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Quantitative Determination of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS in Human Serum by LC/MS/MS

Assay Validation Report

Northwest Bioanalytical (NWB)
A Division of NWT Inc.
1121 East 3900 South
Salt Lake City, UT 84124

PREPARED FOR: 3M
935 Bush Avenue
St. Paul, MN 55133

AUTHOR:

David L. Vollmer, Ph.D., Project Manager DATE: 5/13/99

APPROVED FOR RELEASE BY:

Rodger L. Foltz, Ph.D., Laboratory Director DATE: 5/13/99

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Assay Validation Report

1. INTRODUCTION

Northwest Bioanalytical (NWB) was contracted by 3M to develop and validate a liquid chromatography/tandem mass spectrometry method for the measurement of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS in human serum. For chemical names and structures, see the Analytical Method contained in section 6 of the report.

This report summarizes the analytical results from the validation of the method for quantitation of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS in human serum for 3M. Kris Hansen, Ph.D., at 3M served as the Study Monitor. The following is a list of NWB supervisory personnel involved in the completion of this work: David L. Vollmer, Ph.D. (Project Manager); Brad Coopersmith, Ph.D. (Senior Scientist); Rodger L. Foltz, Ph.D., (NWB Laboratory Director).

3M SOP AMDT-S-12 (effective 12/12/95) and NWB SOPs were used in the conduct of this project and were available to project personnel in electronic or hard copy formats.

Date Study Initiated: January 15, 1999 Date Analyses Completed: February 10, 1999

The method validation study described in this report is not included within the definition of a GLP regulated nonclinical study (Title 21 of the US Code of Federal Regulations Part 58). However, Northwest Bioanalytical conducts all studies within the guidelines of GLP principles.

Background

Early method development experiments demonstrated varying levels of endogenous PFOS, PFOSAA, POAA, and PFHS in several lots of "blank" human serum. These endogenous levels complicate the use of human serum for the preparation of calibration standards and quality control samples.

To circumvent the difficulty of preparing standards and controls in the presence of endogenous levels, several animal matrices were evaluated. Rabbit serum showed the lowest endogenous levels of the aforementioned analytes when directly compared to rat, dog, and monkey serum. Utilizing a calibration curve prepared in rabbit serum, however, did not demonstrate acceptable precision and accuracy for the determination of PFOSA, MeFOSE-OH, and EtFOSE-OH.

A serum matrix that was pre-extracted from human serum to remove any endogenous levels of analytes prior to preparation of the curve was also evaluated. This serum matrix, however, showed greater extraction recoveries for all the analytes in the calibration curve samples than in the human serum samples that were not pre-extracted, and therefore did not demonstrate acceptable accuracy for quantitation of the analytes.

Because the four analytes (PFOS, PFOSAA, POAA, and PFHS) that did show acceptable precision and accuracy with the rabbit serum curve were the same four analytes that showed measurable endogenous concentrations in "blank" human serum, an abbreviated rabbit curve was used to calculate the endogenous levels in specific lots of human serum. Once the endogenous levels were determined for those lots of human serum, those concentrations were added to the spiked concentrations for the human serum QCs and for the human serum calibration standards. This procedure is described in detail within this report.

Principles of the Method

After the addition of HPLC-grade water and sodium dodecyl sulfate (SDS, internal standard) to 0.20 mL of human or rabbit serum, the serum mixture is made basic with the addition of 0.5 M tetrabutylammonium hydrogen sulfate (TBA, pH 10) and 0.25 M carbonate buffer. This mixture is then extracted with methyl-tert-butyl ether. After sufficient mixing, the sample is centrifuged. The organic layer is transferred via pipette into a clean test tube. The organic layer is then evaporated to dryness and the sample is reconstituted into 2 mM ammonium acetate water:methanol (50:50 v/v). The extracts are then analyzed by liquid chromatography/tandem mass spectrometry using negative-ion electrospray ionization and multiple reaction monitoring.

2. VALIDATION SUMMARY

Four separate analytical runs were used in the determination of linearity, precision, and accuracy of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS. The validation included determination of the stability of the analytes maintained under various storage conditions, the extraction efficiencies of the analytes and the internal standard, the system precision of the analytes, and the reproducibility of the analytes for this assay.

All values reported in the validation summary and tables are based upon the actual concentrations of solutions utilized during this validation. Please refer to the *List of Tables* for the location of the validation test data and summary statistics.

2.1. Range of Quantitation

Rabbit Serum Calibration Curve

Each analytical run included calibration standards in duplicate at a minimum of four different concentrations and at least two rabbit serum blanks. Data for PFOSA, N-MeFOSE-OH and N-EtFOSE-OH are not included because there were no endogenous amounts detected in human serum.

	PFOS	PFOSAA	POAA	PFHS
Range (ppb)	1.00 to 50.0	1.00 to 50.0	1.00 to 50.0	1.00 to 50.0
Mean Correlation Coefficient	0.9961	0.9957	0.9987	0.9982

Human Serum Calibration Curve

Each analytical run included calibration standards in duplicate at a minimum of seven different concentrations, a minimum of 10 quality control samples (QCs) (two levels in replicates of five), two "blanks" and two "0-ppb QCs" (QCs containing internal standard, but no spiked analytes).

	PFOS	PFOSA	PFOSAA	N-MeFOSE-OH	N-EtFOSE-OH	POAA	PFHS
Spiked Concentration Range	1.00 to 500 ppb	1.00 to 500 ppb	0.538 to 53.8 ppb	1.00 to 500 ppb	1.00 to 500 ppb	1.00 to 500 ppb	1.00 to 500 ppb
Mean Correlation Coefficient	0.9934	0.9969	0.9966	0.9971	0.9976	0.9972	0.9978

2.2. Precision and Accuracy

The precision and accuracy of the LC/MS/MS method for the quantitation of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS in human serum were determined by analyzing two levels of quality controls in replicates of five on four separate days.

The intra-assay %CV for PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS were less than or equal to 13.0% for each QC concentration.

The inter-assay %CV for PFOS, PFOSA, PFOSAA, POAA and PFHS were less than or equal to 13.3% for each QC concentration. The mean percent theoretical for all levels of quality controls ranged from 95.7 to 106.7.

The inter-assay %CV for N-MeFOSE-OH and N-EtFOSE-OH were less than or equal to 25.5% for each QC concentration. The mean percent theoretical for all levels of quality controls ranged from 93.5 to 99.4.

2.3. Repeatability and Reproducibility Test

A different extractionist prepared samples for run number 32 than the extractionist who had prepared the samples for run numbers 25, 26, and 28. The samples were analyzed under the same operating conditions and with the same instrument. The calibration standards and QCs from run number 32 met the same acceptance criteria used for run numbers 25, 26, and 28.

The analytical procedure demonstrated acceptable reproducibility for all the analytes except N-MeFOSE-OH and N-EtFOSE-OH.

2.4. System Precision Test

The system precision for PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS was determined by injecting unextracted low and high QC samples at the beginning and end of a normal validation run. Instrument response is defined as the peak area ratio (analyte peak area/internal standard peak area). The intra-assay %CV for PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS were less than or equal to 78.2% for each QC concentration.

The unextracted samples did not demonstrate acceptable precision for all the analytes (See section 4 Comments and Conclusions for discussion of system precision.).

2.5. Extraction Efficiency

The extraction efficiencies for PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA, PFHS and SDS (internal standard) from human serum were determined by comparing the peak areas obtained for the following three cases:

1. Both the analyte and internal standard added following the extraction.
2. The analyte added to serum prior to extraction and the internal standard added following extraction.
3. The internal standard added to serum prior to extraction and the analyte added following the extraction.

The extraction efficiencies were then determined by the changes in peak area for the various cases. The mean extraction efficiencies were PFOS (85.9%), PFOSA (82.3%), PFOSAA (71.5%), N-MeFOSE-OH (53.0%), N-EtFOSE-OH (44.6%), POAA (90.0%), PFHS (81.5%) and SDS (71.2%).

2.6. Stability Evaluation

For the following stability evaluations, the mean test QC sample concentrations must fall within 20% of the mean reference QC sample concentrations to demonstrate acceptable stability.

Freeze-thaw Stability

Stability of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS in human serum subjected to freezing and thawing for three cycles before processing was determined for low and high QC samples. Test QCs were compared against reference QC samples subjected to one freeze/thaw cycle.

Samples demonstrated acceptable stability after three freeze/thaw cycles for all the analytes.

Room Temperature Matrix Stability

Stability of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS in human serum stored under normal room temperature and light conditions for up to 4 hours was determined for low and high QC samples. Test QCs were compared against reference QC samples prepared immediately upon thawing.

Samples demonstrated acceptable stability after 4 hours at normal room temperature and light conditions for all the analytes except N-MeFOSE-OH and N-EtFOSE-OH.

Room Temperature Extract Stability

Stability of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS extracts stored at room temperature for approximately 24 hours prior to analysis was determined for low and high QC extracts. Test QCs were compared against reference QC samples analyzed immediately following extraction.

Extracted samples demonstrated acceptable stability after 24 hours at normal room temperature for all the analytes except PFOS, PFOSAA, POAA and PFHS analytes (See section 4 Comments and Conclusions for discussion of room temperature extract stability).

Stock Solution Stability

The stock solution stability was evaluated by comparing the concentrations for a set of unextracted standards made from a stock solution prepared on January 12, 1999 with a set made from a stock solution prepared February 5, 1999. The stock solution stability evaluation was performed in replicates of three at the same level of unextracted standard.

Stock solutions demonstrated acceptable stability after storage at -20 °C for 24 days for all the analytes except N-MeFOSE-OH and N-EtFOSE-OH.

Long-Term Matrix Stability and Reduced Temperature (-20 °C) Extract Stability

These stability tests will be performed and reported at a later date in a separate report.

3. DATA MANAGEMENT

The concentration values for the endogenous levels of PFOS, PFOSAA, POAA and PFHS were calculated using PE Sciex MacQuan software (v. 1.6). All other concentration values were calculated using the Watson® DMLIMS software. Data presented in this report may be formatted to fewer significant figures than may have been used by the computer to generate means, standard deviations and coefficients of variation. Summary statistics may be derived from unrounded data and may differ slightly from the values obtained using the rounded values.

4. COMMENTS AND CONCLUSIONS

During the assay validation, three potential internal standards were evaluated. They were nonanesulfonic acid, octanesulfonic acid and SDS. A fourth internal standard, $C_8H_5F_{13}SO_3$, was evaluated during method development, but was unsuitable for this assay owing to a serum interferent that demonstrated different ionization suppression from lot to lot of human serum. Of the three internal standards, SDS gave the best results for all the analytes, and thus it was used as the internal standard for each analyte.

To calculate the endogenous levels of the analytes in human serum, each validation run included: (1) "blank" human serum samples, prepared in duplicate or greater, from the lot used to prepare

the human serum calibration curve, (2) "blank" human serum samples in five replicates from the lot used to prepare the QCs, and (3) a calibration curve in duplicate prepared using rabbit serum with four different concentrations covering the range 1 to 50 ppb for each analyte. See Tables 5-7 for human serum endogenous concentration data.

If endogenous analyte was detected in the "blank" human serum samples, that concentration was calculated using the rabbit serum calibration curve. The calibration standard and the analytical QC target concentrations were then adjusted to include those endogenous levels.

For example, PFOS was determined to have an average endogenous level of 30.4 ppb in the human serum lot used for the calibration standards on run numbers 26, 28, and 32. The curve range was then adjusted to be 31.4 to 530 ppb; that is, the sum of the endogenous concentration plus the concentration spiked into each calibration standard. The average endogenous PFOS level for the human serum lot used for the analytical QCs was determined to be 22.3 ppb. Thus, the analytical low and high QC target levels were adjusted to 42.3 and 372 ppb (sum of the endogenous concentration plus the concentration spiked into each QC), respectively.

