Quantitative Determination of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in Human Serum by LC/MS/MS

Assay Revalidation Report

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QUALITY ASSURANCE STATEMENT

LABORATORY:	Northwest Bioanalytical (NWB) A Division of NWT, Inc. 1121 East 3900 South Salt Lake City, UT 84124
SPONSOR:	3M Environmental Technology and Services (3M) 935 Bush Avenue St. Paul, MN 55133
COMPOUND(S):	PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570
NWB STUDY NUMBER:	NWBS00-040
SPONSOR STUDY NUMBER:	NA, Assay Validation
NWB STUDY TITLE:	Quantitative Determination of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in Human Serum by LC/MS/MS, Assay Validation

The assay validation study described in this report is not included within the definition of a GLP regulated nonclinical study. However, Northwest Bioanalytical conducts all studies within the guidelines of the U.S. FDA Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (Title 21 CFR Part 58), the OECD Principles of Good Laboratory Practice and the Japanese MHW Good Laboratory Practice Standard Ordinance for Nonclinical Laboratory Studies on the Safety of Drugs (Ordinance No. 21, PAB Notification No. 424.) The following inspections were performed by the NWB QAU per SOP.

Inspection and Reporting Statement.

		Date Inspection Report Issued To	
Inspection Date	Phase of Study	NWB Project Manager	*NWB Management
15 May 2000	Analytical Plan	15 May 2000	31 May 2000
15 – 18 May 2000	Assay Validation	18 May 2000	31 May 2000
07 – 18 Sep 2000	Report Draft/Raw Data	20 Sep 2000	29 Sep 2000
24 Jan 2001	Final Report	24 Jan 2001	31 Jan 2001

*Reports to NWB Management are issued monthly.

As can reasonably be established, the methods and procedures described and the results incorporated into this final report accurately reflect the raw data.

Shaundel Percey, B.S., M.T., ASCP
Quality Assurance Compliance Specialist

24 January 2001
Date

COMPLIANCE STATEMENT

The method validation study described in this report is not included within the definition of a GLP nonclinical regulated study. However, to the best of our knowledge, this study was conducted in accordance with the guidelines of the U.S. FDA Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (Title 21 CFR Part 58) and according to the methods and procedures described within this report. In addition, the study followed the guidelines of the OECD Principles of Good Laboratory Practice and the Japanese MHW Good Laboratory Practice Standard Ordinance for Nonclinical Laboratory Studies on the Safety of Drugs (Ordinance No. 21, PAB Notification No. 424.) Any known circumstances that may have affected the quality or integrity of the study or data are discussed within the report. This report represents an accurate record of the raw data.

Connie O Sakashita DATE: 1/24/01

Connie O. Sakashita, B.S.
NWB Project Manager

Patrick Bennett DATE: 1/24/01

Patrick Bennett, M.S., M.B.A.
NWB Laboratory Director

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Assay Revalidation Report

1. INTRODUCTION

Northwest Bioanalytical (NWB) was contracted by 3M Environmental Technology and Services to develop and validate a liquid chromatography/tandem mass spectrometry method for the measurement of perfluorooctanesulfonate (PFOS), perfluorooctanesulfonamide (PFOSA), N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA), perfluorooctanoate (POAA), perfluorohexanesulfonate (PFHS), perfluorooctanesulfonamidoacetate (M556) and N-methyl perfluorooctanesulfonamidoacetate (M570) in human serum.

The reference material purity for PFOS, PFOSA, PFHS and POAA was not available prior to the conduct of this study. Therefore, all concentrations included in the report for these analytes are based upon an assumed purity of 100%.

After the validation was completed, 3M contracted with Centre Analytical Laboratories, Inc. in State College, Pennsylvania to determine the absolute concentration of PFOS, POAA and PFHS in the stock solutions used to prepare the analytical standards and controls used for this validation and subsequent analyses. Based on the results obtained, the concentrations included in this report should be corrected according to the following table:

Analyte	Correction Factor
PFOS	0.836
POAA	0.909
PFHS	0.855

This report summarizes the accuracy, precision and the Northern Chinese plasma extraction efficiency results from the validation of the method for the quantitation of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in human serum for 3M Environmental Technology and Services. Stability results will be reported in a separate addendum report. Kris Hansen at 3M Environmental Technology and Services served as the Study Monitor. The following is a list of NWB supervisory personnel involved in the completion of this work: Connie O. Sakashita, B.S. (NWB Project Manager); Brad I. Coopersmith, Ph.D. (NWB Senior Scientist); Licong Jiang, Ph.D. (NWB Senior Scientist); Patrick Bennett, M.S., M.B.A. (NWB Laboratory Director); Rodger Foltz, Ph.D. (NWB Technical Director).

NWB SOPs were used in the conduct of this project and were available to project personnel in both electronic and hard copy formats.

Date Study Initiated: April 13, 2000 Date Analyses Completed: July 16, 2000

The method validation study described in this report is not included within the definition of a GLP regulated nonclinical study. However, Northwest Bioanalytical conducts all studies within the guidelines of the U.S. FDA Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (Title 21 CFR Part 58), the OECD Principles of Good Laboratory Practice and the Japanese MHW Good Laboratory Practice Standard Ordinance for Nonclinical Laboratory Studies on the Safety of Drugs (Ordinance No. 21, PAB Notification No. 424). Any changes to or deviations from the original protocol (Analytical Plan) were documented through approved protocol amendments or deviation memos and are retained within the raw data.

Principles of the Method

The newly developed method is a modification of a previous method developed under study NWBS98-082 and reported in NWB report NWBR99-005 [5.1]. The new method was developed to provide improved accuracy, precision and ruggedness using less sample volume, and to add M556 and M570 to the method.

The analytical method consisted of a liquid:liquid extraction procedure followed by evaporation and reconstitution of the extract residue with 30:70 20 mM ammonium acetate in water: 20 mM ammonium acetate in methanol (v/v). The samples were analyzed by liquid chromatography/tandem mass spectrometry using a PE Sciex API 3000. The instrument was operated in the multiple reaction monitoring (MRM) mode under optimized conditions for PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 detection.

2. VALIDATION SUMMARY

Three separate analytical runs were used in the determination of linearity, precision, and accuracy. An additional run was used to determine intra-assay precision and accuracy for PFOSA and PFOSAA. The extraction efficiencies for all analytes and the internal standard 1H,1H,2H,2H-perfluorooctane sulfonic acid (THPFOS) were also determined.

2.1. Persistent Levels of Analytes in Matrix

Because PFOS, PFOSAA, POAA, PFHS, M556 and M570 demonstrate measurable levels in control human serum, a procedure to account for these persistent levels is needed for this method validation.

The sponsor provided NWB with blank matrix (Northern Chinese human plasma). This matrix was tested and demonstrated no quantifiable concentrations for any of the analytes. In order to determine the persistent levels of these analytes in the lot of human serum used for the validation, a partial calibration curve was prepared with the blank Northern Chinese human plasma and extracted along with blank human serum samples. The human serum samples were quantitated against the Northern Chinese human plasma calibration curve to determine the persistent levels of the analytes in the lot of human serum being tested.

Once the persistent level of each analyte was determined, the target concentrations for each calibration standard and quality control sample were adjusted to account for the persistent amount of analyte. For example, if the target concentration at the lower

limit of quantitation was 1.00 ng/mL and the persistent level of the analyte in human serum was 2.00 ng/mL, the target concentration of the LLOQ was adjusted to 3.00 ng/mL. Appendix B outlines the procedure used to calculate persistent levels of analytes in diluted human serum samples.

This procedure to account for persistent levels of the analytes in matrix must be performed whenever a new lot of human serum is used during the course of a study.

2.2. Range of Quantitation

Each analytical run included calibration standards in duplicate at nine different concentrations (eight for M556), a minimum of six quality control samples (QCs) (three levels in replicates of two), two serum blanks and two 0-ng/mL QCs (serum blank with internal standard).

The target calibrator concentrations were approximately 1.00 , 2.50, 10.0, 25.0, 50.0, 100, 250, 400 and 500 ng/mL for all analytes. Each analyte has a different final curve range based upon the persistent levels of the analyte in the human serum used. For the validation study, the calibrator concentrations were as follows:

PFOS	48.1, 49.6, 57.1, 72.1, 97.1, 147, 297, 447, 547
PFOSA	1.00, 2.51, 10.1, 25.1, 50.3, 100, 251, 402, 502
PFOSAA	6.00, 7.50, 15.0, 30.0, 55.0, 105, 255, 405, 505
POAA	5.76, 7.26, 14.8, 29.8, 54.8, 105, 255, 405, 505
PFHS	3.15, 4.65, 12.2, 27.2, 52.2, 102, 252, 402, 502
M556	4.30, 11.8, 26.8, 51.8, 102, 252, 402, 502
M570	5.60, 7.10, 14.6, 29.6, 54.6, 105, 255, 405, 505

	PFOS	PFOSA	PFOSAA	POAA
Range	48.1 to 547 ng/mL	1.00 to 502 ng/mL	6.00 to 505 ng/mL	5.76 to 505 ng/mL
Mean Coefficient of Determination	0.9925 (Table 1)	0.9899 (Table 2)	0.9968 (Table 3)	0.9922 (Table 4)

	PFHS	M556	M570
Range	3.15 to 502 ng/mL	4.30 to 502 ng/mL	5.60 to 505 ng/mL
Mean Coefficient of Determination	0.9954 (Table 5)	0.9935 (Table 6)	0.9967 (Table 7)

The results for the individual calibration standards can be found in Tables 8 – 14.

2.3. Precision and Accuracy

The target concentrations for the quality control samples were 4.00, 150, 400 and 4000 ng/mL for all analytes. All quality control target concentrations were corrected for the persistent levels of the analytes in the human serum used for preparation. For the validation study, the quality control concentrations were as follows:

	Low (ng/mL)	Medium (ng/mL)	High (ng/mL)	Dilution (ng/mL)
PFOS	51.1	197	446	4460
PFOSA	4.00	150	400	4000
PFOSAA	9.00	155	405	4050
POAA	8.74	154	403	4030
PFHS	6.15	152	402	4020
M556	5.80	152	402	4020
M570	8.60	155	405	4050

2.3.1. Precision and Accuracy for PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 Quality Controls

The precision and accuracy of the LC/MS/MS method for the quantitation of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in human serum were determined by analyzing three levels of quality controls in replicates of five on three separate days.

The intra-assay precision (%CV) for PFOS, POAA, PFHS, M556 and M570 were less than or equal to 9.5% for each undiluted QC concentration. The mean accuracy (%Theoretical) for all levels of undiluted quality controls ranged from 84.8% to 111.2% (Tables 15 and 18 – 21). The intra-assay precision (%CV) for PFOSA and PFOSAA were less than or equal to 19.8% for each undiluted QC concentration. The mean accuracy (%Theoretical) for all levels of undiluted QCs ranged from 68.7% to 91.7% (Tables 16 – 17).

The inter-assay precision (%CV) for PFOS, POAA, PFHS, M556 and M570 were less than or equal to 9.7% for each undiluted QC concentration. The mean accuracy (%Theoretical) for all levels of undiluted QCs ranged from 87.4% to 108.5% (Tables 22 and 25 – 28). The inter-assay precision (%CV) for PFOSA and PFOSAA were less than or equal to 14.0% for each undiluted QC concentration. The mean accuracy (%Theoretical) for all levels of undiluted QCs ranged from 73.1% to 88.9% (Tables 23 – 24).

For diluted QCs, the inter-assay precision (%CV) for PFOS, POAA, PFHS, M556 and M570 were less than or equal to 6.6%. The mean accuracy (%Theoretical) for the diluted QCs ranged from 92.8% to 113.9% (Tables 31 and 34 – 37). The inter-assay precision (%CV) for PFOSA and PFOSAA were less than or equal to 9.7% for each diluted QC. The mean accuracy (%Theoretical) for the diluted QCs ranged from 79.0% to 81.3% (Tables 32 – 33).

The slightly high negative bias for the calculated concentrations of the PFOSA and PFOSAA QC samples was a result of the QC preparation procedure. A stock solution containing all the analytes was prepared and evaporated to dryness in order to obtain a concentration high enough for the QC concentration range. This methodology is commonly used when stock solution concentrations are too dilute to obtain the targeted concentration or to reduce the organic content of spiked serum samples. For this preparation, evaporation was required to obtain appropriate concentrations. However, because of the high volatility of PFOSA and PFOSAA, approximately 25% of these analytes evaporated from the solution. The quality control samples from this preparation were biased approximately 25% lower (Tables 23 – 25) than those prepared from a solution that was not evaporated (Tables A.1. – A.2.).

