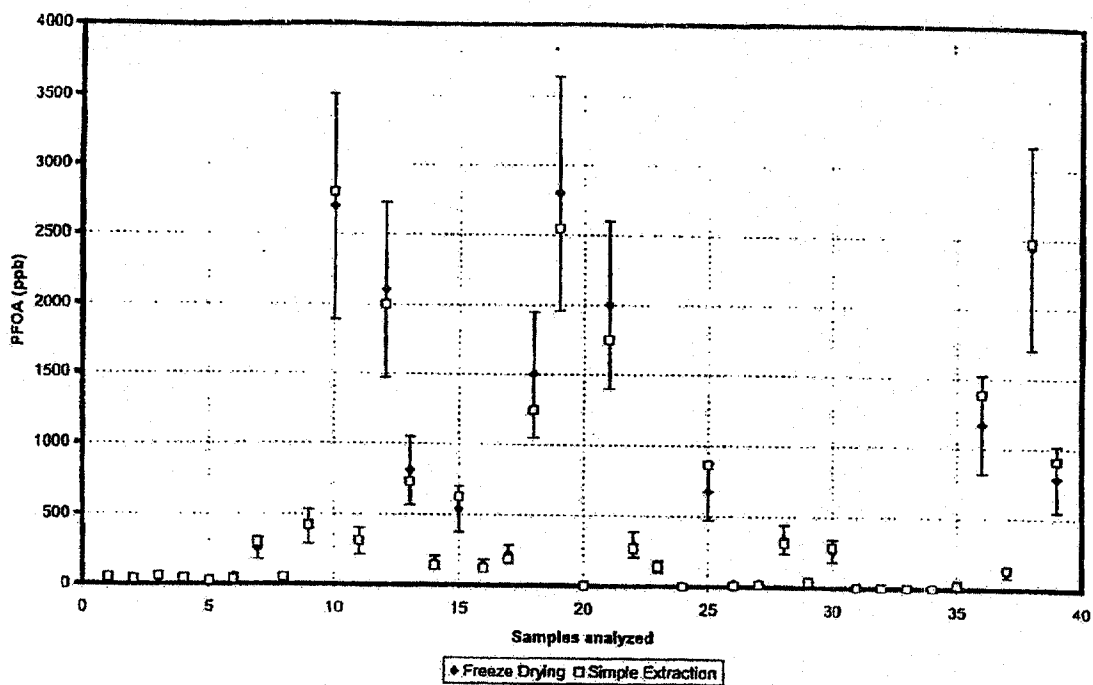
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SD
RD (%)

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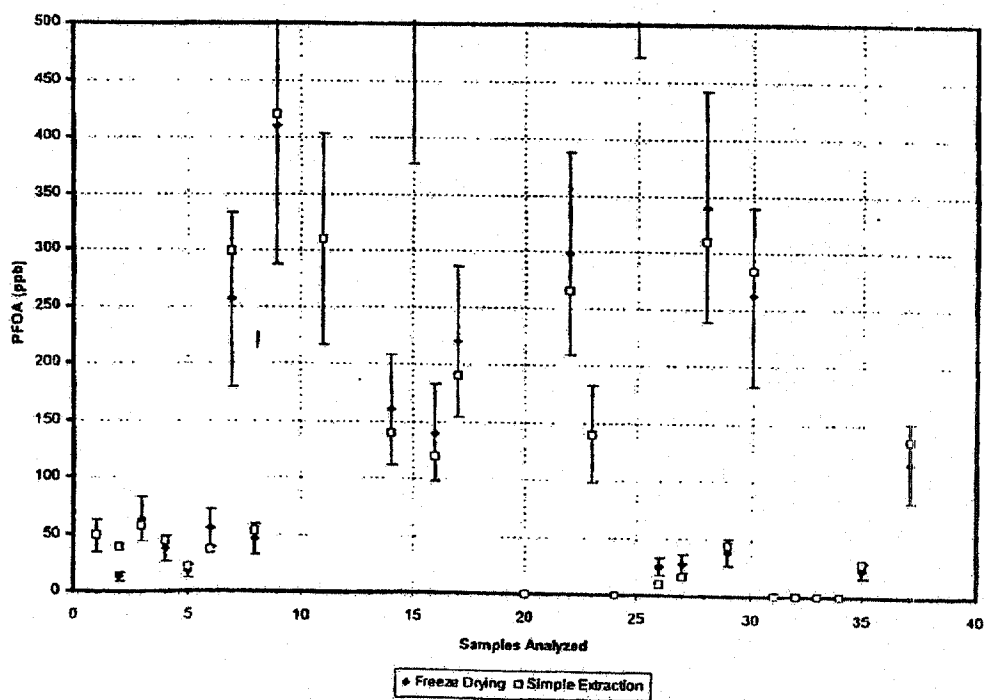
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8