These average endogenous amounts for each analyte were used over the course of the validation except for run number 25. A different lot of human serum was used for the calibration curve on run number 25 than on run numbers 26, 28, and 29, thus yielding a different adjusted human calibration curve range for run number 25. The same human serum lot for the analytical and stability QCs was utilized over the course of the validation.

The method described in this report has been validated for the determination of PFOS, PFOSA, PFOSAA, POAA and PFHS in human serum. The method is not considered validated for N-MeFOSE-OH and N-EtFOSE-OH. The reason the results for N-MeFOSE-OH and N-EtFOSE-OH did not meet the acceptance criteria for validation could be due to the following factors: (1) poor extraction efficiencies, (2) poor ionization efficiencies (i. e., no efficiently ionizable functional groups), and (3) because the only useable MRM transition monitored for these two analytes is not derived from a molecular fragmentation, but rather fragmentation of the acetate adduct of each analyte to the acetate ion (i. e., $[M + CH_3CO_2]^- \rightarrow [CH_3CO_2]^-$).

The poor system precision for all the analytes can be attributed to a "column conditioning" affect. That is, the analytical column used for this assay yields more precise results when it is primed by injection of two or more extracted samples. When unextracted samples are injected onto the column, the condition of the column is affected and the precision decreases. This was verified by injecting two validation runs, with and without unextracted samples. The run without the unextracted samples gave more accurate and precise results than the run that included the unextracted samples.

Acceptable room temperature extract stability was not demonstrated for the high QCs of PFOS, PFOSAA, POAA, and PFHS. Owing to the apparent instability of the room temperature extracts, the samples must be extracted and analyzed on the same day and the analytical run times must be less than 24 hours, which is the longest time period for an accepted validation run.

4.1. Proposed Sample Analysis Acceptance Criteria

Subject Sample Determination

Subject sample concentrations for all the analytes will be determined using the human serum calibration curve, unless an analyte concentration for PFOS, PFOSAA, POAA or PFHS is less that the adjusted LLOQ concentration. If a subject sample has a concentration for any of those analytes that is below the adjusted LLOQ, then the rabbit curve will be used to quantitate that analyte in that sample. In these cases, the accuracy of the rabbit serum curve will be verified by analyzing human serum QCs containing known analyte concentrations between 1 and 50 ppb and calculating their concentrations using the rabbit serum curve.

Calibration Curve

Each run will include calibration standards in duplicate at seven or more concentrations covering the lower to upper limit of quantitation. At least two thirds of the non-LLOQ calibration standard's back-calculated concentrations must be within ± 15% (LLOQ must be within ± 20%) of their individual target concentrations. If a standard is not within the acceptance criteria, it is deactivated. This process starts from the highest standard down to the lowest standard until all the active standards are within the acceptance criteria.

Lower Limit of Quantitation

The back-calculated concentrations of at least one of the duplicate lowest points in the calibration curve must be within ± 20% of the target concentration to qualify as the LLOQ. If this criterion is not met, the next level is subjected to the same test and the LLOQ raised accordingly.

Quality Control Samples

Each analytical run will include low and high QC samples in triplicate. The measured concentrations of at least two-thirds of all analytical QCs must be within ± 20 % of their target concentrations. If study samples require dilution, a dilution QC will be analyzed in triplicate for each dilution level. At least one dilution QC at each level must be within ± 20% of the target concentration. The dilution QC acceptance is independent of the undiluted analytical QC acceptance.

5. REFERENCES

[5.1] L. Clemen, G. Langenburg, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Blood Extracts Using HPLC-Electrospray/Mass Spectrometry," 3M Environmental Laboratory, FACT-M-4.1.

[5.2] L. Clemen, G. Langenburg, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals Compounds from Serum or Other Fluids for Analysis Using HPLC-Electrospray/Mass Spectrometry," 3M Environmental Laboratory, FACT-M-3.1.

[5.3] L. Clemen, "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Sulfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry," 3M Environmental Laboratory, FACT-M-1.0.

The raw data and final report for this study will be stored in the NWB Archives, 1121 East 3900 South, Salt Lake City, UT 84124 per regulations and contract agreement. 3M will be notified concerning final disposition of records at completion of contract obligations.

Table 1. Summary of Rabbit Serum Calibration Curves for PFOS

Linear (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	1.00	5.00	25.0	50.0	Slope	Intercept	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	0.896	5.42	20.0	53.1	1524	266	0.9950	1.00	50.0
		1.03	5.61	24.6	51.4					
01-Feb-1999	26	*0.671	4.44	24.3	47.2	1407	553	0.9950	1.00	50.0
		1.20	4.80	22.7	56.3					
02-Feb-1999	28	0.903	6.22	26.1	47.0	1414	657	0.9977	1.00	50.0
		0.887	4.65	26.3	50.0					
03-Feb-1999	29	0.826	4.96	21.7	51.9	1580	944	0.9966	1.00	50.0
		1.02	6.07	26.8	48.7					
Mean		0.928	5.27	24.1	50.7	1481	605	0.9961		
S.D.		0.146	0.619	2.27	2.95	74	243	0.0011		
%CV		15.8	11.7	9.4	5.8					
n		7	8	8	8	4	4	4		

* sample deactivated – poor instrument response

Table 2. Summary of Rabbit Serum Calibration Curves for PFOSAA

Linear (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	1.00	5.00	25.0	50.0	Slope	Intercept	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	0.857	5.41	19.9	55.0	212	-30	0.9921	1.00	50.0
		2.07	6.16	23.0	50.8					
01-Feb-1999	26	*	4.80	23.2	47.6	215	103	0.9967	1.00	50.0
		1.21	4.25	25.3	54.7					
02-Feb-1999	28	0.724	5.29	25.3	46.8	266	188	0.9962	1.00	50.0
		0.863	6.73	26.8	49.5					
03-Feb-1999	29	0.702	4.98	24.9	47.2	313	159	0.9979	1.00	50.0
		1.09	6.16	25.1	51.8					
Mean		1.07	5.47	24.2	50.4	252	105	0.9957		
S.D.		0.442	0.770	1.98	3.02	41	84	0.0022		
%CV		41.2	14.1	8.2	6.0					
n		7	8	8	8	4	4	4		

* sample deactivated – poor instrument response

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Table 3. Summary of Rabbit Serum Calibration Curves for POAA

Linear (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	1.00	5.00	25.0	50.0	Slope	Intercept	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	1.02	5.57	21.9	52.6	2543	81	0.9976	1.00	50.0
		0.917	5.22	24.2	50.5					
01-Feb-1999	26	*0.643	4.51	23.8	52.2	2782	318	0.9991	1.00	50.0
		1.13	5.01	24.3	50.1					
02-Feb-1999	28	0.835	5.56	23.4	48.2	2983	859	0.9985	1.00	50.0
		1.01	5.31	26.6	51.1					
03-Feb-1999	29	0.857	5.26	24.0	50.3	2988	1141	0.9994	1.00	50.0
		1.00	5.66	24.8	50.1					
Mean		0.925	5.26	24.1	50.6	2824	600	0.9987		
S.D.		0.139	0.352	1.22	1.27	182	421	0.0007		
%CV		15.0	6.7	5.1	2.5					
n		7	8	8	8	4	4	4		

* sample deactivated – poor instrument response

Table 4. Summary of Rabbit Serum Calibration Curves for PFHS

Linear (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	1.00	5.00	25.0	50.0	Slope	Intercept	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	0.833	5.87	22.5	52.9	2677	567	0.9955	1.00	50.0
		0.876	5.50	23.4	52.3					
01-Feb-1999	26	*0.742	4.85	23.2	50.7	2824	701	0.9990	1.00	50.0
		1.06	5.10	24.2	51.9					
02-Feb-1999	28	0.882	5.45	23.2	49.5	3013	629	0.9990	1.00	50.0
		1.08	4.94	25.4	51.6					
03-Feb-1999	29	0.862	5.12	23.8	49.2	3018	974	0.9994	1.00	50.0
		1.03	5.54	25.4	51.0					
Mean		0.921	5.30	23.9	51.1	2883	718	0.9982		
S.D.		0.113	0.326	1.00	1.22	142	155	0.0016		
%CV		12.3	6.2	4.2	2.4					
n		7	8	8	8	4	4	4		

* sample deactivated – poor instrument response

Table 5. Summary Data for Endogenous Analyte Concentrations in Human Serum Calibration Standards for Run 25

	PFOS	PFOSA	PFOSAA	N-MeFOSE-OH	N-EtFOSE-OH	PFHS	POAA
Replicate							
1	16.1	ND	1.60	ND	ND	0.497	3.08
2	15.4	ND	1.94	ND	ND	0.666	3.00
3	16.0	ND	0.943	ND	ND	0.696	3.18
4	17.3	ND	1.34	ND	ND	0.602	3.52
Mean	16.2	N/A	1.46	N/A	N/A	0.615	3.20
S.D.	0.796	N/A	0.421	N/A	N/A	0.088	0.228
% CV	4.9	N/A	28.9	N/A	N/A	14.3	7.1
n	4	4	4	4	4	4	4

ND = none detected
N/A = not applicable

Table 6. Summary Data for Endogenous Analyte Concentrations in Human Serum Calibration Standards for Runs 26, 28 and 29

	PFOS	PFOSA	PFOSAA	N-MeFOSE-OH	N-EtFOSE-OH	POAA	PFHS
Run 26							
1	25.4	ND	0.646	ND	ND	3.91	1.33
2	31.3	ND	1.07	ND	ND	5.04	1.66
3	27.5	ND	2.94	ND	ND	4.59	1.68
4	33.3	ND	1.55	ND	ND	4.79	1.68
Mean	29.4	N/A	1.55	N/A	N/A	4.58	1.59
S.D.	3.61	N/A	0.998	N/A	N/A	0.482	0.172
% CV	12.3	N/A	64.3	N/A	N/A	10.5	10.8
Run 28							
1	36.8	ND	2.88	ND	ND	4.22	1.79
2	35.8	ND	2.30	ND	ND	5.05	1.94
3	39.2	ND	1.25	ND	ND	4.97	2.01
4	27.8	ND	0.953	ND	ND	4.02	1.57
Mean	34.9	N/A	1.85	N/A	N/A	4.56	1.82
S.D.	4.93	N/A	0.901	N/A	N/A	0.521	0.195
% CV	14.1	N/A	48.7	N/A	N/A	11.4	10.7
Run 29							
1	23.4	ND	2.07	ND	ND	3.10	1.24
2	23.9	ND	1.23	ND	ND	2.97	1.26
Mean	23.6	N/A	1.65	N/A	N/A	3.04	1.25
S.D.	0.417	N/A	0.598	N/A	N/A	0.091	0.017
% CV	1.8	N/A	36.3	N/A	N/A	3.0	1.4
Overall Mean	30.4	N/A	1.69	N/A	N/A	4.27	1.61
Overall S.D.	5.66	N/A	0.814	N/A	N/A	0.767	0.269
Overall % CV	18.6	N/A	48.2	N/A	N/A	18.0	16.6

ND = none detected
N/A = not applicable

Table 7. Summary Data for Endogenous Analyte Concentrations in Human Serum Quality Control Samples for Runs 26, 28 and 29