2.3.2. Precision and Accuracy for PFOSA and PFOSAA Specific Quality Controls

Because of the bias demonstrated for PFOSA and PFOSAA calculated concentrations from the original QCs prepared as described in section 2.3.1, a new set of QCs containing only PFOSA and PFOSAA were prepared using concentrated solutions that did not require evaporation.

For the PFOSA and PFOSA specific QCs, the intra-assay precision (%CV) for PFOSA and PFOSAA was less than or equal to 23.8% for each undiluted QC concentration. The mean accuracy (%Theoretical) for all levels of undiluted QCs ranged from 72.9% to 107.7% (Tables 29 – 30).

The PFOSAA LLOQ calibration standards for the intra-assay Run 17 demonstrated unacceptable accuracies resulting in both replicates being rejected and a raised LLOQ of 7.50 ng/mL for the run. The low QCs for PFOSAA also demonstrated variability greater than 20%. This resulted in a negative bias (72.9% of theoretical) for the low PFOSAA QCs. The mid-level and high-level QCs demonstrated acceptable precision and accuracy. Because this bias only appears at the Low QC level and is a result of within-run

variability, study sample analysis acceptance criteria will be maintained at 20% precision and accuracy for QCs.

For the PFOSA and PFOSAA specific QCs containing PFOSA or PFOSAA concentrations above the ULOQ, the intra-assay precision (%CV) for PFOSA and PFOSAA was 19.8% and 20.9%, respectively. The mean accuracy (%Theoretical) for diluted PFOSA and PFOSAA specific QCs were 78.3% and 78.0%, respectively (Tables 38 – 39). The deviation of the calculated concentrations from theoretical concentrations for these QCs diluted with control matrix were both greater than 20%. This indicates that samples with experimentally calculated PFOSA or PFOSAA concentrations above the ULOQ should not be diluted with control matrix. However, the levels of PFOSA and PFOSAA in study samples are expected to be within the range of the calibration curve.

The inter-assay precision and accuracy of the PFOSA and PFOSAA specific QCs was demonstrated with the analytical QC performance during sample analysis study NWBS00-062 (medical surveillance). The results can be found in Appendix A, Tables A.1. and A.2. These tables only contain analyses for reported PFOSA and PFOSAA sample data, and do not include runs that did not meet the acceptance criteria for PFOSA or PFOSAA.

For the PFOSA and PFOSA specific QCs, the inter-assay precision (%CV) was less than or equal to 11.4% for each undiluted QC concentration. The mean accuracy (%Theoretical) for all levels of undiluted QCs ranged from 98.5% to 107.7%.

2.4. Extraction Efficiency

The extraction efficiencies of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 were determined using Northern Chinese human plasma by comparing the area ratios obtained for the following three cases:

1. Both the analyte and internal standard added following the extraction (post-extract).
2. The analyte added to plasma prior to extraction and the internal standard added following extraction (pre-extract analyte).
3. The internal standard added to plasma prior to extraction and the analyte added following the extraction (pre-extract internal standard).

The extraction efficiencies were then determined by the area ratio of the pre-extract samples to the post-extract samples. The extraction efficiency experiments were performed at both low, medium and high concentrations to determine that there was no concentration bias. The mean extraction efficiencies were: PFOS (42.1%), PFOSA (65.3%), PFOSAA (73.4%), POAA (18.0%), PFHS (19.7%), M556 (43.9%), M570 (66.9%). The overall mean extraction efficiency for the internal standard THPFOS was 7.86% (Tables 40 – 46).

The recovery for many of the analytes and for the internal standard are very low. This low recovery is a result of the following factors: a neutral pH was required to minimize any matrix effects that were observed from the previous extraction method which used a basic pH and, the wide range of polarities for the analytes prevented the use of acidic pH during the extraction and prevented the use of an effective SPE extraction for all of the analytes. While the recovery is low, the intra and inter-assay precision and accuracy values demonstrated good reproducibility. The low recovery should not affect the assay performance.

The Chinese plasma containing very low persistent levels of most analytes was used to estimate the extraction recovery of the analytes from matrix. This plasma was used to provide the most accurate results possible at the lower concentrations. The recovery from general population control serum will be evaluated and reported in an addendum report.

2.5. Stability Evaluation

The stability of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 maintained under various storage conditions will be documented in a separate NWB report.

3. DATA MANAGEMENT

PFOS, PFOSA, PFOSAA, POAA, PFHS, M556, M570 chromatographic peaks are integrated using PE Sciex MacQuan software (version 1.6) with a smooth factor of 1. Quantitation is based upon quadratic regression analysis of calibration curves (weighted $1/x^2$) using the area ratio vs. concentration calculated by the Watson® DMLIMS software (version 6.1.1.04).

4. COMMENTS AND CONCLUSIONS

Per agreement with the Sponsor, the regressions were not recalculated based upon the updated purity information for PFOS, POAA and PFHS. Some differences might occur due to the effect of rounding if the regressions were performed with the purity corrected concentrations, but these differences would have a negligible effect on the overall interpretation of the validation results.

Only THPFOS was used as an internal standard because of concerns over the specificity of one of the original internal standards, N-Et-FOSE-OH, which gave an acetate ion as a product.

The volatility of PFOSA and PFOSAA is an important aspect to be cautious of during the evaporation step of the assay. An evaporation step was used for the initial preparation of QC samples to obtain an adequate concentration of the analytes. This set demonstrated acceptable precision, but slightly high negative bias for PFOSA and PFOSAA. A separate set of QCs containing only PFOSA and PFOSAA were prepared without using an evaporation step. The second set (PFOSA and PFOSAA specific QCs) demonstrated

both acceptable precision and accuracy. As a result, two sets of intra-assay and inter-assay precision and accuracy are shown in the report.

The method described in this report has been validated for the determination of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in human serum. However, study samples with experimentally calculated PFOSA or PFOSAA concentrations above the ULOQ should not be diluted with control matrix. Any known circumstances that may have affected the quality or integrity of the data are discussed in this report.

4.1. Proposed Sample Analysis Acceptance Criteria

Calibration Curve

Each run will include in duplicate calibration standards at six or more concentrations covering the lower to upper limit of quantitation. For all analytes except PFOSA, at least three-fourths of the calibration standard's back-calculated concentrations must be within ± 15% (± 20% for LLOQ) of their individual target concentrations. For PFOSA, at least three-fourths of the calibration standard's back-calculated concentrations must be within ± 20% (± 25% for LLOQ) of their individual target concentrations. A calibration standard will be considered a statistical outlier if the back-calculated concentration is greater than two times the acceptance criteria for that standard.

Lower Limit of Quantitation

The back-calculated concentrations of at least one of the duplicate lowest points in the calibration curve must be within ± 25% of the target concentration for PFOSA to qualify as the LLOQ and within ± 20% of the target concentration to qualify as the LLOQ for all other analytes. 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, medium and high QC samples in duplicate. The measured concentrations of at least two-thirds of all analytical QCs must be within ± 20% of their target concentrations (± 25% for PFOSA), and no two QCs at the same concentration can be outside the limit. If study samples require dilution, a dilution QC will be analyzed in triplicate for each dilution level (except for PFOSA and PFOSAA which should not be diluted with control matrix). At least two dilution QC at each level must be within ± 20% of its target concentrations in order to accept diluted study samples at that level. The dilution QC acceptance is independent of the undiluted analytical QC acceptance.

5. REFERENCES

[5.1] D. Vollmer, "Quantitative Determination of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS in Human Serum by LC/MS/MS," NWB study NWBS98-082, NWB report NWBR99-005, May 13, 1999.

6. DATA RETENTION

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 Environmental Technology and Services will be notified concerning final disposition of records at completion of contract obligations.

Table 1. Summary of Calibration Curve Parameters for PFOS

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
29-Jun-2000	13	-0.000009	0.035473	0.368276	0.9877	48.1	547
30-Jun-2000	15	-0.000001	0.018768	0.148749	0.9949	48.1	547
02-Jul-2000	16	-0.000005	0.028829	0.235310	0.9948	48.1	547
Mean		-0.000005	0.027690	0.250778	0.9925		
S.D.		0.000004	0.008411	0.110578	0.0041		
%CV		-80.0	30.4	44.1	0.4		
n		3	3	3	3		

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

Table 2. Summary of Calibration Curve Parameters for PFOSA

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
29-Jun-2000	13	-0.000030	0.093690	0.023501	0.9761	1.00	502
30-Jun-2000	15	-0.000012	0.051196	0.004685	0.9975	1.00	502
02-Jul-2000	16	-0.000020	0.088242	0.025293	0.9960	1.00	502
Mean		-0.000021	0.077709	0.017826	0.9899		
S.D.		0.000009	0.023122	0.011416	0.0119		
%CV		-42.9	29.8	64.0	1.2		
n		3	3	3	3		

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

Table 3. Summary of Calibration Curve Parameters for PFOSAA

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
29-Jun-2000	13	-0.000003	0.023347	-0.035788	0.9969	6.00	505
30-Jun-2000	15	0.000000	0.013890	-0.020748	0.9971	6.00	505
02-Jul-2000	16	0.000001	0.023426	-0.033118	0.9964	6.00	505
Mean		-0.000001	0.020221	-0.029885	0.9968		
S.D.		0.000002	0.005483	0.008024	0.0004		
%CV		-200.0	27.1	-26.8	0.0		
n		3	3	3	3		

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

Table 4. Summary of Calibration Curve Parameters for POAA

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
29-Jun-2000	13	0.000002	0.018721	0.026984	0.9847	5.76	505
30-Jun-2000	15	0.000000	0.012512	0.009407	0.9958	5.76	505
02-Jul-2000	16	-0.000002	0.014490	0.015595	0.9962	5.76	505
Mean		0.000000	0.015241	0.017329	0.9922		
S.D.		0.000002	0.003172	0.008916	0.0065		
%CV		-	20.8	51.5	0.7		
n		3	3	3	3		

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

Table 5. Summary of Calibration Curve Parameters for PFHS

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
29-Jun-2000	13	-0.000006	0.023682	0.021811	0.9947	3.15	502
30-Jun-2000	15	-0.000005	0.016787	0.011507	0.9946	3.15	502
02-Jul-2000	16	-0.000009	0.021344	0.020627	0.9968	3.15	502
Mean		-0.000007	0.020604	0.017982	0.9954		
S.D.		0.000002	0.003507	0.005638	0.0012		
%CV		-28.6	17.0	31.4	0.1		
n		3	3	3	3		

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

Table 6. Summary of Calibration Curve Parameters for M556

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
29-Jun-2000	13	0.000001	0.005000	0.004952	0.9881	4.30	502
30-Jun-2000	15	0.000000	0.002882	0.000748	0.9975	4.30	502
02-Jul-2000	16	0.000000	0.003891	0.000548	0.9949	4.30	502
Mean		0.000000	0.003924	0.002083	0.9935		
S.D.		0.000001	0.001059	0.002487	0.0049		
%CV		-	27.0	119.4	0.5		
n		3	3	3	3		

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

Table 7. Summary of Calibration Curve Parameters for M570

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
29-Jun-2000	13	-0.000002	0.006621	-0.007115	0.9962	5.60	505
30-Jun-2000	15	-0.000001	0.003911	-0.005406	0.9972	5.60	505
02-Jul-2000	16	0.000000	0.006433	-0.009231	0.9967	5.60	505
Mean		-0.000001	0.005655	-0.007251	0.9967		
S.D.		0.000001	0.001513	0.001916	0.0005		
%CV		-100.0	26.8	-26.4	0.1		
n		3	3	3	3		

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

Table 8. Back-Calculated Concentrations of Calibration Standards for PFOS

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	48.1	49.6	57.1	72.1	97.1	147	297	447	547
29-Jun-2000	13	44.9	52.1	56.6	71.7	81.1	153	302	449	515
		46.5	51.5	58.5	80.8	94.6	146	316	445	560
30-Jun-2000	15	46.5	49.8	58.6	72.7	92.2	147	321	438	541
		50.3	48.3	58.4	71.2	98.9	135	306	471	518
02-Jul-2000	16	51.6	50.5	60.3	74.9	94.3	151	299	415	571
		45.2	47.6	54.3	71.2	94.9	148	298	449	547
Mean		47.5	50.0	57.8	73.8	92.7	147	307	445	542
S.D.		2.78	1.77	2.07	3.72	6.07	6.28	9.47	18.2	22.3
%CV		5.9	3.5	3.6	5.0	6.5	4.3	3.1	4.1	4.1
%Bias		-1.2	0.8	1.2	2.4	-4.5	0.0	3.4	-0.4	-0.9
n		6	6	6	6	6	6	6	6	6