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PFOA 30% Error Bars (0-4000 ppb)

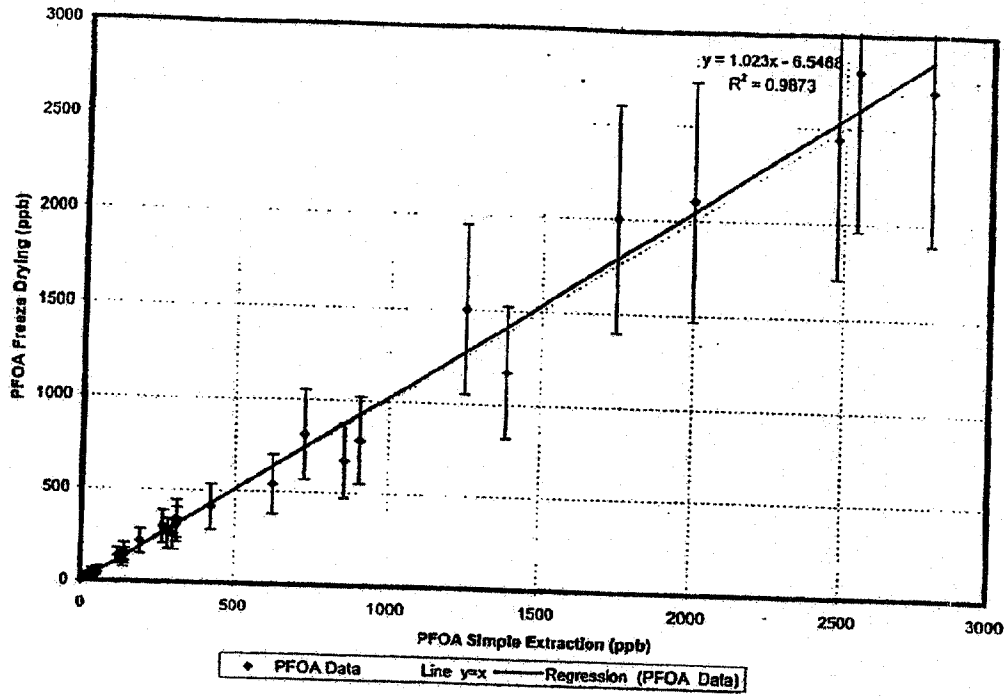


PFOA 30% Error Bars (0-500 ppb)

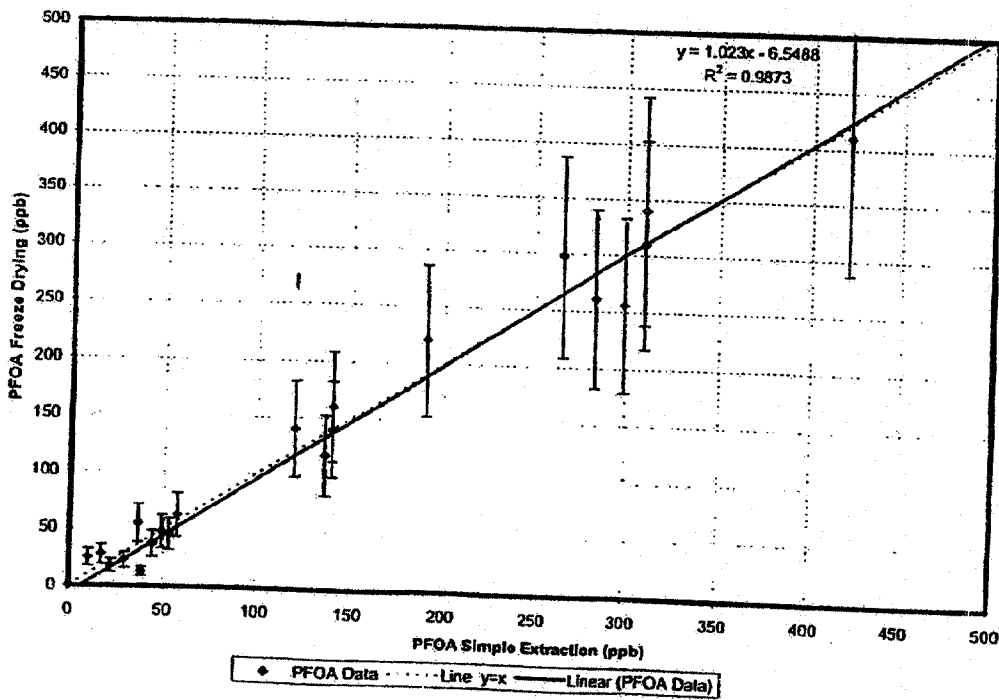


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PFOA Methods Comparison



Pfoa Method Comparison



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