	PFOS	PFOSA	PFOSAA	N-MeFOSE-OH	N-EtFOSE-OH	POAA	PFHS
Run 26							
1	21.6	ND	1.71	ND	ND	1.62	3.22
2	21.4	ND	1.69	ND	ND	1.99	2.85
3	21.0	ND	2.86	ND	ND	1.77	3.54
4	22.5	ND	3.13	ND	ND	2.13	3.32
5	23.4	ND	1.74	ND	ND	2.13	3.38
Mean	22.0	N/A	2.23	N/A	N/A	1.93	3.26
S.D.	0.982	N/A	0.706	N/A	N/A	0.225	0.258
% CV	4.5	N/A	31.7	N/A	N/A	11.7	7.9
Run 28							
1	18.2	ND	1.66	ND	ND	1.66	3.13
2	22.3	ND	1.95	ND	ND	2.02	3.11
3	20.7	ND	1.51	ND	ND	2.04	3.18
4	23.0	ND	1.53	ND	ND	2.13	3.33
5	22.1	ND	2.09	ND	ND	2.07	3.20
Mean	21.3	N/A	1.75	N/A	N/A	1.98	3.19
S.D.	1.91	N/A	0.258	N/A	N/A	0.187	0.087
% CV	0.1	N/A	0.1	N/A	N/A	0.1	0.0
Run 29							
1	21.8	ND	2.41	ND	ND	1.73	3.41
2	23.9	ND	4.01	ND	ND	2.15	3.55
3	22.8	ND	2.59	ND	ND	1.96	2.88
4	23.5	ND	2.50	ND	ND	1.92	3.24
5	26.2	ND	2.80	ND	ND	2.31	3.42
Mean	23.6	N/A	2.86	N/A	N/A	2.01	3.30
S.D.	1.63	N/A	0.658	N/A	N/A	0.224	0.258
% CV	6.9	N/A	23.0	N/A	N/A	11.1	7.8
Overall Mean	22.3	N/A	1.99	N/A	N/A	1.97	3.25
Overall S.D.	1.77	N/A	0.560	N/A	N/A	0.200	0.206
Overall % CV	7.9		28.2			10.2	6.3

ND = none detected
N/A = not applicable

Table 8. Summary of Human Serum Calibration Curve Parameters for PFOS

Quadratic (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	A*	B*	C*	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	-0.000002	0.003075	0.006751	0.9909	17.2	516
01-Feb-1999	26	-0.000002	0.002977	0.000357	0.9930	31.4	530
02-Feb-1999	28	-0.000001	0.001649	0.007710	0.9937	31.4	530
10-Feb-1999	32	-0.000003	0.004162	-0.009869	0.9960	31.4	530
Mean		-0.000002	0.002966	0.001237	0.9934		
S.D.		0.000001	0.001029	0.008092	0.0021		
n		4	4	4	4		

* A, B, and C are coefficients used to define the quadratic curve.

Table 9. Summary of Human Serum Calibration Curve Parameters for PFOSA

Quadratic (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	A*	B*	C*	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	-0.000002	0.003444	0.000622	0.9946	1.00	500
01-Feb-1999	26	-0.000002	0.003308	0.000757	0.9965	1.00	500
02-Feb-1999	28	-0.000001	0.001834	0.000789	0.9982	1.00	500
10-Feb-1999	32	-0.000002	0.003945	0.001714	0.9982	1.00	500
Mean		-0.000002	0.003133	0.000971	0.9969		
S.D.		0.000001	0.000908	0.000501	0.0017		
n		4	4	4	4		

* A, B, and C are coefficients used to define the quadratic curve.

Table 10. Summary of Human Serum Calibration Curve Parameters for PFOSAA

Quadratic (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	A*	B*	C*	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	0.000000	0.000676	-0.000208	0.9964	1.99	270
01-Feb-1999	26	-0.000001	0.000764	-0.000653	0.9971	2.23	271
02-Feb-1999	28	0.000000	0.000469	-0.000089	0.9963	2.23	271
10-Feb-1999	32	-0.000001	0.001170	-0.000458	0.9965	2.23	271
Mean		0.000000	0.000770	-0.000352	0.9966		
S.D.		0.000001	0.000294	0.000253	0.0004		
n		4	4	4	4		

* A, B, and C are coefficients used to define the quadratic curve.

Table 11. Summary of Human Serum Calibration Curve Parameters for N-MeFOSE-OH

Quadratic (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	A*	B*	C*	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	-0.000007	0.021767	-0.001921	0.9981	1.00	500
01-Feb-1999	26	-0.000004	0.012302	0.005797	0.9962	1.00	500
02-Feb-1999	28	0.000001	0.007315	0.001464	0.9970	1.00	500
10-Feb-1999	32	-0.000010	0.019016	0.003235	0.9970	1.00	500
Mean		-0.000005	0.015100	0.002144	0.9971		
S.D.		0.000005	0.006538	0.003241	0.0008		
n		4	4	4	4		

* A, B, and C are coefficients used to define the quadratic curve.

Table 12. Summary of Human Serum Calibration Curve Parameters for N-EtFOSE-OH

Quadratic (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	A*	B*	C*	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	-0.000002	0.015048	-0.003325	0.9994	1.00	500
01-Feb-1999	26	-0.000002	0.008104	0.002172	0.9954	1.00	500
02-Feb-1999	28	0.000000	0.005031	-0.005001	0.9986	5.00**	500
10-Feb-1999	32	-0.000004	0.011968	-0.001308	0.9969	1.00	500
Mean		-0.000002	0.010038	-0.001866	0.9976		
S.D.		0.000002	0.004383	0.003086	0.0018		
n		4	4	4	4		

* A, B, and C are coefficients used to define the quadratic curve.
** Both Standards at 1.00 ppb were statistical outliers, so the LLOQ was raised to 5.00 ppb.

Table 13. Summary of Human Serum Calibration Curve Parameters for POAA

Quadratic (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	A*	B*	C*	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	-0.000003	0.005601	0.000463	0.9972	4.20	503
01-Feb-1999	26	-0.000004	0.006274	-0.001033	0.9963	5.27	504
02-Feb-1999	28	-0.000002	0.004036	0.003976	0.9981	5.27	504
10-Feb-1999	32	-0.000005	0.008652	-0.001660	0.9970	5.27	504
Mean		-0.000004	0.006141	0.000437	0.9972		
S.D.		0.000001	0.001919	0.002522	0.0007		
n		4	4	4	4		

* A, B, and C are coefficients used to define the quadratic curve.

Table 14. Summary of Human Serum Calibration Curve Parameters for PFHS

Quadratic (weighted 1/x). All concentrations are expressed as ppb.

Run Date	Run Number	A*	B*	C*	Multiple Rsquared	LLOQ (ppb)	ULOQ (ppb)
30-Jan-1999	25	-0.000004	0.005961	0.002977	0.9979	1.62	501
01-Feb-1999	26	-0.000004	0.006088	0.000767	0.9977	2.61	502
02-Feb-1999	28	-0.000002	0.003814	0.003933	0.9982	2.61	502
10-Feb-1999	32	-0.000006	0.007750	0.002353	0.9973	2.61	502
Mean		-0.000004	0.005903	0.002508	0.9978		
S.D.		0.000002	0.001614	0.001330	0.0004		
n		4	4	4	4		

* A, B, and C are coefficients used to define the quadratic curve.

Table 15. Back-Calculated Concentrations of Human Serum Calibration Standards for PFOS

All concentrations are expressed as ppb.

Run Date	Run Number	17.2	21.2	26.2	31.4	35.4	40.4	41.2	55.4	66.2	80.4	116	130	280	516	530
30-Jan-1999	25	16.6	19.9	27.2				44.2		65.1		103			460	
		16.4	18.3	29.4				45.0		69.3		120			584	
01-Feb-1999	26				35.6	37.2	39.6		50.8		73.0		130			498
					32.8	32.4	*32.6		53.0		*56.4		143			563
02-Feb-1999	28				28.4	36.6	39.1		53.6		76.1		126	288		566
					34.6	35.7	42.1		62.6		68.5		135	290		486
10-Feb-1999	32				29.7	33.6	43.3		57.7		*65.5		140	278		501
					32.4	33.8	40.5		56.7		73.6		135	262		580
Mean		16.5	19.1	28.3	32.3	34.9	40.9	44.6	55.7	67.2	72.8	112	135	280	522	532
S.D.		0.141	1.13	1.56	2.77	1.90	1.75	0.566	4.20	2.97	3.17	12.0	6.24	12.8	87.7	41.6
%CV		0.9	5.9	5.5	8.6	5.4	4.3	1.3	7.5	4.4	4.4	10.7	4.6	4.6	16.8	7.8
%Bias		-4.1	-9.9	8.0	2.9	-1.4	1.2	8.3	0.5	1.5	-9.5	-3.4	3.8	0.0	1.2	0.4
n		2	2	2	6	6	5	2	6	2	4	2	6	4	2	6

* sample deactivated-statistical outlier

Table 16. Back-Calculated Concentrations of Human Serum Calibration Standards for PFOSA

All concentrations are expressed as ppb.

Run Date	Run Number	1.00	5.00	10.0	25.0	50.0	100	250	500
30-Jan-1999	25	0.818	4.39	10.0	25.3	51.0	86.9		456
		1.14	5.02	*11.8	28.4	54.3	103		550
01-Feb-1999	26	1.19	5.06	10.1	23.2	48.3	107		454
		0.929	4.93	9.82	23.4	*40.6	*120		550
02-Feb-1999	28	1.00	5.01	10.2	23.9	47.7	99.9	262	525
		1.05	4.80	*12.2	26.4	47.0	107	236	476
10-Feb-1999	32	0.998	4.72	*11.8	23.9	*41.4	*118	247	471
		0.989	4.94	11.0	24.8	49.7	108	241	538
Mean		1.01	4.86	10.2	24.9	49.7	102	247	503
S.D.		0.116	0.222	0.456	1.77	2.69	7.99	11.3	42.2
%CV		11.5	4.6	4.5	7.1	5.4	7.8	4.6	8.4
%Bias		1.0	-2.8	2.0	-0.4	-0.6	2.0	-1.2	0.6
n		8	8	5	8	6	6	4	8

* sample deactivated-statistical outlier

Table 17. Back-Calculated Concentrations of Human Serum Calibration Standards for PFOSAA

All concentrations are expressed as ppb.

Run Date	Run Number	1.99	2.23	4.15	4.38	6.84	7.07	14.9	15.1	28.4	28.6	55.3	55.5	136	270	271
30-Jan-1999	25	1.74		4.03		7.29		13.7		27.3		53.2			250	
		2.10		4.37		7.06		*20.1		30.6		56.7			291	
01-Feb-1999	26		*3.80		4.16		6.75		14.2		26.4		54.6			264
			2.47		4.53		7.95		13.3		*22.7		61.5			278
02-Feb-1999	28		2.08		4.39		*9.11		*18.3		28.1		57.0	140		294
			2.35		4.29		*8.77		*18.4		29.2		57.8	126		252
10-Feb-1999	32		2.19		3.94		7.94		13.0		25.4		63.5	131		264
			2.29		4.62		7.02		16.0		28.5		56.7	132		282
Mean		1.92	2.28	4.20	4.32	7.18	7.42	13.7	14.1	29.0	27.5	55.0	58.5	132	271	272
S.D.		0.255	0.149	0.240	0.249	0.163	0.622		1.35	2.33	1.57	2.47	3.32	5.80	29.0	15.1
%CV		13.3	6.5	5.7	5.8	2.3	8.4		9.6	8.0	5.7	4.5	5.7	4.4	10.7	5.6
%Bias		-3.5	2.2	1.2	-1.4	5.0	5.0	-8.1	-6.6	2.1	-3.8	-0.5	5.4	-2.9	0.4	0.4
n		2	5	2	6	2	4	1	4	2	5	2	6	4	2	6

* sample deactivated-statistical outlier

Table 18. Back-Calculated Concentrations of Human Serum Calibration Standards for N-MeFOSE-OH

All concentrations are expressed as ppb.