Table 9. Back-Calculated Concentrations of Calibration Standards for PFOSA

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	1.00	2.51	10.1	25.1	50.3	100	251	402	502
29-Jun-2000	13	1.00	2.44	10.6	25.7	30.8	104	280	420	483
		1.03	2.23	11.1	33.3	45.7	95.0	256	379	507
30-Jun-2000	15	1.03	2.46	9.90	25.7	48.6	98.5	255	401	518
		0.957	2.68	9.71	25.7	50.4	94.8	277	418	447
02-Jul-2000	16	0.952	2.48	11.5	26.2	47.6	99.0	244	391	506
		1.04	2.57	9.79	22.7	48.7	107	237	431	501
Mean		1.00	2.48	10.4	26.6	45.3	99.7	258	407	494
S.D.		0.0388	0.150	0.753	3.54	7.27	4.90	17.3	19.7	25.6
%CV		3.9	6.0	7.2	13.3	16.0	4.9	6.7	4.8	5.2
%Bias		0.0	-1.2	3.0	6.0	-9.9	-0.3	2.8	1.2	-1.6
n		6	6	6	6	6	6	6	6	6

Table 10. Back-Calculated Concentrations of Calibration Standards for PFOSAA

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	6.00	7.50	15.0	30.0	55.0	105	255	405	505
29-Jun-2000	13	5.90	7.74	14.4	31.3	*31.8	110	262	412	476
		5.92	7.85	14.1	30.8	49.4	106	266	400	512
30-Jun-2000	15	6.00	7.82	14.9	29.0	53.3	105	271	403	494
		5.58	7.81	15.7	30.1	53.3	99.9	271	426	475
02-Jul-2000	16	5.92	7.22	15.8	28.2	49.8	102	255	392	501
		6.15	7.65	14.8	30.2	55.8	119	260	413	504
Mean		5.91	7.68	15.0	29.9	52.3	107	264	408	494
S.D.		0.187	0.237	0.683	1.15	2.69	6.83	6.37	11.9	15.2
%CV		3.2	3.1	4.6	3.8	5.1	6.4	2.4	2.9	3.1
%Bias		-1.5	2.4	0.0	-0.3	-4.9	1.9	3.5	0.7	-2.2
n		6	6	6	6	5	6	6	6	6

* Sample deactivated as an outlier (> 2 times the acceptance criteria). Result not included in summary statistics.

Table 11. Back-Calculated Concentrations of Calibration Standards for POAA

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	5.76	7.26	14.8	29.8	54.8	105	255	405	505
29-Jun-2000	13	5.15	8.55	14.6	30.9	*36.6	104	246	377	450
		6.41	6.41	13.4	25.3	56.6	116	287	423	541
30-Jun-2000	15	6.26	7.24	14.0	29.5	54.4	108	280	390	517
		5.67	6.80	15.0	29.7	55.2	97.6	276	406	466
02-Jul-2000	16	5.36	7.09	15.2	28.2	50.7	103	250	385	532
	205	*11.8	7.74	15.9	32.2	54.9	106	255	394	521
Mean		5.77	7.31	14.7	29.3	54.4	106	266	396	505
S.D.		0.550	0.754	0.891	2.38	2.20	6.12	17.4	16.4	37.3
%CV		9.5	10.3	6.1	8.1	4.0	5.8	6.5	4.1	7.4
%Bias		0.2	0.7	-0.7	-1.7	-0.7	1.0	4.3	-2.2	0.0
n		5	6	6	6	5	6	6	6	6

* Sample deactivated as an outlier (> 2 times the acceptance criteria). Result not included in summary statistics.

Table 12. Back-Calculated Concentrations of Calibration Standards for PFHS

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	3.15	4.65	12.2	27.2	52.2	102	252	402	502
29-Jun-2000	13	3.28	5.17	11.8	28.0	55.3	107	252	377	448
		2.85	4.59	11.7	27.3	48.8	97.6	250	414	574
30-Jun-2000	15	2.96	5.08	13.2	26.2	47.6	101	264	395	515
		3.00	4.70	13.8	26.6	50.4	94.6	255	415	484
02-Jul-2000	16	3.21	4.70	12.9	28.4	50.0	101	244	382	545
		3.12	4.26	13.5	26.5	50.3	99.7	249	399	509
Mean		3.07	4.75	12.8	27.2	50.4	100	252	397	513
S.D.		0.162	0.333	0.880	0.887	2.63	4.14	6.77	15.8	44.3
%CV		5.3	7.0	6.9	3.3	5.2	4.1	2.7	4.0	8.6
%Bias		-2.5	2.2	4.9	0.0	-3.4	-2.0	0.0	-1.2	2.2
n		6	6	6	6	6	6	6	6	6

Table 13. Back-Calculated Concentrations of Calibration Standards for M556

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	4.30	11.8	26.8	51.8	102	252	402	502
29-Jun-2000	13	5.11	11.3	30.6	*33.3	111	266	396	468
		3.78	9.88	24.0	49.7	101	272	395	514
30-Jun-2000	15	4.25	11.6	27.4	49.8	107	273	403	499
		4.39	11.7	26.1	51.1	95.1	266	412	466
02-Jul-2000	16	3.78	11.5	25.6	47.5	98.2	250	379	513
		4.74	13.0	26.9	53.6	110	254	408	506
Mean		4.34	11.5	26.8	50.3	104	264	399	494
S.D.		0.527	0.996	2.22	2.23	6.56	9.46	11.8	21.9
%CV		12.1	8.7	8.3	4.4	6.3	3.6	3.0	4.4
%Bias		0.9	-2.5	0.0	-2.9	2.0	4.8	-0.7	-1.6
n		6	6	6	5	6	6	6	6

* Sample deactivated as an outlier (> 2 times the acceptance criteria). Result not included in summary statistics.

Table 14. Back-Calculated Concentrations of Calibration Standards for M570

Quadratic weighted $1/x^2$. All concentrations are expressed as ng/mL.

Run Date	Run Number	5.60	7.10	14.6	29.6	54.6	105	255	405	505
29-Jun-2000	13	5.50	7.08	13.7	31.6	*34.5	107	264	418	466
		5.47	7.61	14.6	30.5	50.5	98.9	269	388	530
30-Jun-2000	15	5.26	7.11	14.8	29.1	55.2	101	266	408	498
		6.00	6.94	14.5	30.7	54.7	97.0	272	422	474
02-Jul-2000	16	5.67	6.55	15.7	27.5	49.8	108	255	394	509
		5.82	6.98	14.9	29.5	56.3	113	251	401	513
Mean		5.62	7.05	14.7	29.8	53.3	104	263	405	498
S.D.		0.266	0.342	0.648	1.44	2.94	6.16	8.18	13.4	24.4
%CV		4.7	4.9	4.4	4.8	5.5	5.9	3.1	3.3	4.9
%Bias		0.4	-0.7	0.7	0.7	-2.4	-1.0	3.1	0.0	-1.4
n		6	6	6	6	5	6	6	6	6

* Sample deactivated as an outlier (> 2 times the acceptance criteria). Result not included in summary statistics.

Table 15. Intra-Assay Precision and Accuracy for PFOS Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 51.1 ng/mL	Medium QC 197 ng/mL	High QC 446 ng/mL
29-Jun-2000	13	45.9	191	392
		44.8	177	403
		47.6	183	429
		48.8	175	383
		48.6	179	377
Mean		47.1	181	397
S.D.		1.74	6.32	20.5
%CV		3.7	3.5	5.2
%Theoretical		92.2	91.9	89.0
n		5	5	5
Run Date	**Run Number**	**Low QC 51.1 ng/mL**	**Medium QC 197 ng/mL**	**High QC 446 ng/mL**
30-Jun-2000	15	48.8	173	382
		46.7	174	395
		47.8	175	402
		45.8	166	382
		47.1	176	406
Mean		47.2	173	393
S.D.		1.13	3.96	11.1
%CV		2.4	2.3	2.8
%Theoretical		92.4	87.8	88.1
n		5	5	5

Table 15. Intra-Assay Precision and Accuracy for PFOS Quality Control Samples (continued)

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 51.1 ng/mL	Medium QC 197 ng/mL	High QC 446 ng/mL
02-Jul-2000	16	45.2	170	380
		48.7	160	393
		46.4	166	385
		47.7	169	387
		44.0	172	361
Mean		46.4	167	381
S.D.		1.88	4.67	12.2
%CV		4.1	2.8	3.2
%Theoretical		90.8	84.8	85.4
n		5	5	5

Table 16. Intra-Assay Precision and Accuracy for PFOSA Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 4.00 ng/mL	Medium QC 150 ng/mL	High QC 400 ng/mL
29-Jun-2000	13	3.43	133	325
		3.21	122	345
		3.39	128	357
		3.58	129	322
		3.67	139	342
Mean		3.46	130	338
S.D.		0.178	6.30	14.6
%CV	✓	5.1	4.8	4.3
%Theoretical		86.5	86.7	84.5
n		5	5	5
Run Date	**Run Number**	**Low QC 4.00 ng/mL**	**Medium QC 150 ng/mL**	**High QC 400 ng/mL**
30-Jun-2000	15	* 2.88	* 100	* 278
		* 2.76	* 105	* 291
		* 2.89	* 103	318
		* 2.78	* 97.8	* 297
		* 2.77	* 110	314
Mean		2.82	103	300
S.D.		0.0635	4.71	16.5
%CV	✓	2.3	4.6	5.5
%Theoretical		70.5	68.7	75.0
n		5	5	5

* > ±25% theoretical

Fortification problem see p. 14

Repeated Table 29

Table 16. Intra-Assay Precision and Accuracy for PFOSA Quality Control Samples (continued)

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 4.00 ng/mL	Medium QC 150 ng/mL	High QC 400 ng/mL
02-Jul-2000	16	* 2.58	* 105	* 289
		* 2.96	* 102	311
		3.50	* 103	315
		3.94	* 102	304
		* 2.51	* 112	305
Mean		3.10	105	305
S.D.		0.613	4.21	9.91
%CV		19.8	4.0	3.2
%Theoretical		77.5	70.0	76.3
n		5	5	5

* > ±25% theoretical

Table 17. Intra-Assay Precision and Accuracy for PFOSAA Quality Control Samples

LLOQ = 6.00

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 9.00 ng/mL	Medium QC 155 ng/mL	High QC 405 ng/mL
29-Jun-2000	13	7.98	129	* 272
		7.43	* 120	* 303
		8.05	* 123	* 317
		8.81	* 121	* 260
		8.46	127	* 286
Mean		8.15	124	288
S.D.		0.522	3.87	23.0
%CV		6.4	3.1	8.0
%Theoretical		90.6	80.0	71.1
n		5	5	5
Run Date	**Run Number**	**Low QC 9.00 ng/mL**	**Medium QC 155 ng/mL**	**High QC 405 ng/mL**
30-Jun-2000	15	7.65	* 112	* 285
		7.76	* 117	* 300
		7.73	* 116	* 315
		7.29	* 104	* 290
		7.52	* 117	* 313
Mean		7.59	113	301
S.D.		0.192	5.54	13.4
%CV		2.5	4.9	4.5
%Theoretical		84.3	72.9	74.3
n		5	5	5

* > ±20% theoretical

Fortification problems (p. 14) Repeated table 20

Table 17. Intra-Assay Precision and Accuracy for PFOSAA Quality Control Samples (continued)

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 9.00 ng/mL	Medium QC 155 ng/mL	High QC 405 ng/mL
02-Jul-2000	16	7.37	* 117	* 287
		8.11	* 107	* 309
		8.47	* 111	* 301
		9.67	* 114	* 312
		7.63	* 116	* 291
Mean		8.25	113	300
S.D.		0.900	4.06	10.9
%CV		10.9	3.6	3.6
%Theoretical		91.7	72.9	74.1
n		5	5	5

* > ±20% theoretical

Table 18. Intra-Assay Precision and Accuracy for POAA Quality Control Samples

All concentrations are expressed as ng/mL. LLOQ: 5.79

Run Date	Run Number	Low QC 8.74 ng/mL	Medium QC 154 ng/mL	High QC 403 ng/mL
29-Jun-2000	13	7.74	157	404
		8.83	156	401
		9.28	154	434
		10.1	153	422
		9.01	163	377
Mean		8.99	157	408
S.D.		0.852	3.91	21.8
%CV	✓	9.5	2.5	5.3
%Theoretical		102.9	101.9	101.2
n		5	5	5
Run Date	**Run Number**	**Low QC 8.74 ng/mL**	**Medium QC 154 ng/mL**	**High QC 403 ng/mL**
30-Jun-2000	15	8.79	144	374
		8.00	153	389
		8.26	147	382
		7.59	136	376
		7.88	139	398
Mean		8.10	144	384
S.D.		0.453	6.69	9.86
%CV	✓	5.6	4.6	2.6
%Theoretical		92.7	93.5	95.3
n		5	5	5

Table 18. Intra-Assay Precision and Accuracy for POAA Quality Control Samples (continued)

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 8.74 ng/mL	Medium QC 154 ng/mL	High QC 403 ng/mL
02-Jul-2000	16	7.15	150	401
		8.15	136	393
		8.42	140	398
		8.72	148	405
		7.12	141	376
Mean		7.91	143	395
S.D.		0.737	5.83	11.3
%CV		9.3	4.1	2.9
%Theoretical		90.5	92.9	98.0
n		5	5	5

Table 19. Intra-Assay Precision and Accuracy for PFHS Quality Control Samples

All concentrations are expressed as ng/mL. LLOQ= 2.15

Run Date	Run Number	Low QC 6.15 ng/mL	Medium QC 152 ng/mL	High QC 402 ng/mL
29-Jun-2000	13	7.03	175	454
		6.44	166	431
		6.77	171	468
		6.89	158	434
		7.05	165	424
Mean		6.84	167	442
S.D.		0.249	6.44	18.2
%CV	✓	3.6	3.9	4.1
%Theoretical		111.2	109.9	110.0
n		5	5	5
Run Date	**Run Number**	**Low QC 6.15 ng/mL**	**Medium QC 152 ng/mL**	**High QC 402 ng/mL**
30-Jun-2000	15	6.53	163	429
		6.22	157	455
		6.06	158	463
		6.00	149	429
		6.31	164	447
Mean		6.22	158	445
S.D.		0.211	5.97	15.3
%CV	✓	3.4	3.8	3.4
%Theoretical		101.1	103.9	110.7
n		5	5	5

Table 19. Intra-Assay Precision and Accuracy for PFHS Quality Control Samples (continued)

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 6.15 ng/mL	Medium QC 152 ng/mL	High QC 402 ng/mL
02-Jul-2000	16	6.43	154	408
		6.52	150	441
		6.23	152	422
		6.86	153	430
		5.93	151	409
Mean		6.39	152	422
S.D.		0.345	1.58	14.1
%CV		5.4	1.0	3.3
%Theoretical		103.9	100.0	105.0
n		5	5	5

Table 20. Intra-Assay Precision and Accuracy for M556 Quality Control Samples

LLOQ = 4.30

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 5.80 ng/mL	Medium QC 152 ng/mL	High QC 402 ng/mL
29-Jun-2000	13	** 8.98	165	427
		6.34	159	429
		** 7.98	161	439
		6.55	163	413
		5.86	170	366
Mean		6.25	164	415
S.D.		0.354	4.22	28.8
%CV	✓	5.7	2.6	6.9
%Theoretical		107.8	107.9	103.2
n		3	5	5
Run Date	**Run Number**	**Low QC 5.80 ng/mL**	**Medium QC 152 ng/mL**	**High QC 402 ng/mL**
30-Jun-2000	15	5.74	143	380
		5.89	147	395
		5.46	152	408
		5.33	139	390
		5.61	153	407
Mean		5.61	147	396
S.D.		0.221	5.93	11.8
%CV	✓	3.9	4.0	3.0
%Theoretical		96.7	96.7	98.5
n		5	5	5

** Sample deactivated due to injector carryover (not included in summary statistics)

Table 20. Intra-Assay Precision and Accuracy for M556 Quality Control Samples (continued)

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 5.80 ng/mL	Medium QC 152 ng/mL	High QC 402 ng/mL
02-Jul-2000	16	6.23	149	391
		6.17	137	418
		6.17	149	390
		6.33	152	417
		5.61	150	378
Mean		6.10	147	399
S.D.		0.283	5.94	17.8
%CV	✓	4.6	4.0	4.5
%Theoretical		105.2	96.7	99.3
n		5	5	5

Table 21. Intra-Assay Precision and Accuracy for M570 Quality Control Samples

LLOQ: 5.60

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 8.60 ng/mL	Medium QC 155 ng/mL	High QC 405 ng/mL
29-Jun-2000	13	8.23	168	393
		8.07	155	403
		8.34	164	424
		8.87	153	378
		8.04	170	377
Mean		8.31	162	395
S.D.		0.336	7.65	19.5
%CV	✓	4.0	4.7	4.9
%Theoretical		96.6	104.5	97.5
n		5	5	5
Run Date	**Run Number**	**Low QC 8.60 ng/mL**	**Medium QC 155 ng/mL**	**High QC 405 ng/mL**
30-Jun-2000	15	8.17	151	396
		9.09	149	396
		8.72	148	412
		7.71	138	388
		7.88	150	413
Mean		8.31	147	401
S.D.		0.579	5.26	11.0
%CV	✓	7.0	3.6	2.7
%Theoretical		96.6	94.8	99.0
n		5	5	5

Table 21. Intra-Assay Precision and Accuracy for M570 Quality Control Samples (continued)

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 8.60 ng/mL	Medium QC 155 ng/mL	High QC 405 ng/mL
02-Jul-2000	16	8.67	148	382
		8.49	137	391
		9.73	148	384
		9.69	145	412
		7.95	151	368
Mean		8.91	146	387
S.D.		0.780	5.36	16.1
%CV		8.8	3.7	4.2
%Theoretical		103.6	94.2	95.6
n		5	5	5

Table 22. Inter-Assay Precision for PFOS Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 51.1 ng/mL	Medium QC 197 ng/mL	High QC 446 ng/mL
29-Jun-2000	13	45.9	191	392
		44.8	177	403
		47.6	183	429
		48.8	175	383
		48.6	179	377
30-Jun-2000	15	48.8	173	382
		46.7	174	395
		47.8	175	402
		45.8	166	382
		47.1	176	406
02-Jul-2000	16	45.2	170	380
		48.7	160	393
		46.4	166	385
		47.7	169	387
		44.0	172	361
Mean		46.9	174	390
S.D.		1.55	7.46	15.7
%CV		3.3	4.3	4.0
%Theoretical		91.8	88.3	87.4
%Bias		-8.2	-11.7	-12.6
n		15	15	15

Table 23. Inter-Assay Precision for PFOSA Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 4.00 ng/mL	Medium QC 150 ng/mL	High QC 400 ng/mL
29-Jun-2000	13	3.43	133	325
		3.21	122	345
		3.39	128	357
		3.58	129	322
		3.67	139	342
30-Jun-2000	15	*2.88	*100	*278
		*2.76	*105	*291
		*2.89	*103	318
		*2.78	*97.8	*297
		*2.77	*110	314
02-Jul-2000	16	*2.58	*105	*289
		*2.96	*102	311
		3.50	*103	315
		3.94	*102	304
		*2.51	*112	305
Mean		3.12	113	314
S.D.		0.437	13.7	21.9
%CV		14.0	12.1	7.0
%Theoretical		78.0	75.3	78.5
%Bias		-22.0	-24.7	-21.5
n		15	15	15

* > ±25% theoretical

Table 24. Inter-Assay Precision for PFOSAA Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 9.00 ng/mL	Medium QC 155 ng/mL	High QC 405 ng/mL
29-Jun-2000	13	7.98	129	*272
		7.43	*120	*303
		8.05	*123	*317
		8.81	*121	*260
		8.46	127	*286
30-Jun-2000	15	7.65	*112	*285
		7.76	*117	*300
		7.73	*116	*315
		7.29	*104	*290
		7.52	*117	*313
02-Jul-2000	16	7.37	*117	*287
		8.11	*107	*309
		8.47	*111	*301
		9.67	*114	*312
		7.63	*116	*291
Mean		8.00	117	296
S.D.		0.640	6.79	16.6
%CV		8.0	5.8	5.6
%Theoretical		88.9	75.5	73.1
%Bias		-11.1	-24.5	-26.9
n		15	15	15

* > ±20% theoretical

Table 25. Inter-Assay Precision for POAA Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 8.74 ng/mL	Medium QC 154 ng/mL	High QC 403 ng/mL
29-Jun-2000	13	7.74	157	404
		8.83	156	401
		9.28	154	434
		10.1	153	422
		9.01	163	377
30-Jun-2000	15	8.79	144	374
		8.00	153	389
		8.26	147	382
		7.59	136	376
		7.88	139	398
02-Jul-2000	16	7.15	150	401
		8.15	136	393
		8.42	140	398
		8.72	148	405
		7.12	141	376
Mean		8.34	148	395
S.D.		0.812	8.27	17.4
%CV		9.7	5.6	4.4
%Theoretical		95.4	96.1	98.0
%Bias		-4.6	-3.9	-2.0
n		15	15	15

Table 26. Inter-Assay Precision for PFHS Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 6.15 ng/mL	Medium QC 152 ng/mL	High QC 402 ng/mL
29-Jun-2000	13	7.03	175	454
		6.44	166	431
		6.77	171	468
		6.89	158	434
		7.05	165	424
30-Jun-2000	15	6.53	163	429
		6.22	157	455
		6.06	158	463
		6.00	149	429
		6.31	164	447
02-Jul-2000	16	6.43	154	408
		6.52	150	441
		6.23	152	422
		6.86	153	430
		5.93	151	409
Mean		6.48	159	436
S.D.		0.368	7.96	18.1
%CV		5.7	5.0	4.2
%Theoretical		105.4	104.6	108.5
%Bias		5.4	4.6	8.5
n		15	15	15

Table 27. Inter-Assay Precision for M556 Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 5.80 ng/mL	Medium QC 152 ng/mL	High QC 402 ng/mL
29-Jun-2000	13	**8.98	165	427
		6.34	159	429
		**7.98	161	439
		6.55	163	413
		5.86	170	366
30-Jun-2000	15	5.74	143	380
		5.89	147	395
		5.46	152	408
		5.33	139	390
		5.61	153	407
02-Jul-2000	16	6.23	149	391
		6.17	137	418
		6.17	149	390
		6.33	152	417
		5.61	150	378
Mean		5.95	153	403
S.D.		0.381	9.49	21.0
%CV		6.4	6.2	5.2
%Theoretical		102.6	100.7	100.2
%Bias		2.6	0.7	0.2
n		13	15	15

** Sample deactivated due to injector carryover (not included in summary statistics)

Table 28. Inter-Assay Precision for M570 Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 8.60 ng/mL	Medium QC 155 ng/mL	High QC 405 ng/mL
29-Jun-2000	13	8.23	168	393
		8.07	155	403
		8.34	164	424
		8.87	153	378
		8.04	170	377
30-Jun-2000	15	8.17	151	396
		9.09	149	396
		8.72	148	412
		7.71	138	388
		7.88	150	413
02-Jul-2000	16	8.67	148	382
		8.49	137	391
		9.73	148	384
		9.69	145	412
		7.95	151	368
Mean		8.51	152	394
S.D.		0.621	9.51	15.8
%CV		7.3	6.3	4.0
%Theoretical		99.0	98.1	97.3
%Bias		-1.0	-1.9	-2.7
n		15	15	15

Table 29. Intra-Assay Precision and Accuracy for PFOSA Specific Quality Controls

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC (4.00 ng/mL)	Medium QC (150 ng/mL)	High QC (400 ng/mL)
16-Jul-2000	17	3.55	145	322
		3.58	135	324
		3.84	171	326
		4.01	149	330
		3.46	144	341
Mean		3.69	149	329
S.D.		0.229	13.4	7.54
%CV		6.2	9.0	2.3
%Theoretical		92.3	99.3	82.3
n		5	5	5

Note: The following calibration curve and standard statistics are included for analytical run review. (not included in overall validation summary statistics)
Quadratic weighted $1/x^2$.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
16-Jul-2000	17	0.000002	0.020014	0.006490	0.9985	1.00	502
n		1	1	1	1		

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

Run Date	Run Number	1.00	2.51	10.1	25.1	50.3	100	251	402	502
16-Jul-2000	17	0.998	2.48	10.1	24.5	52.6	96.2	244	390	531
		0.980	2.67	10.4	24.2	50.4	104	239	399	505
Mean		0.989	2.58	10.3	24.4	51.5	100	242	395	518
%Bias		-1.1	2.8	2.0	-2.8	2.4	0.0	-3.6	-1.7	3.2
n		2	2	2	2	2	2	2	2	2