Run Date	Run Number	1.00	5.00	10.0	25.0	50.0	100	250	500
30-Jan-1999	25	1.18	4.54	9.73	22.8	47.7	*63.1		478
		0.862	*4.04	11.3	*30.7	53.4	101		522
01-Feb-1999	26	1.10	4.69	10.1	23.6	52.8	*132		463
		1.04	4.77	*7.56	24.2	*39.4	*152		538
02-Feb-1999	28	0.971	4.61	10.2	24.9	45.1	87.0	252	493
		1.13	4.72	11.3	*29.5	*37.3	106	265	*385
10-Feb-1999	32	0.945	*3.84	11.4	21.8	*40.2	103	268	460
		1.19	4.41	10.6	21.9	*36.5	98.6	240	537
Mean		1.05	4.62	10.7	23.2	49.8	99.1	256	499
S.D.		0.118	0.133	0.678	1.25	4.02	7.30	12.9	33.6
%CV		11.2	2.9	6.3	5.4	8.1	7.4	5.0	6.7
%Bias		5.0	-7.6	7.0	-7.2	-0.4	-0.9	2.4	-0.2
n		8	6	7	6	4	5	4	7

* sample deactivated-statistical outlier

Table 19. Back-Calculated Concentrations of Human Serum Calibration Standards for N-EtFOSE-OH

All concentrations are expressed as ppb.

Run Date	Run Number	1.00	5.00	10.0	25.0	50.0	100	250	500
30-Jan-1999	25	1.19	5.01	10.1	23.4	49.8	*60.9		491
		0.963	4.25	*12.0	*30.1	52.4	100		509
01-Feb-1999	26	1.11	4.65	10.0	23.7	52.5	*133		461
		1.03	4.75	*7.79	24.5	*38.1	*153		540
02-Feb-1999	28	*1.88	5.20	9.64	22.5	*40.9	*78.6	244	497
		*2.06	4.98	10.9	25.5	*34.0	96.1	264	*386
10-Feb-1999	32	1.16	4.38	*11.7	22.8	42.6	107	272	477
		1.14	4.75	11.3	22.2	*37.5	97.5	246	513
Mean		1.10	4.75	10.4	23.5	49.3	100	257	498
S.D.		0.0861	0.321	0.687	1.17	4.65	4.84	13.7	25.7
%CV		7.8	6.8	6.6	5.0	9.4	4.8	5.3	5.2
%Bias		10.0	-5.0	4.0	-6.0	-1.4	0.0	2.8	-0.4
n		6	8	5	7	4	4	4	7

* sample deactivated-statistical outlier

Table 20. Back-Calculated Concentrations of Human Serum Calibration Standards for POAA

All concentrations are expressed as ppb.

Run Date	Run Number	4.2	5.27	8.2	9.27	13.2	14.3	28.2	29.3	53.2	54.3	103	104	254	503	504
30-Jan-1999	25	4.49		7.26		13.4		27.6		51.7		100			461	
		4.30		7.69		14.0		29.6		53.3		107			549	
01-Feb-1999	26		6.09		9.12		13.9		*18.9		48.7		105			500
			5.14		9.55		14.1		26.7		*44.3		113			
02-Feb-1999	28		4.92		9.49		14.8		27.2		53.2		104	270		528
			5.05		9.39		15.6		31.0		52.1		106	239		481
10-Feb-1999	32		5.47		8.49		*16.9		27.3		*44.5		113	241		489
			5.20		9.59		14.4		28.7		55.6		113	235		541
Mean		4.40	5.31	7.48	9.27	13.7	14.6	28.6	28.2	52.5	52.4	104	109	246	505	508
S.D.		0.134	0.423	0.304	0.418	0.424	0.673	1.41	1.74	1.13	2.87	4.95	4.43	16.0	62.2	25.7
%CV		3.0	8.0	4.1	4.5	3.1	4.6	4.9	6.2	2.2	5.5	4.8	4.1	6.5	12.3	5.1
%Bias		4.8	0.8	-8.8	0.0	3.8	2.1	1.4	-3.8	-1.3	-3.5	1.0	4.8	-3.1	0.4	0.8
n		2	6	2	6	2	5	2	5	2	4	2	6	4	2	5

* sample deactivated-statistical outlier

Table 21. Back-Calculated Concentrations of Human Serum Calibration Standards for PFHS

All concentrations are expressed as ppb.

Run Date	Run Number	1.62	2.61	5.62	6.61	10.6	11.6	25.6	26.6	50.6	51.6	101	102	252	501	502
30-Jan-1999	25	1.49		5.65		11.3		26.2		49.2		98.1			458	
		1.50		5.31		12.1		26.1		50.5		102			554	
01-Feb-1999	26		2.68		7.13		12.0		24.0		48.7		102			478
			2.65		6.74		11.4		24.3		*39.0		111			525
02-Feb-1999	28		*1.82		6.86		12.2		25.3		50.3		103	250		522
			2.16		6.55		12.4		29.2		48.6		111	238		494
10-Feb-1999	32		2.28		6.27		13.3		25.6		*42.5		112	243		475
			2.59		6.79		12.2		25.6		50.0		106	234		566
Mean		1.50	2.47	5.48	6.72	11.7	12.3	26.2	25.7	49.9	49.4	100	108	241	506	510
S.D.		0.00707	0.236	0.240	0.291	0.566	0.619	0.0707	1.86	0.919	0.876	2.76	4.42	6.90	67.9	34.7
%CV		0.5	9.6	4.4	4.3	4.8	5.0	0.3	7.2	1.8	1.8	2.8	4.1	2.9	13.4	6.8
%Bias		-7.4	-5.4	-2.5	1.7	10.4	6.0	2.3	-3.4	-1.4	-4.3	-1.0	5.9	-4.4	1.0	1.6
n		2	5	2	6	2	6	2	6	2	4	2	6	4	2	6

* sample deactivated-statistical outlier

Table 22. Intra-Assay Precision for PFOS Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Number: 26 Run Date: 01-Feb-1999	Low QC (42.3 ppb)	High QC (372 ppb)
Replicate		
1	42.0	412
2	44.6	* 459
3	45.0	324
4	41.4	392
5	43.5	443
Mean	43.3	406
S.D.	1.57	52.8
CV%	3.6	13.0
%Theoretical	102.4	109.1
n	5	5

* > 20% theoretical

Table 23. Intra-Assay Precision for PFOSA Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Number: 26 Run Date: 01-Feb-1999	Low QC (20.0 ppb)	High QC (350 ppb)
Replicate		
1	21.8	387
2	*26.1	410
3	23.2	324
4	21.5	374
5	23.2	409
Mean	23.2	381
S.D.	1.82	35.2
CV%	7.8	9.2
%Theoretical	116.0	108.9
n	5	5

* > 20% theoretical

Table 24. Intra-Assay Precision for PFOSAA Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Number: 26 **Run Date: 01-Feb-1999**	**Low QC (12.7 ppb)**	**High QC (190 ppb)**
Replicate		
1	12.0	187
2	14.0	200
3	13.2	163
4	13.9	189
5	13.9	196
Mean	13.4	187
S.D.	0.846	14.4
%CV	6.3	7.7
%Theoretical	105.5	98.4
n	5	5

Table 25. Intra-Assay Precision for N-MeFOSE-OH Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Number: 26 Run Date: 01-Feb-1999	Low QC (20.0 ppb)	High QC (350 ppb)
Replicate		
1	23.2	*424
2	22.1	*425
3	23.2	336
4	21.7	*426
5	21.5	407
Mean	22.3	404
S.D.	0.814	38.6
CV%	3.7	9.6
%Theoretical	111.5	115.4
n	5	5

* > 20% theoretical

Table 26. Intra-Assay Precision for N-EtFOSE-OH Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Number: 26 Run Date: 01-Feb-1999	Low QC (20.0 ppb)	High QC (350 ppb)
Replicate		
1	23.1	*431
2	22.2	*431
3	22.7	341
4	21.4	*440
5	22.1	403
Mean	22.3	409
S.D.	0.644	40.6
CV%	2.9	9.9
%Theoretical	111.5	116.9
n	5	5

* > 20% theoretical

Table 27. Intra-Assay Precision for POAA Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Number: 26 Run Date: 01-Feb-1999	Low QC (23.3 ppb)	High QC (353 ppb)
Replicate		
1	21.8	366
2	24.0	374
3	22.2	*281
4	22.2	341
5	18.8	365
Mean	21.8	345
S.D.	1.88	38.1
CV%	8.6	11.0
%Theoretical	93.6	97.7
n	5	5

* > 20% theoretical

Table 28. Intra-Assay Precision for PFHS Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Number: 26 Run Date: 01-Feb-1999	Low QC (22.0 ppb)	High QC (352 ppb)
Replicate		
1	22.9	343
2	25.2	384
3	23.3	*278
4	23.3	332
5	24.9	360
Mean	23.9	339
S.D.	1.05	39.5
CV%	4.4	11.7
%Theoretical	108.6	96.3
n	5	5

* > 20% theoretical

Table 29. Inter-Assay Precision for PFOS Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (42.3 ppb)	High QC (372 ppb)
30-Jan-1999	25	40.9	414
		45.2	409
		**33.6	408
		**29.2	354
		41.2	398
01-Feb-1999	26	42.0	412
		44.6	**459
		45.0	324
		41.4	392
		43.5	443
02-Feb-1999	28	*19.0	**503
		38.9	401
		44.7	418
		43.2	397
		36.9	435
10-Feb-1999	32	40.7	352
		43.3	340
		41.9	377
		38.6	344
		41.5	355
Mean		40.9	397
S.D.		4.08	44.3
%CV		10.0	11.2
%Theoretical		96.7	106.7
%Bias		-3.3	6.7
n		19	20

* sample deactivated – poor instrument response
** > 20% theoretical

Table 30. Inter-Assay Precision for PFOSA Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (20.0 ppb)	High QC (350 ppb)
30-Jan-1999	25	23.9	386
		**24.7	357
		17.8	410
		**14.2	343
		21.5	381
01-Feb-1999	26	21.8	387
		**26.1	410
		23.2	324
		21.5	374
		23.2	409
02-Feb-1999	28	*10.5	**456
		20.5	342
		20.9	370
		22.2	339
		19.2	388
10-Feb-1999	32	20.7	338
		23.4	329
		20.7	347
		17.0	331
		20.1	317
Mean		21.2	367
S.D.		2.81	36.5
%CV		13.3	9.9
%Theoretical		106.0	104.9
%Bias		6.0	4.9
n		19	20

* sample deactivated– poor instrument response
** > 20% theoretical

Table 31. Inter-Assay Precision for PFOSAA Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (12.7 ppb)	High QC (190 ppb)
30-Jan-1999	25	**16.6	223
		13.6	225
		11.1	**246
		**9.97	208
		14.0	213
01-Feb-1999	26	12.0	187
		14.0	200
		13.2	163
		13.9	189
		13.9	196
02-Feb-1999	28	*9.11	212
		12.2	186
		14.8	194
		13.9	177
		11.8	189
10-Feb-1999	32	13.4	202
		14.3	195
		12.5	205
		10.9	192
		12.2	175
Mean		13.1	199
S.D.		1.56	19.1
%CV		11.9	9.6
%Theoretical		103.1	104.7
%Bias		3.1	4.7
n		19	20

* sample deactivated– poor instrument response
** > 20% theoretical

Table 32. Inter-Assay Precision for N-MeFOSE-OH Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (20.0 ppb)	High QC (350 ppb)
30-Jan-1999	25	16.4	310
		19.0	316
		**15.2	355
		**12.2	301
		17.4	311
01-Feb-1999	26	23.2	**424
		22.1	**425
		23.2	336
		21.7	**426
		21.5	407
02-Feb-1999	28	*11.6	**446
		23.6	352
		**28.8	383
		**26.5	335
		22.8	401
10-Feb-1999	32	**14.8	**252
		16.0	**244
		**15.0	328
		**11.5	315
		**14.7	292
Mean		19.2	348
S.D.		4.90	58.9
%CV		25.5	16.9
%Theoretical		96.0	99.4
%Bias		-4.0	-0.6
n		19	20