Table 30. Intra-Assay Precision and Accuracy for PFOSAA Specific Quality Controls

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC (9.00 ng/mL)	Medium QC (155 ng/mL)	High QC (405 ng/mL)
16-Jul-2000	17	7.30	161	368
		*4.93	176	382
		*5.65	*190	395
		8.36	159	365
		**21.4	151	346
Mean		6.56	167	371
S.D.		1.56	15.5	18.5
%CV		23.8	9.3	5.0
%Theoretical		72.9	107.7	91.6
n		4	5	5

* > ±20% theoretical

** Deactivated as an outlier (not included in summary statistics)

Note: The following calibration curve and standard statistics are included for analytical run review. (not included in overall validation summary statistics)

Quadratic weighted $1/x^2$.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
16-Jul-2000	17	0.000001	0.005669	0.008710	0.9764	7.50	505
n		1	1	1	1		

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

Run Date	Run Number	6.00	7.50	15.0	30.0	55.0	105	255	405	505
16-Jul-2000	17	**8.89	8.60	*17.8	32.5	*65.3	107	287	413	559
		**3.30	6.49	*12.1	*25.4	47.6	104	231	372	465
Mean		-	7.55	15.0	29.0	56.5	106	259	393	512
%Bias		-	0.7	0.0	-3.3	2.7	1.0	1.6	-3.0	1.4
n		-	2	2	2	2	2	2	2	2

* > ±15% theoretical

** Sample deactivated as an outlier (> 2 times the acceptance criteria). Result not included in summary statistics.

Table 31. PFOS Dilution Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Dilution QC 4460 ng/mL
29-Jun-2000	13	4310
		4170
		4420
		3900
		4110
30-Jun-2000	15	4490
		3990
		4180
		4050
		4400
02-Jul-2000	16	4160
		4160
		4020
		3790
		3970
Mean		4140
S.D.		199
%CV		4.8
%Theoretical		92.8
%Bias		-7.2
n		15

Table 32. PFOSA Dilution Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Dilution QC 4000 ng/mL
29-Jun-2000	13	3720
		3570
		3720
		3380
		3690
30-Jun-2000	15	3020
		*2890
		3100
		*2750
		*2860
02-Jul-2000	16	3270
		3130
		3130
		3210
		3240
Mean		3250
S.D.		315
%CV		9.7
%Theoretical		81.3
%Bias		-18.8
n		15

* > ±25% theoretical

Table 33. PFOSAA Dilution Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Dilution QC 4050 ng/mL
29-Jun-2000	13	3490
		3280
		3270
		*3170
		3390
30-Jun-2000	15	3420
		*3030
		3240
		*3060
		*3120
02-Jul-2000	16	*3220
		*3110
		*3210
		*2960
		*3000
Mean		3200
S.D.		157
%CV		4.9
%Theoretical		79.0
%Bias		-21.0
n		15

* > ±20% theoretical

Table 34. POAA Dilution Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Dilution QC 4030 ng/mL
29-Jun-2000	13	4220
		3820
		4250
		4040
		4000
30-Jun-2000	15	4550
		3850
		4010
		4000
		4020
02-Jul-2000	16	4170
		4090
		4230
		3910
		4040
Mean		4080
S.D.		184
%CV		4.5
%Theoretical		101.2
%Bias		1.2
n		15

Table 35. PFHS Dilution Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Dilution QC 4020 ng/mL
29-Jun-2000	13	4830
		4430
		4530
		4150
		4190
30-Jun-2000	15	*4980
		4500
		4550
		4490
		4790
02-Jul-2000	16	4700
		*5030
		4550
		4260
		4760
Mean		4580
S.D.		267
%CV		5.8
%Theoretical		113.9
%Bias		13.9
n		15

* > ±20% theoretical

Table 36. M556 Dilution Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Dilution QC 4020 ng/mL
29-Jun-2000	13	4330
		4050
		4260
		3940
		4000
30-Jun-2000	15	4490
		3980
		4130
		4000
		4170
02-Jul-2000	16	4270
		3990
		4240
		3890
		3980
Mean		4110
S.D.		172
%CV		4.2
%Theoretical		102.2
%Bias		2.2
n		15

Table 37. M570 Dilution Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Dilution QC 4050 ng/mL
29-Jun-2000	13	4710
		4590
		4550
		4100
		4490
30-Jun-2000	15	4610
		3980
		4080
		4080
		4280
02-Jul-2000	16	4270
		3950
		4120
		3890
		3960
Mean		4240
S.D.		278
%CV		6.6
%Theoretical		104.7
%Bias		4.7
n		15

Table 38. PFOSA Specific Dilution Quality Controls

All concentrations are expressed as ng/mL.

Run Date	Run Number	Dilution QC 4000 ng/mL
16-Jul-2000	17	*2630
		*2570
		3110
		3250
		4110
Mean		3130
S.D.		620
%CV		19.8
%Theoretical		78.3
%Bias		-21.8
n		5

* > ±25% theoretical

Table 39. PFOSAA Specific Dilution Quality Controls

All concentrations are expressed as ng/mL.

Run Date	Run Number	Dilution QC 4050 ng/mL
16-Jul-2000	17	*2560
		*2650
		*2900
		3640
		4070
Mean		3160
S.D.		661
%CV		20.9
%Theoretical		78.0
%Bias		-22.0
n		5

* > ±20% theoretical

Table 40. PFOS Extraction Efficiency

Run 7

Low Concentration = 2.50 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.00936	107
		0.0101	99.2
		0.0103	97.5
	Mean	0.00992	101
2. Analyte prior and IS after extraction		0.00532	
		0.00568	
		0.00517	
	Mean	0.00539	
3. IS prior and Analyte after extraction			6.94
			7.51
			8.42
	Mean		7.62

Mean extraction efficiency for the analyte = 54.3%
Mean extraction efficiency for the internal standard = 7.54%

Medium Concentration = 100 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.326	3.07
		0.297	3.37
		0.312	3.21
	Mean	0.312	3.22
2. Analyte prior and IS after extraction		0.117	
		0.121	
		0.113	
	Mean	0.117	
3. IS prior and Analyte after extraction			0.263
			0.269
			0.280
	Mean		0.271

Mean extraction efficiency for the analyte = 37.5%
Mean extraction efficiency for the internal standard = 8.42%

High Concentration = 400 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		1.17	0.855
		1.25	0.797
		1.21	0.828
	Mean	1.21	0.827
2. Analyte prior and IS after extraction		0.435	
		0.398	
		0.418	
	Mean	0.417	
3. IS prior and Analyte after extraction			0.0692
			0.0652
			0.0668
	Mean		0.0671

Mean extraction efficiency for the analyte = 34.5%
Mean extraction efficiency for the internal standard = 8.11%

Overall mean extraction efficiency for analyte = 42.1%
Overall mean extraction efficiency for internal standard = 8.02%

Table 41. PFOSA Extraction Efficiency

Run 7

Low Concentration = 2.50 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.00603	166
		0.00611	164
		0.00587	170
	Mean	0.00600	167
2. Analyte prior and IS after extraction		0.00375	
		0.00434	
		0.00383	
	Mean	0.00397	
3. IS prior and Analyte after extraction			11.4
			11.2
			13.4
	Mean		12.0

Mean extraction efficiency for the analyte = 66.2%
Mean extraction efficiency for the internal standard = 7.19%

Medium Concentration = 100 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.240	4.16
		0.203	4.92
		0.214	4.68
	Mean	0.219	4.59
2. Analyte prior and IS after extraction		0.148	
		0.128	
		0.152	
	Mean	0.143	
3. IS prior and Analyte after extraction			0.331
			0.357
			0.358
	Mean		0.349

Mean extraction efficiency for the analyte = 65.3%
Mean extraction efficiency for the internal standard = 7.60%

High Concentration = 400 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.950	1.05
		0.901	1.11
		0.973	1.03
	Mean	0.941	1.06
2. Analyte prior and IS after extraction		0.595	
		0.574	
		0.648	
	Mean	0.606	
3. IS prior and Analyte after extraction			0.0880
			0.0778
			0.0817
	Mean		0.0825

Mean extraction efficiency for the analyte = 64.4%
Mean extraction efficiency for the internal standard = 7.78%

Overall mean extraction efficiency for analyte = 65.3%
Overall mean extraction efficiency for internal standard = 7.52%

Table 42. PFOSAA Extraction Efficiency

Run 7

Low Concentration = 2.50 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.00526	190
		0.00589	170
		0.00559	179
	Mean	0.00558	180
2. Analyte prior and IS after extraction		0.00401	
		0.00427	
		0.00399	
	Mean	0.00409	
3. IS prior and Analyte after extraction			12.8
			14.1
			15.6
	Mean		14.2

Mean extraction efficiency for the analyte = 73.3%
Mean extraction efficiency for the internal standard = 7.89%

Medium Concentration = 100 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.373	2.68
		0.325	3.08
		0.342	2.93
	Mean	0.347	2.90
2. Analyte prior and IS after extraction		0.254	
		0.270	
		0.249	
	Mean	0.258	
3. IS prior and Analyte after extraction			0.234
			0.238
			0.248
	Mean		0.240

Mean extraction efficiency for the analyte = 74.4%
Mean extraction efficiency for the internal standard = 8.28%

High Concentration = 400 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.864	1.16
		0.900	1.11
		0.889	1.12
	Mean	0.884	1.13
2. Analyte prior and IS after extraction		0.642	
		0.624	
		0.658	
	Mean	0.641	
3. IS prior and Analyte after extraction			0.0944
			0.0878
			0.0885
	Mean		0.0902

Mean extraction efficiency for the analyte = 72.5%
Mean extraction efficiency for the internal standard = 7.98%

Overall mean extraction efficiency for analyte = 73.4%
Overall mean extraction efficiency for internal standard = 8.05%

Table 43. POAA Extraction Efficiency

Run 7

Low Concentration = 250 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.00921	109
		0.00982	102
		0.0105	95.0
	Mean	0.00984	102
2. Analyte prior and IS after extraction		0.00209	
		0.00213	
		0.00224	
	Mean	0.00215	
3. IS prior and Analyte after extraction			7.02
			7.35
			7.97
	Mean		7.45

Mean extraction efficiency for the analyte = 21.8%
Mean extraction efficiency for the internal standard = 7.30%

Medium Concentration = 100 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.358	2.79
		0.318	3.15
		0.336	2.98
	Mean	0.337	2.97
2. Analyte prior and IS after extraction		0.0526	
		0.0570	
		0.0516	
	Mean	0.0537	
3. IS prior and Analyte after extraction			0.227
			0.237
			0.248
	Mean		0.237

Mean extraction efficiency for the analyte = 15.9%
Mean extraction efficiency for the internal standard = 7.98%

High Concentration = 400 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		1.19	0.841
		1.31	0.762
		1.29	0.775
	Mean	1.26	0.793
2. Analyte prior and IS after extraction		0.205	
		0.201	
		0.216	
	Mean	0.207	
3. IS prior and Analyte after extraction			0.0638
			0.0595
			0.0627
	Mean		0.0620

Mean extraction efficiency for the analyte = 16.4%
Mean extraction efficiency for the internal standard = 7.82%

Overall mean extraction efficiency for analyte = 18.0%
Overall mean extraction efficiency for internal standard = 7.70%

Table 44. PFHS Extraction Efficiency

Run 7

Low Concentration = 2.50 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.0158	63.2
		0.0168	59.6
		0.0169	59.3
	Mean	0.0165	60.7
2. Analyte prior and IS after extraction		0.00371	
		0.00384	
		0.00399	
	Mean	0.00385	
3. IS prior and Analyte after extraction			4.43
			4.47
			5.27
	Mean		4.72

Mean extraction efficiency for the analyte = 23.3%
Mean extraction efficiency for the internal standard = 7.78%

Medium Concentration = 100 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.632	1.58
		0.567	1.76
		0.598	1.67
	Mean	0.599	1.67
2. Analyte prior and IS after extraction		0.105	
		0.113	
		0.108	
	Mean	0.109	
3. IS prior and Analyte after extraction			0.134
			0.146
			0.146
	Mean		0.142

Mean extraction efficiency for the analyte = 18.2%
Mean extraction efficiency for the internal standard = 8.50%