* sample deactivated-- poor instrument response
** > 20% theoretical

Table 33. Inter-Assay Precision for N-EtFOSE-OH Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (20.0 ppb)	High QC (350 ppb)
30-Jan-1999	25	**15.5	316
		18.7	308
		**15.0	340
		**12.1	294
		17.3	316
01-Feb-1999	26	23.1	**431
		22.2	**431
		22.7	341
		21.4	**440
		22.1	403
02-Feb-1999	28	*11.4	**436
		23.0	329
		23.5	363
		23.1	332
		21.2	378
10-Feb-1999	32	**15.1	**251
		16.8	**254
		**15.4	339
		**12.5	314
		**14.9	301
Mean		18.7	346
S.D.		3.97	57.6
%CV		21.2	16.6
%Theoretical		93.5	98.9
%Bias		-6.5	-1.1
n		19	20

* sample deactivated– poor instrument response
** > 20% theoretical

Table 34. Inter-Assay Precision for POAA Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (23.3 ppb)	High QC (353 ppb)
30-Jan-1999	25	**28.0	349
		24.4	396
		**18.3	377
		**17.4	318
		24.2	379
01-Feb-1999	26	21.8	366
		24.0	374
		22.2	**281
		22.2	341
		18.8	365
02-Feb-1999	28	*11.6	376
		23.1	325
		24.8	345
		22.0	324
		19.1	351
10-Feb-1999	32	23.5	348
		25.2	321
		22.1	321
		19.5	326
		22.6	302
Mean		22.3	344
S.D.		2.70	29.5
%CV		12.1	8.6
%Theoretical		95.7	97.5
%Bias		-4.3	-2.5
n		19	20

* sample deactivated– poor instrument response
** > 20% theoretical

Table 35. Inter-Assay Precision for PFHS Human Serum Quality Control Samples

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (22.0 ppb)	High QC (352 ppb)
30-Jan-1999	25	**26.6	379
		24.4	391
		18.7	370
		**17.1	328
		24.0	361
01-Feb-1999	26	22.9	343
		25.2	384
		23.3	**278
		23.3	332
		24.9	360
02-Feb-1999	28	*11.9	393
		23.1	359
		23.1	382
		23.3	366
		21.0	377
10-Feb-1999	32	21.7	357
		22.6	344
		22.6	354
		20.8	300
		21.4	308
Mean		22.6	353
S.D.		2.22	31.1
%CV		9.8	8.8
%Theoretical		102.7	100.3
%Bias		2.7	0.3
n		19	20

* sample deactivated– statistical outlier
** > 20% theoretical

Table 36. System Precision for PFOS

Run Date	Run Number	Unextracted Low QC (20.0 ppb)	Unextracted High QC (350 ppb)
		Instrument Response	
10-Feb-1999	32	0.101895	1.622610
		0.141723	1.870562
		0.169329	1.990953
Mean		0.137649	1.828042
S.D.		0.033901	0.187817
%CV		24.6	10.3
n		3	3

Table 37. System Precision for PFOSA

Run Date	Run Number	Unextracted Low QC (20.0 ppb)	Unextracted High QC (350 ppb)
		Instrument Response	
10-Feb-1999	32	0.006447	0.207807
		0.006939	0.558348
		0.018200	0.347834
Mean		0.010529	0.371330
S.D.		0.006648	0.176448
%CV		63.1	47.5
n		3	3

Table 38. System Precision for PFOSAA

Run Date	Run Number	Unextracted Low QC (12.7 ppb)	Unextracted High QC (190 ppb)
		Instrument Response	
10-Feb-1999	32	0.017247	0.247510
		0.015952	0.338792
		0.029981	0.357733
Mean		0.021060	0.314678
S.D.		0.007753	0.058935
%CV		36.8	18.7
n		3	3

Table 39. System Precision for N-MeFOSE-OH

Run Date	Run Number	Unextracted Low QC (20.0 ppb)	Unextracted High QC (350 ppb)
		Instrument Response	
10-Feb-1999	32	0.015063	0.583610
		0.010676	2.230467
		0.032584	1.338583
Mean		0.019441	1.384220
S.D.		0.011592	0.824376
%CV		59.6	59.6
n		3	3

Table 40. System Precision for N-EtFOSE-OH

Run Date	Run Number	Unextracted Low QC (20.0 ppb)	Unextracted High QC (350 ppb)
		Instrument Response	
10-Feb-1999	32	0.011565	0.525858
		0.008637	2.211409
		0.034819	1.323620
Mean		0.018340	1.353629
S.D.		0.014346	0.843176
%CV		78.2	62.3
n		3	3

Table 41. System Precision for POAA

Run Date	Run Number	Unextracted Low QC (20.0 ppb)	Unextracted High QC (350 ppb)
		Instrument Response	
10-Feb-1999	32	0.148240	2.629881
		0.197618	3.363888
		0.313308	3.570008
Mean		0.219722	3.187926
S.D.		0.084725	0.494147
%CV		38.6	15.5
n		3	3

Table 42. System Precision for PFHS

Run Date	Run Number	Unextracted Low QC (20.0 ppb)	Unextracted High QC (350 ppb)
		Instrument Response	
10-Feb-1999	32	0.179242	2.770742
		0.244403	3.162425
		0.295965	3.428575
Mean		0.239870	3.120581
S.D.		0.058493	0.330907
%CV		24.4	10.6
n		3	3

Table 43. PFOS Extraction Efficiency

Time of Spiking	Analyte Peak Areas	Istd Peak Areas
1. Analyte and IS after extraction	81470	539547
	86784	554615
	77797	523549
Mean	82017	539237
2. Analyte prior and IS after extraction	61442	
	94927	
	54973	
Mean	70447	
3. IS prior and Analyte after extraction		200162
		461938
		490278
Mean		384126

Mean extraction efficiency for the analyte = 85.9%
Mean extraction efficiency for the internal standard = 71.2%

Table 44. PFOSA Extraction Efficiency

Time of Spiking	Analyte Peak Areas	Istd Peak Areas
1. Analyte and IS after extraction	45280	539547
	45068	554615
	38724	523549
Mean	43024	539237
2. Analyte prior and IS after extraction	33229	
	47251	
	25711	
Mean	35397	
3. IS prior and Analyte after extraction		200162
		461938
		490278
Mean		384126

Mean extraction efficiency for the analyte = 82.3%
Mean extraction efficiency for the internal standard = 71.2%

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Table 45. PFOSAA Extraction Efficiency

Time of Spiking	Analyte Peak Areas	Istd Peak Areas
1. Analyte and IS after extraction	6484	539547
	6197	554615
	6383	523549
Mean	6355	539237
2. Analyte prior and IS after extraction	4229	
	5443	
	3961	
Mean	4544	
3. IS prior and Analyte after extraction		200162
		461938
		490278
Mean		384126

Mean extraction efficiency for the analyte = 71.5%
Mean extraction efficiency for the internal standard = 71.2%

Table 46. N-MeFOSE-OH Extraction Efficiency

Time of Spiking	Analyte Peak Areas	Istd Peak Areas
1. Analyte and IS after extraction	257654	539547
	262358	554615
	154951	523549
Mean	224988	539237
2. Analyte prior and IS after extraction	85560	
	226651	
	45573	
Mean	119261	
3. IS prior and Analyte after extraction		200162
		461938
		490278
Mean		384126

Mean extraction efficiency for the analyte = 53.0%
Mean extraction efficiency for the internal standard = 71.2%

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Table 47. N-EtFOSE-OH Extraction Efficiency

Time of Spiking	Analyte Peak Areas	Istd Peak Areas
1. Analyte and IS after extraction	191529	539547
	190650	554615
	122094	523549
Mean	168091	539237
2. Analyte prior and IS after extraction	52797	
	139842	
	32402	
Mean	75014	
3. IS prior and Analyte after extraction		200162
		461938
		490278
Mean		384126

Mean extraction efficiency for the analyte = 44.6%
Mean extraction efficiency for the internal standard = 71.2%

Table 48. POAA Extraction Efficiency

Time of Spiking	Analyte Peak Areas	Istd Peak Areas
1. Analyte and IS after extraction	87822	539547
	84427	554615
	75701	523549
Mean	82650	539237
2. Analyte prior and IS after extraction	76904	
	83445	
	62754	
Mean	74368	
3. IS prior and Analyte after extraction		200162
		461938
		490278
Mean		384126

Mean extraction efficiency for the analyte = 90.0%
Mean extraction efficiency for the internal standard = 71.2%

Table 49. PFHS Extraction Efficiency

Time of Spiking	Analyte Peak Areas	Istd Peak Areas
1. Analyte and IS after extraction	87124	539547
	86589	554615
	82852	523549
Mean	85522	539237
2. Analyte prior and IS after extraction	66595	
	81391	
	61113	
Mean	69700	
3. IS prior and Analyte after extraction		200162
		461938
		490278
Mean		384126

Mean extraction efficiency for the analyte = 81.5%
Mean extraction efficiency for the internal standard = 71.2%

Table 50. Freeze/Thaw Stability for PFOS

All concentrations are expressed as ppb.

Run Number	25
Run Date	30-Jan-1999
	LOW QC
	After 3 Freeze/Thaw cycles
1	43.3
2	33.1
3	40.5
Mean	39.0
S.D.	5.27
% CV	13.5%
Control (ng/mL)	38.0
Mean % Dev. from Control	**2.54%**
	HIGH QC
	After 3 Freeze/Thaw cycles
1	330
2	315*
3	410
Mean	370
S.D.	56.6
% CV	15.3%
Control (ng/mL)	397
Mean % Dev. from Control	**-6.80%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 51. Freeze/Thaw Stability for PFOSA

All concentrations are expressed as ppb.

Run Number	25
Run Date	30-Jan-1999
	LOW QC
	After 3 Freeze/Thaw cycles
1	23.5
2	19.9
3	20.0
Mean	21.1
S.D.	2.05
% CV	9.70%
Control (ng/mL)	20.4
Mean % Dev. from Control	**3.59%**
	HIGH QC
	After 3 Freeze/Thaw cycles
1	336
2	248*
3	401
Mean	369
S.D.	46.0
% CV	12.5%
Control (ng/mL)	375
Mean % Dev. from Control	**-1.73%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 52. Freeze/Thaw Stability for PFOSAA

All concentrations are expressed as ppb.

Run Number	**25**
Run Date	**30-Jan-1999**
	LOW QC
	After 3 Freeze/Thaw cycles
1	13.6
2	12.5
3	12.0
Mean	12.7
S.D.	0.819
% CV	6.45%
Control (ng/mL)	13.1
Mean % Dev. from Control	**-3.05%**
	HIGH QC
	After 3 Freeze/Thaw cycles
1	205
2	*180
3	**237
Mean	221
S.D.	22.6
% CV	10.2%
Control (ng/mL)	223
Mean % Dev. from Control	**-0.897%**

* sample deactivated – poor instrument response
** > ±20% theoretical
Control = mean of reference QC samples

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Table 53. Freeze/Thaw Stability for N-MeFOSE-OH

All concentrations are expressed as ppb.

Run Number	25
Run Date	30-Jan-1999
	LOW QC
	After 3 Freeze/Thaw cycles
1	18.9
2	11.8
3	15.5
Mean	15.4
S.D.	3.55
% CV	23.1%
Control (ng/mL)	16.0
Mean % Dev. from Control	**-3.75%**
	HIGH QC
	After 3 Freeze/Thaw cycles
1	320
2	213*
3	324
Mean	322
S.D.	2.83
% CV	0.878%
Control (ng/mL)	319
Mean % Dev. from Control	**0.940%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 54. Freeze/Thaw Stability for N-EtFOSE-OH

All concentrations are expressed as ppb.