High Concentration = 400 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		2.07	0.484
		2.25	0.444
		2.15	0.465
	Mean	2.16	0.464
2. Analyte prior and IS after extraction		0.387	
		0.363	
		0.385	
	Mean	0.378	
3. IS prior and Analyte after extraction			0.0394
			0.0360
			0.0365
	Mean		0.0373

Mean extraction efficiency for the analyte = 17.5%
Mean extraction efficiency for the internal standard = 8.04%

Overall mean extraction efficiency for analyte = 19.7%
Overall mean extraction efficiency for internal standard = 8.11%

Table 45. M556 Extraction Efficiency

Run 7

Low Concentration = 2.50 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.00635	157
		0.00659	152
		0.00684	146
	Mean	0.00659	152
2. Analyte prior and IS after extraction		0.00293	
		0.00299	
		0.00279	
	Mean	0.00290	
3. IS prior and Analyte after extraction			10.7
			10.7
			12.4
	Mean		11.3

Mean extraction efficiency for the analyte = 44.0%
Mean extraction efficiency for the internal standard = 7.43%

Medium Concentration = 100 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.270	3.70
		0.240	4.17
		0.252	3.97
	Mean	0.254	3.95
2. Analyte prior and IS after extraction		0.106	
		0.124	
		0.111	
	Mean	0.114	
3. IS prior and Analyte after extraction			0.309
			0.320
			0.325
	Mean		0.318

Mean extraction efficiency for the analyte = 44.9%
Mean extraction efficiency for the internal standard = 8.05%

High Concentration = 400 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		1.02	0.984
		1.07	0.934
		1.05	0.953
	Mean	1.05	0.957
2. Analyte prior and IS after extraction		0.444	
		0.441	
		0.463	
	Mean	0.449	
3. IS prior and Analyte after extraction			0.0800
			0.0744
			0.0765
	Mean		0.0770

Mean extraction efficiency for the analyte = 42.8%
Mean extraction efficiency for the internal standard = 8.05%

Overall mean extraction efficiency for analyte = 43.9%
Overall mean extraction efficiency for internal standard = 7.84%

Table 46. M570 Extraction Efficiency

Run 7

Low Concentration = 2.50 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.00470	213
		0.00487	205
		0.00530	189
	Mean	0.00496	202
2. Analyte prior and IS after extraction		0.00317	
		0.00355	
		0.00320	
	Mean	0.00331	
3. IS prior and Analyte after extraction			14.4
			14.0
			15.8
	Mean		14.7

Mean extraction efficiency for the analyte = 66.7%
Mean extraction efficiency for the internal standard = 7.28%

Medium Concentration = 100 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.217	4.61
		0.191	5.24
		0.204	4.90
	Mean	0.204	4.92
2. Analyte prior and IS after extraction		0.130	
		0.143	
		0.136	
	Mean	0.136	
3. IS prior and Analyte after extraction			0.391
			0.396
			0.422
	Mean		0.403

Mean extraction efficiency for the analyte = 66.7%
Mean extraction efficiency for the internal standard = 8.19%

High Concentration = 400 ng/mL for Analyte

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.772	1.29
		0.831	1.20
		0.796	1.26
	Mean	0.800	1.25
2. Analyte prior and IS after extraction		0.533	
		0.521	
		0.563	
	Mean	0.539	
3. IS prior and Analyte after extraction			0.102
			0.0970
			0.0984
	Mean		0.0991

Mean extraction efficiency for the analyte = 67.4%
Mean extraction efficiency for the internal standard = 7.93%

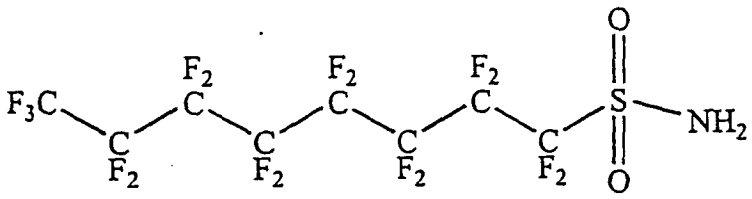
Overall mean extraction efficiency for analyte = 66.9%
Overall mean extraction efficiency for internal standard = 7.80%

7. ANALYTICAL METHOD

Principles of the Method

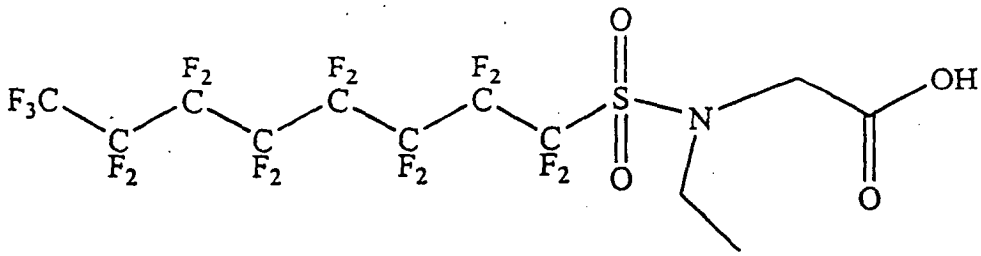
The analytical method consisted of a liquid:liquid extraction procedure followed by evaporation and reconstitution of the extract residue with 30:70 (v/v) 20 mM ammonium acetate in water: 20 mM ammonium acetate in methanol. The samples were analyzed by liquid chromatography/tandem mass spectrometry using a PE Sciex API 3000. The instrument was operated in the multiple reaction monitoring (MRM) mode under optimized conditions for PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 detection.

CHEMICAL STRUCTURES



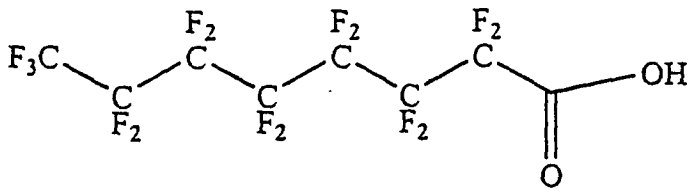
PFOSA

MW=499.0
[M-H]=498.0



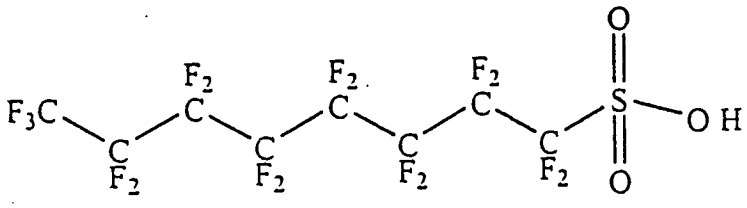
PFOSAA

MW=585.0
[M-H]=584.0



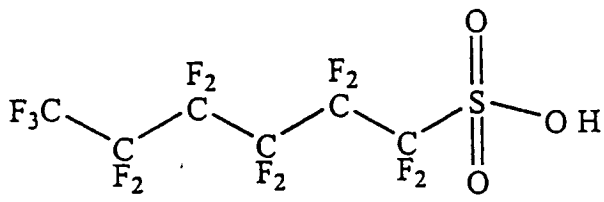
POAA

MW=414.0
[M-H]=413.0



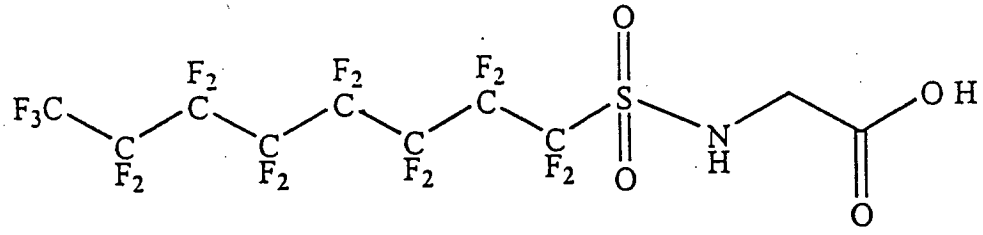
PFOS

MW=499.9
[M-H]=498.9



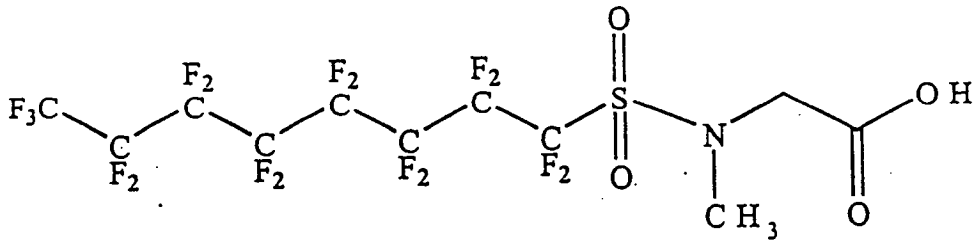
PFHS

MW=399.9
[M-H]=398.9



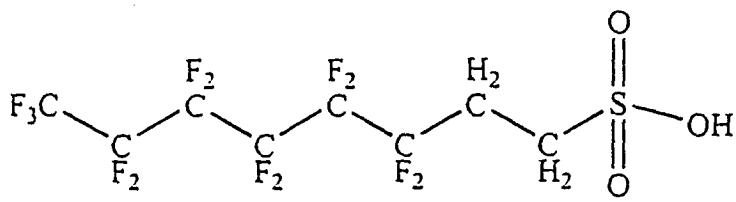
M556

MW=556.9
[M-H]=555.9



M570

MW=571.0
[M-H]=570.0



THPFOS
MW=428

7.1. Reference Materials and Matrices

Analyte	*Lot Number*	*Purity*	*Expiration Date*	*Source*	*Storage Conditions*
PFOS (FC-95)	193	100%	12/31/2010	3M	Room Temperature
PFOSA	214	100%	12/31/2010	3M	Room Temperature
PFOSAA (FC-129)	617	53.8%	12/31/2010	3M	Room Temperature
POAA (FC-143)	245	100%	12/31/2010	3M	Room Temperature**
PFHS*	S398-182	100%	12/31/2010	3M	-20°C
M556	NB113047-8D	99.89%	12/31/2010	3M	Room Temperature
M570	118506-26	99.75%	12/31/2010	3M	Room Temperature
THPFOS	59909	90%	12/31/2010	3M	Room Temperature

* Received as a 6,200 ppm solution in methanol. ** Kept dry.

The reference material purity for PFOS, PFOSA, PFHS and POAA was not available prior to the conduct of this study. Therefore, all concentrations included in the report for these analytes are based upon an assumed purity of 100%.

Prior to the completion of this final report, 3M contracted with Centre Analytical Laboratories, Inc. in State College, Pennsylvania to determine the absolute concentration of PFOS, POAA and PFHS in the stock standard solution prepared using seven reference materials (PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and

M570) at a target concentration of 5000 ppb. Absolute determination of the concentration of PFOSA in the stock standard solutions cannot be made until a full purity determination of these reference materials is completed.

Based on the results obtained, the concentrations included in this report should be corrected according to the following table:

Analyte	Correction Factor
PFOS	0.836
POAA	0.909
PFHS	0.855

Matrix	*Lot Numbers*
Human serum	BC30399-4
Human plasma (Northern Chinese)	C5186; C4929; C5517; C5200

7.2. Chemicals and Equipment

Chemicals
Ammonium acetate, 99.9%
Di (ethylene glycol) methyl ether [may be written as 2-(2-methoxyethoxy) ethanol]
Methanol, HPLC-grade
Methyl-*Tert*-Butyl Ether (MTBE), HPLC-grade
Water, HPLC-grade

Equipment/Supplies	
Name	**Source**
Autosampler: PE Series 200	Perkin Elmer, Norwalk, CT
Balance: Mettler Toledo MT5	Mettler-Toledo, Inc., Hightstown, NJ

Equipment/Supplies	
Name	**Source**
Centrifuge: Beckman GS-6R	Beckman Instruments, Fullerton, CA
Evaporator: Turbo Vap LV, Model 43750	Zymark Corp., Hopkinton, MA
Hematology/Chemistry Mixer, Model 346	Fisher Scientific, Pittsburgh, PA
HPLC Chromatographic column: Genesis Lightning, C_{18}, 4 µm, 2 x 50 mm	Jones Chromatography, Lakewood, CA
Liquid Chromatograph: Hewlett Packard 1100	Agilent (Hewlett Packard), Palo Alto, CA
Liquid Chromatograph: PE Sciex Series 200	Perkin Elmer, Norwalk, CT
Liquid Chromatograph: Shimadzu SCL-10A controller with CL-10AD pump and CTO-10A column oven	Shimadzu, Columbia, MD
Mass Spectrometer: Perkin Elmer Sciex API 3000	PE Sciex, Concord, Ontario
Multi-tube Vortexer	VWR Scientific Products, Bridgeport, NJ
Pipettes: Eppendorf Repeater Pipette	Brinkman Instruments, Inc., Westbury, NY
Pipettes: Finnpipette: Digital 1-5 mL	Fisher Scientific, Pittsburgh, PA
Pipettes: Rainin EDP Digital 100-1000µL ™	Rainin Instrument Co., Woburn, MA
Pipettes: Rainin M Volumes: adjustable 20-50, 10-100, 100-1000 µL	Rainin Instrument Co., Woburn, MA
Pipettes: Rainin RL Volumes: adjustable 5-20, 20-100, 40-200, 200-1000 µL	Rainin Instrument Co., Woburn, MA
Sonicator, Branson	Branson, Danbury, CT
Sonicator, Fisher	Fisher Scientific, Fair Lawn, NJ
Vortex: Fisher Genie 2	Fisher Scientific, Fair Lawn, NJ
Water Pro Plus	Labconco, Co., Kansas City, MO

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

The calibrator, quality control and stock solution preparations listed below serve as a general guideline for attaining the targeted concentrations. Due to the fact that PFHS was received at NWB as a solution, both the calibration standard and quality control stocks are from the same solution and are not from separate weighings of reference material as per NWB SOP LABOP003. However, separate aliquots were used for calibration standard and QC stock solution preparation.