Run Number	25
Run Date	30-Jan-1999
	LOW QC
	After 3 Freeze/Thaw cycles
1	19.1
2	11.4
3	15.9
Mean	15.5
S.D.	3.87
% CV	25.0%
Control (ng/mL)	15.7
Mean % Dev. from Control	**-1.49%**
	HIGH QC
	After 3 Freeze/Thaw cycles
1	302
2	214*
3	307
Mean	305
S.D.	3.54
% CV	1.16%
Control (ng/mL)	315
Mean % Dev. from Control	**-3.33%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 55. Freeze/Thaw Stability for POAA

All concentrations are expressed as ppb.

Run Number	**25**
Run Date	**30-Jan-1999**
	LOW QC
	After 3 Freeze/Thaw cycles
1	25.3
2	23.8
3	25.5
Mean	24.9
S.D.	0.929
% CV	3.74%
Control (ng/mL)	22.5
Mean % Dev. from Control	**10.5%**
	HIGH QC
	After 3 Freeze/Thaw cycles
1	330
2	144*
3	385
Mean	358
S.D.	38.9
% CV	10.9%
Control (ng/mL)	364
Mean % Dev. from Control	**-1.79%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 56. Freeze/Thaw Stability for PFHS

All concentrations are expressed as ppb.

Run Number	25
Run Date	30-Jan-1999
	LOW QC
	After 3 Freeze/Thaw cycles
1	24.4
2	21.7
3	23.5
Mean	23.2
S.D.	1.37
% CV	5.93%
Control (ng/mL)	22.2
Mean % Dev. from Control	**4.50%**
	HIGH QC
	After 3 Freeze/Thaw cycles
1	310
2	145*
3	365
Mean	338
S.D.	38.9
% CV	11.5%
Control (ng/mL)	366
Mean % Dev. from Control	**-7.79%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 57. Room Temperature Matrix Stability for PFOS

All concentrations are expressed as ppb.

Run Number	32	
Run Date	10-Feb-1999	
	LOW QC	
	2 Hour	*4 Hour*
1	39.5	38.4
2	41.0	36.9
3	40.8	37.5
Mean	40.4	37.6
S.D.	0.814	0.755
% CV	2.01%	2.01%
Control (ng/mL)	41.2	41.2
Mean % Dev. from Control	**-1.94%**	**-8.74%**
	HIGH QC	
	2 Hour	*4 Hour*
1	322	343
2	271	173*
3	276	349
Mean	290	346
S.D.	28.1	4.24
% CV	9.69%	1.23%
Control (ng/mL)	354	354
Mean % Dev. from Control	**-18.1%**	**-2.26%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 58. Room Temperature Matrix Stability for PFOSA

All concentrations are expressed as ppb.

Run Number	32	
Run Date	10-Feb-1999	
	LOW QC	
	2 Hour	*4 Hour*
1	20.0	18.7
2	20.3	17.2
3	20.7	18.2
Mean	20.3	18.0
S.D.	0.351	0.764
% CV	1.73%	4.24%
Control (ng/mL)	20.4	20.4
Mean % Dev. from Control	**-0.490%**	**-11.8%**
	HIGH QC	
	2 Hour	*4 Hour*
1	310	317
2	261	174*
3	276	336
Mean	282	327
S.D.	25.1	13.4
% CV	8.89%	4.1%
Control (ng/mL)	332	332
Mean % Dev. from Control	**-15.0%**	**-1.51%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 59. Room Temperature Matrix Stability for PFOSAA

All concentrations are expressed as ppb.

Run Number	32	
Run Date	10-Feb-1999	
	LOW QC	
	2 Hour	*4 Hour*
1	12.8	11.5
2	13.3	11.5
3	13.3	10.3
Mean	13.1	11.1
S.D.	0.289	0.693
% CV	2.21%	6.24%
Control (ng/mL)	12.7	12.7
Mean % Dev. from Control	**3.15%**	**-12.6%**
	HIGH QC	
	2 Hour	*4 Hour*
1	179	200
2	**138	*103
3	152	185
Mean	156	193
S.D.	20.8	10.6
% CV	13.3%	5.49%
Control (ng/mL)	194	194
Mean % Dev. from Control	**-19.6%**	**-0.515%**

* sample deactivated – poor instrument response
** > ±20% theoretical
Control = mean of reference QC samples

Table 60. Room Temperature Matrix Stability for N-MeFOSE-OH

All concentrations are expressed as ppb.

Run Number	**32**	
Run Date	**10-Feb-1999**	
	LOW QC	
	2 Hour	***4 Hour***
1	14.3	10.5
2	14.9	12.5
3	13.0	10.8
Mean	14.1	11.3
S.D.	0.971	1.08
% CV	6.90%	9.57%
Control (ng/mL)	14.4	14.4
Mean % Dev. from Control	**-2.31%**	**-21.8%**
	HIGH QC	
	2 Hour	***4 Hour***
1	248	218
2	199	131*
3	197	335
Mean	215	277
S.D.	28.9	82.7
% CV	13.5%	29.9%
Control (ng/mL)	286	286
Mean % Dev. from Control	**-24.9%**	**-3.15%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 61. Room Temperature Matrix Stability for N-EtFOSE-OH

All concentrations are expressed as ppb.

Run Number	32	
Run Date	10-Feb-1999	
	LOW QC	
	2 Hour	*4 Hour*
1	15.1	11.9
2	14.8	12.7
3	13.1	12.0
Mean	14.3	12.2
S.D.	1.08	0.436
% CV	7.52%	3.57%
Control (ng/mL)	14.9	14.9
Mean % Dev. from Control	**-3.80%**	**-18.12%**
	HIGH QC	
	2 Hour	*4 Hour*
1	255	235
2	224	139*
3	210	339
Mean	230	287
S.D.	23.0	73.5
% CV	10.0%	25.6%
Control (ng/mL)	292	292
Mean % Dev. from Control	**-21.3%**	**-1.71%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 62. Room Temperature Matrix Stability for POAA

All concentrations are expressed as ppb.

Run Number	**32**	
Run Date	**10-Feb-1999**	
	LOW QC	
	2 Hour	***4 Hour***
1	22.3	20.9
2	22.8	20.1
3	24.4	20.1
Mean	23.2	20.4
S.D.	1.10	0.462
% CV	4.74%	2.27%
Control (ng/mL)	22.6	22.6
Mean % Dev. from Control	**2.51%**	**-9.88%**
	HIGH QC	
	2 Hour	***4 Hour***
1	313	327
2	221	171*
3	280	308
Mean	271	318
S.D.	46.6	13.4
% CV	17.2%	4.21%
Control (ng/mL)	324	324
Mean % Dev. from Control	**-16.3%**	**-1.85%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 63. Room Temperature Matrix Stability for PFHS

All concentrations are expressed as ppb.

Run Number	32	
Run Date	10-Feb-1999	
	LOW QC	
	2 Hour	*4 Hour*
1	20.8	21.5
2	21.3	19.5
3	23.9	21.1
Mean	22.0	20.7
S.D.	1.66	1.06
% CV	7.57%	5.11%
Control (ng/mL)	21.8	21.8
Mean % Dev. from Control	**0.917%**	**-5.05%**
	HIGH QC	
	2 Hour	*4 Hour*
1	333	341
2	251	172*
3	281	306
Mean	288	324
S.D.	41.5	24.7
% CV	14.4%	7.62%
Control (ng/mL)	333	333
Mean % Dev. from Control	**-13.4%**	**-2.70%**

* sample deactivated – poor instrument response
Control = mean of reference QC samples

Table 64. Room Temperature Extract Stability for PFOS

All concentrations are expressed as ppb.

Run Number	32
Run Date	10-Feb-1999
	LOW QC *After 24 Hours*
1	35.9
2	36.5
3	32.3
Mean	34.9
S.D.	2.27
% CV	6.51%
Control (ng/mL)	41.2
Mean % Dev. from Control	**-15.3%**
	HIGH QC *After 24 Hours*
1	300
2	235
3	271
Mean	269
S.D.	32.6
% CV	12.1%
Control (ng/mL)	354
Mean % Dev. from Control	**-24.1%**

Control = mean of reference QC samples

Table 65. Room Temperature Extract Stability for PFOSA

All concentrations are expressed as ppb.

Run Number	**32**
Run Date	**10-Feb-1999**
	LOW QC *After 24 Hours*
1	19.7
2	19.4
3	16.1
Mean	18.4
S.D.	1.997
% CV	10.9%
Control (ng/mL)	20.4
Mean % Dev. from Control	**-9.80%**
	HIGH QC *After 24 Hours*
1	311
2	239
3	270
Mean	273
S.D.	36.12
% CV	13.2%
Control (ng/mL)	332
Mean % Dev. from Control	**-17.7%**

Control = mean of reference QC samples

Table 66. Room Temperature Extract Stability for PFOSAA

All concentrations are expressed as ppb.

Run Number	32
Run Date	10-Feb-1999
	LOW QC *After 24 Hours*
1	12.6
2	11.7
3	10.2
Mean	11.5
S.D.	1.21
% CV	10.5%
Control (ng/mL)	12.7
Mean % Dev. from Control	-9.45%
	HIGH QC *After 24 Hours*
1	167
2	*133
3	*147
Mean	149
S.D.	17.1
% CV	11.5%
Control (ng/mL)	194
Mean % Dev. from Control	-23.2%

* > ±20% theoretical
Control = mean of reference QC samples

Table 67. Room Temperature Extract Stability for N-MeFOSE-OH

All concentrations are expressed as ppb.

Run Number	32
Run Date	10-Feb-1999
	LOW QC *After 24 Hours*
1	12.8
2	13.5
3	10.1
Mean	12.1
S.D.	1.80
% CV	14.8%
Control (ng/mL)	14.4
Mean % Dev. from Control	-15.7%
	HIGH QC *After 24 Hours*
1	276
2	257
3	242
Mean	258
S.D.	17.0
% CV	6.60%
Control (ng/mL)	286
Mean % Dev. from Control	-9.67%

Control = mean of reference QC samples

000077

Table 68. Room Temperature Extract Stability for N-EtFOSE-OH

All concentrations are expressed as ppb.

Run Number	**32**
Run Date	**10-Feb-1999**
	LOW QC *After 24 Hours*
1	12.8
2	13.5
3	10.5
Mean	12.3
S.D.	1.57
% CV	12.8%
Control (ng/mL)	14.9
Mean % Dev. from Control	-17.7%
	HIGH QC *After 24 Hours*
1	289
2	265
3	247
Mean	267
S.D.	21.1
% CV	7.89%
Control (ng/mL)	292
Mean % Dev. from Control	**-8.56%**

Control = mean of reference QC samples

Table 69. Room Temperature Extract Stability for POAA

All concentrations are expressed as ppb.

Run Number	32
Run Date	10-Feb-1999
	LOW QC *After 24 Hours*
1	21.7
2	22.4
3	19.1
Mean	21.1
S.D.	1.74
% CV	8.25%
Control (ng/mL)	22.6
Mean % Dev. from Control	-6.78%
	HIGH QC *After 24 Hours*
1	290
2	222
3	247
Mean	253
S.D.	34.4
% CV	13.6%
Control (ng/mL)	324
Mean % Dev. from Control	-21.9%

Control = mean of reference QC samples

000079

Table 70. Room Temperature Extract Stability for PFHS

All concentrations are expressed as ppb.

Run Number	**32**
Run Date	**10-Feb-1999**
	LOW QC *After 24 Hours*
1	20.7
2	19.9
3	18.1
Mean	19.6
S.D.	1.33
% CV	6.81%
Control (ng/mL)	21.8
Mean % Dev. from Control	**-10.2%**
	HIGH QC *After 24 Hours*
1	283
2	216
3	241
Mean	247
S.D.	33.9
% CV	13.7%
Control (ng/mL)	333
Mean % Dev. from Control	**-25.9%**

Control = mean of reference QC samples

Table 71. Stock Solution Stability for PFOS

	Analyte Peak Area
Prepared day of analytical run	121195
	128494
	125134
Mean	124941
SD	3653
% CV	2.92
n	3
Stored for 24 days	143208
	132499
	122485
Mean	132731
SD	10363
% CV	7.81
n	3
% Difference	6.23%

Table 72. Stock Solution Stability for PFOSA

	Analyte Peak Area
Prepared day of analytical run	41058
	34117
	84507
Mean	53227
SD	27310
% CV	51.3
n	3
Stored for 24 days	63582
	58872
	46572
Mean	56342
SD	8783
% CV	15.6
n	3
% Difference	5.85%

Table 73. Stock Solution Stability for PFOSAA

	Analyte Peak Area
Prepared day of analytical run	14044
	15654
	15150
Mean	14949
SD	824
% CV	5.51
n	3
Stored for 24 days	17264
	16136
	13572
Mean	15657
SD	1892
% CV	12.1
n	3
% Difference	4.74%

Table 74. Stock Solution Stability for N-MeFOSE-OH

	Analyte Peak Area
Prepared day of analytical run	115190
	90190
	52253
Mean	85878
SD	31689
% CV	36.9
n	3
Stored for 24 days	169447
	175317
	125816
Mean	156860
SD	27045
% CV	17.2
n	3
% Difference	82.7%

Table 75. Stock Solution Stability for N-EtFOSE-OH

	Analyte Peak Area
Prepared day of analytical run	113772
	87610
	52034
Mean	84472
SD	30988
% CV	36.7
n	3
Stored for 24 days	160189
	166768
	115012
Mean	147323
SD	28175
% CV	19.1
n	3
% Difference	74.4%

Table 76. Stock Solution Stability for POAA

	Analyte Peak Area
Prepared day of analytical run	200388
	206992
	205145
Mean	204175
SD	3407
% CV	1.67
n	3
Stored for 24 days	200970
	200039
	183527
Mean	194845
SD	9813
% CV	5.04
n	3
% Difference	-4.57%

Table 77. Stock Solution Stability for PFHS

	Analyte Peak Area
Prepared day of analytical run	212505
	221778
	218307
Mean	217530
SD	4685
% CV	2.15
n	3
Stored for 24 days	230603
	224907
	200109
Mean	218540
SD	16214
% CV	7.42
n	3
% Difference	0.464%

6. ANALYTICAL METHOD

Principles of the Method

After the addition of HPLC-grade water and sodium dodecyl sulfate (SDS, internal standard) to 0.20 mL of human or rabbit serum, the serum mixture is made basic with the addition of 0.5 M tetrabutylammonium hydrogen sulfate (TBA, pH 10) and 0.25 M carbonate buffer. This mixture is then extracted with methyl-tert-butyl ether. After sufficient mixing, the sample is centrifuged. The organic layer is transferred via pipette into a clean test tube. The organic layer is then evaporated to dryness and the sample is reconstituted into 2 mM ammonium acetate water:methanol (50:50 v/v). The extracts are then analyzed by liquid chromatography/tandem mass spectrometry using negative-ion electrospray ionization and multiple reaction monitoring.

CHEMICAL FORMULAS

Abbreviation	Chemical Name	Formula
PFOS	Perfluorooctane sulfonate	$C_8F_{17}SO_3^-$
PFOSA	Perfluorooctane sulfonylamide	$C_8F_{17}SO_2NH_2$
PFOSAA	Perfluorooctane sulfonylamido(ethyl)acetate	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$
PFHS	Perfluorohexane sulfonate	$C_6F_{13}SO_3^-$
POAA	Perfluorooctanoate	$C_7F_{15}CO_2^-$
N-EtFOSE-OH	2(N-ethylperfluorooctanesulfonamidoethanol	$C_8F_{17}\ SO_2\ N(CH_2CH_3)(CH_2CH_2OH)$
N-MeFOSE-OH	2(N-methylperfluorooctanesulfonamidoethanol	$C_8F_{17}\ SO_2\ N\ (CH_3)(CH_2CH_2OH)$
SDS	Dodecyl sulfate (sodium)	$CH_3(CH_2)_{10}\ CH_2\ OSO_3^-$

6.1. Reference Materials and Matrices

Reference Material	*Lot Number*	*Purity*	*Expiration Date*	*Source*	*Storage Conditions*
PFOS	193	100%	12/31/2010	3M	Room Temperature
PFOSA	214	100%	12/31/2010	3M	Room Temperature
PFOSAA	617	53.8%	12/31/2010	3M	Room Temperature
N-MeFOSE-OH	212	100%	12/31/2010	3M	Room Temperature
N-EtFOSE-OH	936	100%	12/31/2010	3M	Room Temperature

Reference Material	*Lot Number*	*Purity*	*Expiration Date*	*Source*	*Storage Conditions*
POAA	245	100%	12/31/2010	3M	Room Temperature
PFHS (in methanol)	S398-182	100% (6200 ppm)	12/31/2010	3M	- 20 °C
SDS (sodium dodecyl sulfate)	17H0459	91.0%	1/16/2001	Sigma	Room Temperature

Matrix	*Source*
Human serum	Biochemed
Rabbit serum	Biochemed

6.2. Chemicals and Equipment

Chemicals
Ammonium Acetate, 99.9 %
Water, HPLC-grade
Methanol, HPLC-grade
Methyl-*tert*-Butyl Ether, HPLC-grade
Sodium Hydroxide, 98.4%
Sodium Carbonate, 101.0%
Sodium Bicarbonate, 100.3%
Tetrabutylammonium Hydrogen Sulfate (TBA), approx. 97%

Equipment / Supplies	
Model	*Manufacturer*
Balance: Mettler Toledo AT261	Mettler-Toledo, Inc., Hightstown, NJ
Balance: Mettler Toledo MT5	Mettler-Toledo, Inc., Hightstown, NJ
Centrifuge: Beckman GS-6R	Beckman Instruments, Fullerton, CA
Evaporator: Turbo Vap LV, Model 43750	Zymark Corp., Hopkinton, MA
Liquid Chromatograph: Hewlett Packard 1100	Hewlett Packard, Palo Alto, CA
Mass Spectrometer: Perkin Elmer Sciex API 3000	Perkin Elmer Sciex, Thornhill,

Equipment / Supplies	
Model	*Manufacturer*
	Ontario
pH Meter: Orion 230A	Orion, Boston, MA
Pipettes: Finnpipette: Digital 1-5 mL	Fisher Scientific, Pittsburgh, PA
Pipettes: Rainin Pipetman™ Volumes: fixed20; adjust.: 20, 100; 200; 1000 µL	Rainin Instrument Co., Woburn, MA
Sonicator	Branson, Danbury, CT
Stir Plate: Nuova II	Thermolyne Corp., Dubuque, IA
Vortex: Fisher Genie 2	Fisher Scientific, Fair Lawn, NJ

6.3. Reagents, Calibration Standard and Quality Control (QC) Solutions

The calibrator, quality control and stock solution concentrations listed below are reported as they were prepared for the validation study and serve as a general guideline for future preparations.

Reagents

All reagent solutions are stored at room temperature.

- *0.25 M carbonate buffer*

  Add approximately 500 mL HPLC-grade water to an appropriate 1-L container. Add 26.5 g of sodium carbonate (Na_2CO_3) to the 1-L container. Add 21.0 g of sodium bicarbonate ($NaHCO_3$) to the 1-L container. Fill to volume with HPLC-grade water. Use a stir plate to mix.

- *10 N sodium hydroxide*

  Add 400 g of sodium hydroxide (NaOH) to an appropriate 1-L container. ***Slowly*** add approximately 900 mL of HPLC-grade water while mixing. After the NaOH is completely dissolved, allow the solution to cool to room temperature. Fill to volume with HPLC-grade water and mix. ***Caution:*** The solution will become very hot as water is added! Prepare in the hood.

- *0.5 M Tetrabutylammonium Hydrogen Sulfate (TBA, pH 10)*

  Add approximately 50 mL of HPLC-grade water to an appropriate 100-mL container. Add 16.9 g of TBA to the container. Adjust the pH with 10 N sodium hydroxide. Fill to volume with HPLC-grade water and mix. ***Note:*** Be sure to check the pH daily when in use.

- *2 mM ammonium acetate in water*

  Add 1 mL of 1 M ammonium acetate to a 500-mL container approximately half full with HPLC-grade water. QS to volume with HPLC-grade water and mix by inversion.

- *2 mM ammonium acetate in methanol*

  Fill an appropriate 1-L container approximately half full with methanol. Weigh out 0.154 g of ammonium acetate (NH_4 OAc) and transfer to the container. Use a stir plate to mix. Adjust the pH if desired and fill to volume with methanol.

- *2 mM ammonium acetate water:methanol (50:50 v/v)*

  Fill an appropriate 1-L container with 500 mL of 2 mM ammonium acetate in water and 2 mM ammonium acetate in methanol.

Calibration Standard and Quality Control (QC) Solutions

All calibration standard solutions are transferred to 16 x 100 mm polypropylene screw-cap tubes and stored in a -20 °C freezer.

- *PFOS Stock Standard (1053 ppm)*

  Weigh 10.527 mg of PFOS and transfer to a 10-mL volumetric flask. QS to volume with methanol. Sonicate for approximately 5 minutes.

- *PFOSA Stock Standard (1078 ppm)*

  Weigh 10.778 mg of PFOSA and transfer to a 10-mL volumetric flask. QS to volume with methanol. Sonicate for approximately 5 minutes.

- *PFOSAA Stock Standard (1031 ppm)*

  Weigh 10.310 mg of PFOSAA and transfer to a 10-mL volumetric flask. QS to volume with methanol. Sonicate for approximately 5 minutes.

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- *N-MeFOSE-OH Stock Standard (1210 ppm)*

  Weigh 12.103 mg of N-MeFOSE-OH and transfer to a 10-mL volumetric flask. QS to volume with methanol. Sonicate for approximately 5 minutes.

- *N-EtFOSE-OH Stock Standard (1127 ppm)*

  Weigh 11.266 mg of N-EtFOSE-OH and transfer to a 10-mL volumetric flask. QS to volume with methanol. Sonicate for approximately 5 minutes.

- *POAA Stock Standard (1090 ppm)*

  Weigh 10.9 mg of POAA and transfer to a 10-mL volumetric flask. QS to volume with methanol. Sonicate for approximately 5 minutes.

- *PFHS Stock Standard (1000 ppm)*

  Add 807 µL of PFHS (6200 ppm) to a 5-mL volumetric flask. QS to volume with methanol. Mix by inversion.

- *Diluted Stock Solution (10.0 ppm)*

  Add 95 µL of PFOS Stock Solution (1053 ppm), 93 µL of PFOSA Stock Solution (1078 ppm), 97 µL of PFOSAA Stock Solution (1031 ppm), 83 µL of N-MeFOSE-OH Stock Solution (1210 ppm), 89 µL of N-EtFOSE-OH Stock Solution (1127 ppm), 92 µL of POAA Stock Solution (1090 ppm) and 100 µL of PFHS Stock Solution (1000 ppm) to a 10-mL volumetric flask. QS to volume with methanol. Mix by inversion.

- *Spiking Standard 8 (5.00 ppm)*

  Add 500 µL of methanol and 500 µL of Diluted Stock Solution (10.0 ppm) to a 13 x 100 mm silanized glass tube. Mix by vortexing.