Reagents

All reagent solutions are stored at room temperature unless otherwise noted.

- *50 mM ammonium acetate in water (unadjusted: pH ~6.9)*
  Fill a 1-L volumetric flask approximately half full with water. Weigh out 3.85 g of ammonium acetate and transfer to the flask. Use a stir plate to mix and fill to volume with water.
- *20 mM ammonium acetate in water (unadjusted: pH ~6.9)*
  Fill a 1-L volumetric flask approximately half full with water. Weigh out 1.54 g of ammonium acetate and transfer to the flask. Use a stir plate to mix and fill to volume with water.
- *20 mM ammonium acetate in methanol*
  Prepare as described above, substituting methanol for water.
- *30:70 20 mM ammonium acetate in water (unadjusted: pH ~6.9): 20 mM ammonium acetate in methanol (v/v)*
- *50:50 water:methanol (v/v)*

Calibration Standard Solutions

All calibration standard solutions are transferred to polypropylene containers and stored in a –20 °C freezer. The PFHS reference material was provided as a 6200 ppm solution.

- *PFOS Stock Standard (0.05904 mg/mL)*

  Weigh 1.476 mg of PFOS and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *PFOSA Stock Standard (0.07652 mg/mL)*

  Weigh 1.913 mg of PFOSA and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *PFOSAA Stock Standard (0.02264 mg/mL)*

  Weigh 1.052 mg of PFOSAA and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *POAA Stock Standard (0.03868 mg/mL)*

  Weigh 0.967 mg of POAA and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *M556 Stock Standard (0.04579 mg/mL)*

  Weigh 1.146 mg of M556 and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *M570 Stock Standard (0.04796 mg/mL)*

  Weigh 1.202 mg of M570 and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *Spiking Standard 9 (5000 ng/mL for PFOS, PFOSAA, POAA, PFHS, M556 and M570; 5020 ng/mL for PFOSA)*

  Add 2.117 mL of PFOS Stock Standard (0.05904 mg/mL), 1.639 mL of PFOSA

Stock Standard (0.07652 mg/mL), 5.521 mL of PFOSAA Stock Standard (0.02264 mg/mL), 3.232 mL of POAA Stock Standard (0.03868 mg/mL), 20.16 µL of PFHS (6200 ppm), 2.73 mL of M556 Stock Standard (0.04579 mg/mL) and 2.606 mL of M570 Stock Standard (0.04796 mg/mL) to a 25-mL volumetric flask. Fill to volume with 50:50 water:methanol and mix by inverting 10 times and sonicating for approximately 10 minutes.

- *Spiking Standard 8 (4000 ng/mL for PFOS, PFOSAA, POAA, PFHS, M556, M570; 4020 ng/mL for PFOSA)*

  Add 8.00 mL of Spiking Standard 9 to a 16 x 100 mm polypropylene tube and add 2.00 mL of 50:50 water:methanol. Mix by inverting 20 times.

- *Spiking Standard 7 (2500 ng/mL for PFOS, PFOSAA, POAA, PFHS, M556,M570; 2510 ng/mL for PFOSA)*

  Add 5.00 mL of Spiking Standard 9 to a 16 x 100 mm polypropylene tube and add 5.00 mL of 50:50 water:methanol. Mix by inverting 20 times.

- *Spiking Standard 6 (1000 ng/mL for PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570)*

  Add 2.00 mL of Spiking Standard 9 to a 16 x 100 mm polypropylene tube and add 8.00 mL of 50:50 water:methanol. Mix by inverting 20 times.

- *Spiking Standard 5 (500 ng/mL for PFOS, PFOSAA, POAA, PFHS, M556, M570; 503ng/mL for PFOSA)*

  Add 1.25 mL of Spiking Standard 8 to a 16 x 100 mm polypropylene tube and add 8.75 mL of 50:50 water:methanol. Mix by inverting 20 times.

- *Spiking Standard 4 (250 ng/mL for PFOS, PFOSAA, POAA, PFHS, M556, M570; 251 ng/mL for PFOSA)*

  Add 0.500 mL of Spiking Standard 9 to a 16 x 100 mm polypropylene tube and add 9.50 mL of 50:50 water:methanol. Mix by inverting 20 times.

- *Spiking Standard 3 (100 ng/mL for PFOS, PFOSAA, POAA, PFHS, M556, M570; 101 ng/mL for PFOSA)*

  Add 0.250 mL of Spiking Standard 8 to a 16 x 100 mm polypropylene tube and add 9.75 mL of 50:50 water:methanol. Mix by inverting 20 times.

- *Spiking Standard 2 (25.0 ng/mL for PFOS, PFOSAA, POAA, PFHS, M556, M570; 25.1 ng/mL for PFOSA)*

  Add 0.100 mL of Spiking Standard 7 to a 16 x 100 mm polypropylene tube and add 9.90 mL of 50:50 water:methanol. Mix by inverting 20 times.

- *Spiking Standard 1 (10.0 ng/mL for PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570)*

  Add 0.100 mL of Spiking Standard 6 to a 16 x 100 mm polypropylene tube and add 9.90 mL of 50:50 water:methanol. Mix by inverting 20 times.

Internal Standard Solutions

All internal standard solutions are transferred to polypropylene containers and stored in a –20 °C freezer.

- *THPFOS Stock Solution (0.04842 mg/mL)*

  Weigh 0.2690 mg of THPFOS and add to a 5-mL volumetric flask. Fill to volume with 50:50 water:methanol and mix by inverting 5 times and sonicating approximately 10 minutes.

- *Working Internal Standard (400 ng/mL THPFOS)*

  Add 413 µL of the THPFOS Stock Solution (0.04842 mg/mL) to a 50-mL volumetric flask and fill to volume with 50:50 water:methanol. Mix by inverting 5 to 10 times and sonicating approximately 5 to 10 minutes.

Quality Control (QC) Solutions

All quality control solutions are transferred to polypropylene containers and stored in a –20 °C freezer.

- *PFOS QC Stock (0.07732 mg/mL)*

  Weigh 1.933 mg of PFOS and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *PFOSA QC Stock (0.04876 mg/mL)*

  Weigh 1.219 mg of PFOSA and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *PFOSAA QC Stock (0.02920 mg/mL)*

  Weigh 1.357 mg of PFOSAA and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *POAA QC Stock (0.04228 mg/mL)*

  Weigh 1.057 mg of POAA and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *PFHS QC Stock (1000 ppm)*

  Aliquot 807µL of PFHS stock solution (6200 ppm) to a 5-mL volumetric flask. Fill to volume with methanol and mix by inversion.

- *M556 QC Stock (0.05230 mg/mL)*

  Weigh 1.309 mg of M556 and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *M570 QC Stock (0.05474 mg/mL)*

  Weigh 1.372 mg of M570 and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *PFOS Concentrated QC Solution (997,000 ng/mL)*
  Evaporate 1.69 mL of PFOS *(0.07732 mg/mL)* at 20 °C and below 5 P.S.I. nitrogen. Reconstitute with 131 µL of methanol.

- *PFOSA Concentrated QC Solution (1,000,000 ng/mL)*
  Evaporate 2.05 mL of PFOSA *(0.04876 mg/mL)* at 20 °C and below 5 P.S.I. nitrogen. Reconstitute with 100 µL of methanol.

- *PFOSAA Concentrated QC Solution (1,000,000 ng/mL)*
  Evaporate 4.42 mL of PFOSAA *(0.02920 mg/mL)* at 20 °C and below 5 P.S.I. nitrogen. Reconstitute with 129 µL of methanol.

- *POAA Concentrated QC Solution (996,000 ng/mL)*
  Evaporate 2.59 mL of POAA *(0.04228 mg/mL)* at 20 °C and below 5 P.S.I. nitrogen. Reconstitute with 110 µL of methanol.

- *PFHS Concentrated QC Solution*
  No PFHS concentrated solution was prepared.

- *M556 Concentrated QC Solution (1,000,000 ng/mL)*
  Evaporate 2.18 mL of M556 *(0.05230 mg/mL)* at 20 °C and below 5 P.S.I. nitrogen. Reconstitute with 114 µL of methanol.

- *M570 Concentrated QC Solution (1,000,000 ng/mL)*
  Evaporate 2.09 mL of M570 *(0.05474 mg/mL)* at 20 °C and below 5 P.S.I. nitrogen. Reconstitute with 114.4 µL of methanol.

7.4. Preparation of Validation Quality Control Samples

All quality control target concentrations will be corrected for the persistent levels of the analytes in human serum.

Dilution Quality Control (3990 ng/mL PFOS, 4000 ng/mL PFOSA, 4000 ng/mL PFOSAA, 3980 ng/mL POAA, 4000 ng/mL PFHS, 4000 ng/mL M556 and 4000 ng/mL M570)

Transfer 100 µL of each Concentrated QC Solution (997,000 ng/mL PFOS, 1,000,000 ng/mL PFOSA, 1,000,000 ng/mL PFOSAA, 996,000 ng/mL POAA, 1,000,000 ng/mL M556, and 1,000,000 ng/mL M570) and 100 µL of the 1000 ppm PFHS QC Stock into a 25-mL volumetric flask. Fill to volume with human serum and mix by inversion. Sonicate for approximately 10 minutes and equilibrate for approximately 10 minutes.

High Quality Control (399 ng/mL PFOS, 400 ng/mL PFOSA, 400 ng/mL PFOSAA, 398 ng/mL POAA, . 400 ng/mL PFHS, 400 ng/mL M556 and 400 ng/mL M570)

Transfer 5.00 mL of the Dilution Quality Control into a 50-mL volumetric flask. Fill to volume with human serum and mix by inversion. Sonicate for approximately 10 minutes and equilibrate for approximately 10 minutes.

Medium Quality Control (150 ng/mL PFOS, 150 ng/mL PFOSA, 150 ng/mL PFOSAA, 149 ng/mL POAA, 150 ng/mL PFHS, 150 ng/mL M556 and 150 ng/mL M570)

Transfer 1.875 mL of the Dilution Quality Control into a 50-mL volumetric flask. Fill to volume with human serum and mix by inversion. Sonicate for approximately 10 minutes and equilibrate for approximately 10 minutes.

Low Quality Control (3.99 ng/mL PFOS, 4.00 ng/mL PFOSA, 4.00 ng/ mL PFOSAA, 3.98 ng/mL POAA, 4.00 ng/mL PFHS, 4.00 ng/mL M556 and 4.00 ng/mL M570)

Transfer 0.500 mL of the High Quality Control into a 50-mL volumetric flask. Fill to volume with human serum and mix by inversion. Sonicate for approximately 10 minutes and equilibrate for approximately 10 minutes.

Storage of QC Samples

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

7.5. Preparation of PFOSA and PFOSAA Specific Validation Quality Control Samples

Quality Control (QC) Solutions

All quality control solutions are transferred to polypropylene containers and stored in a –20 °C freezer.

- *PFOSA QC Stock (0.2166 mg/mL)*
  Weigh 5.414 mg of PFOSA and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

- *PFOSAA QC Stock (0.2322 mg/mL)*
  Weigh 10.790 mg of PFOSAA and add to a 25-mL volumetric flask. Fill to volume with methanol, mix by inverting 5 times and sonicating for approximately 10 minutes.