- *Spiking Standard 7 (2.50 ppm)*

  Add 500 µL of methanol and 500 µL of Spiking Standard 8 (5.00 ppm) to a 13 x 100 mm silanized glass tube. Mix by vortexing.

- *Spiking Standard 6 (1.00 ppm)*

  Add 800 µL of methanol and 200 µL of Spiking Standard 7 (5.00 ppm) to a 13 x 100 mm silanized glass tube. Mix by vortexing.

- *Spiking Standard 5 (0.500 ppm)*

  Add 900 µL of methanol and 100 µL of Spiking Standard 7 (5.00 ppm) to a 13 x 100 mm silanized glass tube. Mix by vortexing.

- *Spiking Standard 4 (0.250 ppm)*

  Add 950 µL of methanol and 50 µL of Spiking Standard 7 (5.00 ppm) to a 13 x 100 mm silanized glass tube. Mix by vortexing.

- *Spiking Standard 3 (0.100 ppm)*

  Add 900 µL of methanol and 100 µL of Spiking Standard 6 (1.00 ppm) to a 13 x 100 mm silanized glass tube. Mix by vortexing

- *Spiking Standard 2 (0.0500 ppm)*

  Add 900 µL of methanol and 100 µL of Spiking Standard 5 (0.500 ppm) to a 13 x 100 mm silanized glass tube. Mix by vortexing

- *Spiking Standard 1 (0.0100 ppm)*

  Add 900 µL of methanol and 100 µL of Spiking Standard 3 (0.100 ppm) to a 13 x 100 mm silanized glass tube. Mix by vortexing

Internal Standard Solutions

All internal standard solutions are transferred to 16 x 100 mm polypropylene screw-cap tubes and stored in a -20 °C freezer.

- *SDS Stock Solution (1091 ppm)*

  Weigh 5.986 mg of SDS and transfer to a 5-mL volumetric flask. QS to volume with methanol. Sonicate for 10 minutes.

- *SDS Diluted Stock Solution (10.0 ppm)*

  Add 91.7 µL of SDS Stock Solution (1091 ppm) to a 10-mL volumetric flask. QS to volume with methanol and mix by inversion.

- *SDS Working Internal Standard (1.00 ppm)*

  Add 2.50 mL of SDS Diluted Stock Solution (10 ppm) to a 25-mL class A volumetric flask. QS to volume with methanol and mix by inversion.

6.4. Preparation of Quality Control Samples

High Quality Control (350 ppb spiked)

Quantitatively transfer 1.05 mL of the Diluted Stock Solution (10.0 ppm) and 28.95 mL of blank human serum into a polypropylene tube. Sonicate for approximately 10 minutes and equilibrate for approximately 10 minutes.

Low Quality Control (20.0 ppb spiked)

Quantitatively transfer 60 µL of the Diluted Stock Solution (10.0 ppm) and 29.94 mL of blank human serum into a polypropylene tube. Sonicate for approximately 10 minutes and equilibrate for approximately 10 minutes.

Storage of QC Samples

After preparation, place aliquots of the low and high QC pools into 2-mL cryogenic vials, and store in a -20 °C freezer.

6.5. Preparation of Calibration Standards

If endogenous analyte concentrations in human serum need to be calculated, a rabbit serum calibration curve is prepared by spiking standards as shown in the table below. The calibration curve range is 1.00 ppb to 50.0 ppb.

Standard Number	Concentration of Spiking Solution (ppm)	Volume of Spiking Solution (µL)	Volume of Blank Rabbit Serum (mL)	Final Spiked Concentration (ppb)
5	0.500	20	0.20	50.0
4	0.250	20	0.20	25.0
2	0.0500	20	0.20	5.00
1	0.0100	20	0.20	1.00

The human serum calibration curve is prepared on the day of each run by spiking standards as shown in the table below. The unadjusted calibration curve range is 1.00 ppb to 500 ppb.

Standard Number	Concentration of Spiking Solution (ppm)	Volume of Spiking Solution (µL)	Volume of Blank Human Serum (mL)	Final Spiked Concentration (ppb)
8	5.00	20	0.20	500
7	2.50	20	0.20	250
6	1.00	20	0.20	100
5	0.500	20	0.20	50.0
4	0.250	20	0.20	25.0
3	0.100	20	0.20	10.0
2	0.0500	20	0.20	5.00
1	0.0100	20	0.20	1.00

6.6. Sample Preparation

Calibration Curve Samples (prepare in duplicate)

Rabbit Serum Curve

Transfer 0.20 mL of HPLC-grade water and 0.20 mL rabbit serum into appropriately labeled 13 x 100 mm polypropylene tubes. Add 20 µL of Calibration Standard Spiking Solutions 1, 2, 4 and 5 to the corresponding labeled tube.

Human Serum Curve

Transfer 0.20 mL of HPLC-grade water and 0.20 mL human serum into appropriately labeled 13 x 100 mm polypropylene tubes. Add 20 µL of Calibration Standard Spiking Solutions 1-8 to the corresponding labeled tube.

Quality Control Samples (prepare in duplicate)

Aliquot 0.20 mL of each of the Low and High controls into appropriately labeled 13 x 100 mm polypropylene tubes.

Aliquot 0.20 mL of blank human serum into separate 13 x 100 mm polypropylene tubes and label as QC0.

Aliquot 0.20 mL of blank human serum into separate 13 x 100 mm polypropylene tubes and label as BLANK

Aliquot 0.20 mL of blank rabbit serum into separate 13 x 100 mm polypropylene tubes and label as RABBIT BLANK.

Study Samples

Transfer 0.20-mL aliquots of each study sample into appropriately labeled 13 x 100 mm polypropylene tubes.

Extraction Procedure

1. Add 0.20 mL of HPLC-grade water (this should be performed before adding the calibration standard spiking solutions to calibration curve samples).
2. Add 20 µL of SDS internal standard (1.00 ppm) to all samples, excluding BLANKS.
3. Vortex samples for at least 5 seconds.
4. Add 0.40 mL 0.5 M tetrabutylammonium hydrogen sulfate (TBA), pH 10, and 0.40 mL of 0.25 M carbonate buffer. NOTE: Check the pH of TBA daily.
5. Add 3.0 mL methyl-*tert*-butyl ether.

6. Cap each sample, vortex and rotate for approximately 10 minutes.

7. Centrifuge at approximately 3500 rpm for 10 minutes (or until the layers are well separated).

8. Transfer the organic layer to a clean 13 x 100 mm polypropylene tube with a disposable transfer pipet.

9. Dry samples under nitrogen at 45 °C until dry (approximately 10 minutes).

10. Add 75 µL of 2 mM ammonium acetate water:methanol (50:50 v/v) to each tube.

11. Vortex for at least 5 seconds.

12. Transfer into autosampler vial insert.

13. Cap and store extracts at 4 °C until analysis.

6.7. LC/MS/MS Conditions

LC Conditions

Column I.D.	Betasil C-18
Mobile Phase(s)	A: 2mM ammonium acetate in water
	B: 2mM ammonium acetate in methanol
Flow Rate	300 µL/min
Column Temperature	50 °C
Injection Volume	5 - 15 µL
LC Conditions	Gradient

The HPLC gradient required is shown below.

Time (min.)	% Mobile Phase A	% Mobile Phase B
0.00	50	50
4.50	50	50
11.0	2.7	97.3
12.0	2.7	97.3
13.0	50	50

MS Conditions

Post Column Split	2:1
Source	Turbo Ion Spray
Source Temp.	300 °C
Analysis type	Multiple reaction monitoring (MRM)

Compounds	Transitions monitored	Dwell Time (ms)	Collision Energy (v)
PFOS	499 / 80	100	91
PFOSA	498 / 78	100	38
PFOSAA	584 / 419	200	30
N-MeFOSE-OH	616 / 59	100	52
N-EtFOSE-OH	630 / 59	100	58
POAA	413 / 369	100	15
PFHS	399 / 80	100	48
SDS	265 / 97	100	41

The prepared standards and QCs are loaded onto the autosampler tray in a random order and injected into the instrument LC/MS/MS system.

6.8. Quantitation

PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA, PFHS and SDS chromatographic peaks are integrated using the instrument manufacturer supplied software with a smooth factor of 1. Quantitation of the rabbit calibration curve is based upon linear regression analysis of calibration curves (weighted 1/x) using the analyte peak areas. This quantitation is performed using the instrument manufacturer supplied software. Quantitation of the human calibration curve is based upon quadratic regression analysis of calibration curves (weighted 1/x) using the peak area ratio (analyte peak area/internal standard peak area) vs. concentration. This quantitation is performed using the Watson® DMLIMS software.

Figure 1. Total Ion Chromatogram of Rabbit Serum Blank

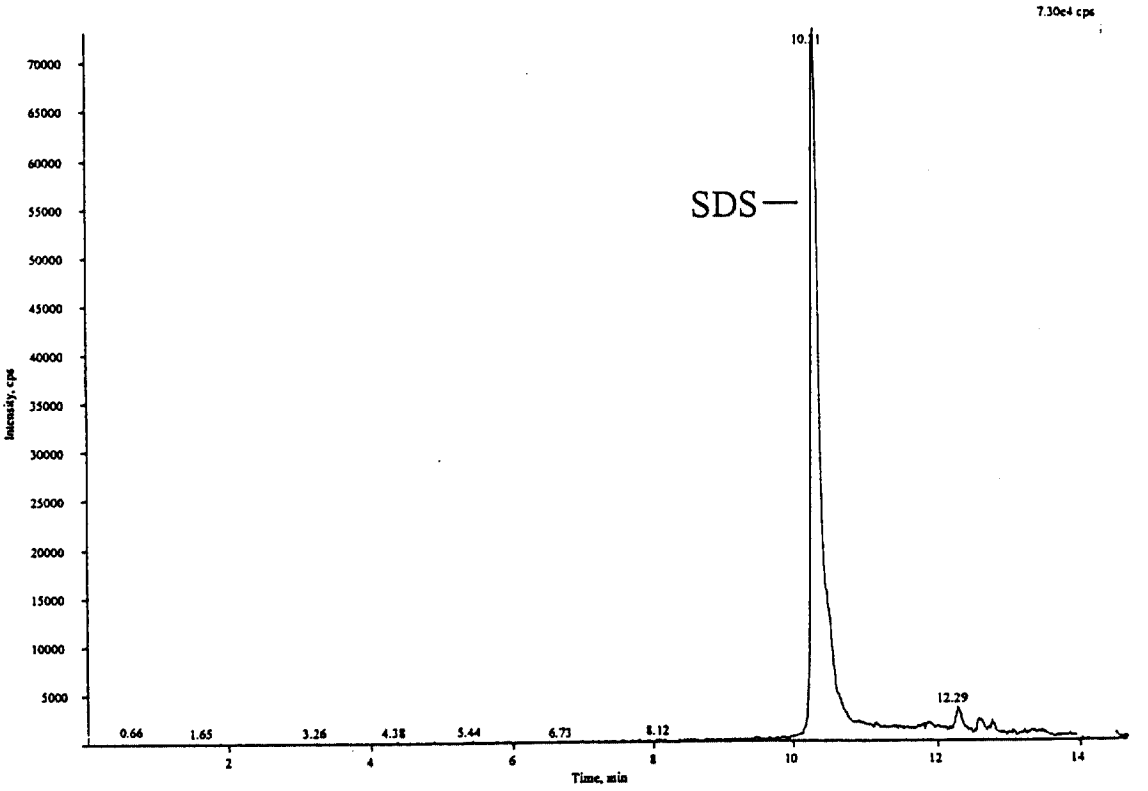

Figure 3. Total Ion Chromatogram of High Standard (500 ppb)