Preparation of Quality Control Samples

All quality control target concentrations will be corrected for the persistent levels of the analytes in human serum.

Dilution Quality Control (4000 ng/mL PFOSA and PFOSAA)

Transfer 0.462 mL of the PFOSA QC Stock Solution (*0.2166 mg/mL)* and 0.431 mL of the PFOSAA QC Stock Solution (*0.2322 mg/mL*) into a 25-mL volumetric flask. Fill to volume with human serum and mix by inverting approximately 10 times. Sonicate for 5 minutes and equilibrate for approximately 5 minutes.

High Quality Control (400 ng/mL PFOSA and PFOSAA)

Transfer 2.50 mL of the Dilution Quality Control into a 25-mL volumetric flask. Fill to volume with human serum and mix by inverting approximately 10 times. Sonicate for 5 minutes and equilibrate for approximately 5 minutes.

Medium Quality Control (150 ng/mL PFOSA and PFOSAA)

Transfer 0.938 mL of the Dilution Quality Control into a 25-mL volumetric flask. Fill to volume with human serum and mix by inverting approximately 10 times. Sonicate for 5 minutes and equilibrate for approximately 5 minutes.

Low Quality Control (4.00 ng/mL PFOSA and PFOSAA)

Transfer 0.250 mL of the High Quality Control into a 25-mL volumetric flask. Fill to volume with human serum and mix by inverting approximately 10 times. Sonicate for 5 minutes and equilibrate for approximately 5 minutes.

Storage of QC Samples

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

7.6. Recommended Calibration Standard and Quality Control Preparation for Analysis

While concentrated solutions of the analytes can be used to prepare calibration standards and quality control samples, the evaporation of stock solutions to prepare concentrated stock solutions is not recommended for PFOSA and PFOSAA.

7.7. Preparation of Calibration Standards

The calibration curve is prepared on the day of each run by adding 100 µL blank human serum and 400 µL of 50 mM ammonium acetate in water (unadjusted: pH ~6.9) to 13 x 100 mm polypropylene tubes. After a brief vortex mixing, spike 10.0 µL of the appropriate spiking solution into the tubes as shown in the table below.

Standard Number	Concentration of Spiking Solution (ng/mL) PFOS, PFOSAA, POAA, PFHS, M556, M570 / PFOSA	Volume of Spiking Solution (µL)	Volume of Blank Human Serum (µL)	Final Concentration* (ng/mL)
9	5000 / 5020	10.0	100	500 / 502
8	4000 / 4020	10.0	100	400 / 402
7	2500 / 2510	10.0	100	250 / 251

Standard Number	Concentration of Spiking Solution (ng/mL) PFOS, PFOSAA, POAA, PFHS, M556, M570 / PFOSA	Volume of Spiking Solution (µL)	Volume of Blank Human Serum (µL)	Final Concentration* (ng/mL)
6	1000	10.0	100	100
5	500 / 503	10.0	100	50.0 / 50.3
4	250 / 251	10.0	100	25.0 / 25.1
3	100 / 101	10.0	100	10.0 / 10.1
2	25.0 / 25.1	10.0	100	2.50 / 2.51
1	10.0	10.0	100	1.00

* The target calibration curve range is 1.00 ng/mL to 500 ng/mL. Each analyte has a different final curve range based upon the persistent levels of the analyte in the human serum used.

7.8. Sample Preparation

Calibration Curve Samples (prepare in duplicate) as indicated above

Quality Control Samples (prepare in duplicate)

Aliquot 100 µL of each of the Low, Medium and High controls into separate 13 x 100 mm polypropylene tubes. If needed, prepare a dilution control (in triplicate) according to the following formula: for DF=X, aliquot (0.500 mL/X) of dilution control into [0.500 mL – (0.500 mL/X)] of blank human serum. Aliquot 100 µL of the prepared dilution control into separate 13 x 100 mm polypropylene tubes.

Blank Control Samples (prepare in duplicate)

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

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

Study Samples

Transfer 100-µL aliquots of each study sample into appropriately labeled 13 x 100 mm polypropylene tubes. If necessary, dilute study samples in the same manner as the Dilution QC.

Extraction Procedure

1. Add 400 µL of 50 mM ammonium acetate in water (unadjusted: pH ~6.9) to each sample except calibrators and vortex mix briefly.
2. Add 50.0 µL of Working Internal Standard to each sample (except Blanks) and vortex mix for approximately 30 seconds.
3. Centrifuge samples at 3000 rpm for approximately 2 minutes.
4. Add 3 mL of methyl-*tert*-butyl ether to each tube, cap and vortex mix for approximately 30 seconds.
5. Rotate samples for approximately 10 minutes.
6. Centrifuge samples at 3000 rpm for approximately 10 minutes.
7. Transfer the top MTBE layer into clean 13 x 100 mm polypropylene tubes using a transfer pipette.
8. Evaporate the organic layer to dryness in a TurboVap set at 25°C under gentle nitrogen stream (setting ≤5 psi) for approximately 40 minutes.
9. Reconstitute in 100 µL of 30:70 20 mM ammonium acetate in water (unadjusted: pH ~6.9): 20 mM ammonium acetate in methanol and vortex mix for approximately 15 seconds.
10. Transfer samples to autosampler vials and centrifuge at 3000 rpm for approximately 2 minutes

7.9. LC/MS/MS Conditions

LC Conditions

HPLC Column	Genesis Lightning C_{18}, 4 µm, 2x50 mm
Mobile Phases	A: 20 mM ammonium acetate in water (unadjusted: pH ~6.9) B: 20 mM ammonium acetate in methanol
LC Conditions	Gradient

Time (min.)	% B
0	50
1	50
7	97.5
9	97.5
9.1	50
11	end

Flow Rate	300 µL/minute
Post Column Addition	50 µL/minute of 2-(2-methoylethoxy) ethanol
Column Temperature	40°C
Injection Volume	2 – 20 µL

MS Conditions

Post Column Split	None
Source	TurboIonSpray™ (flow rate 8 L/min.)
Source Temp.	400 °C
Ionization Mode	Negative Ion
Analysis type	Multiple reaction monitoring (MRM)

	Transitions monitored (±0.3)	Dwell Time (ms)	Collision Energy (eV)
PFOS	499 → 80	70	80
PFOSA	498 → 78	70	65

	Transitions monitored (±0.3)	Dwell Time (ms)	Collision Energy (eV)
PFOSAA	584 → 419	70	29
POAA	413 → 169	70	25
PFHS	399 → 80	70	70
M556	556 → 498	400	40
M570	570 → 419	70	42
THPFOS	427 → 407	70	42

The prepared standards and QCs were injected into the instrument LC/MS/MS system in a systematic order.

7.10. Quantitation

PFOS, PFOSA, PFOSAA, POAA, PFHS, M556, M570 and THPFOS chromatographic peaks are integrated using PE Sciex MacQuan software (version 1.6) with a smooth factor of 1. Several of the analytes possess iosmers. The chromatographic conditions of this method can achieve baseline separation of these isomers. Because the goal of this method is to determine the total amount for each analyte, all isomer peaks are integrated together and the peak areas are treated as a single peak by MacQuan. Quantitation is based upon quadratic regression analysis of calibration curves (weighted $1/x^2$) using the area ratio vs. concentration by the Watson® DMLIMS software (version 6.1.1.04)

Figure 1. Representative Calibration Curve for PFOS

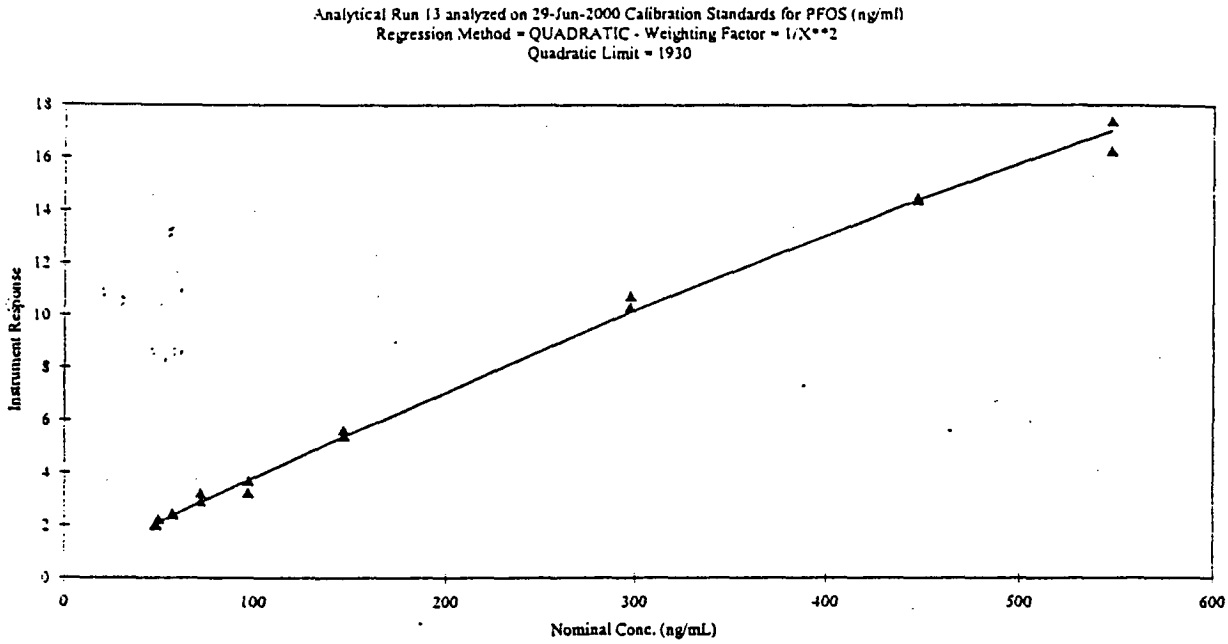


Figure 2. Representative Calibration Curve for PFOSA

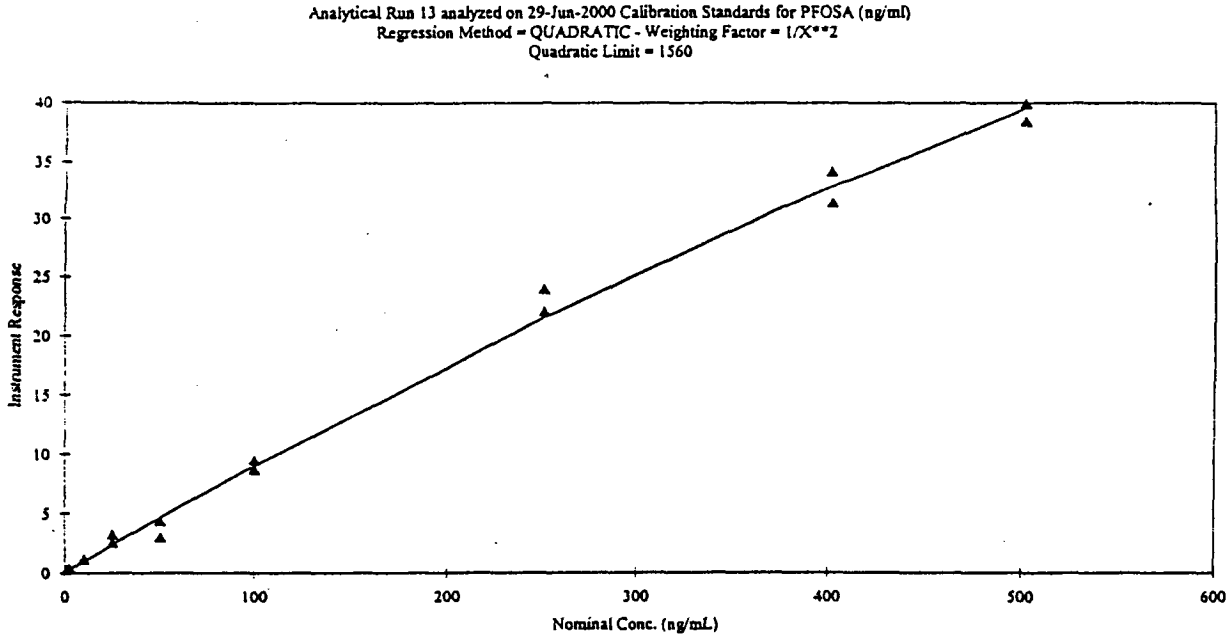


Figure 3. Representative Calibration Curve for PFOSAA

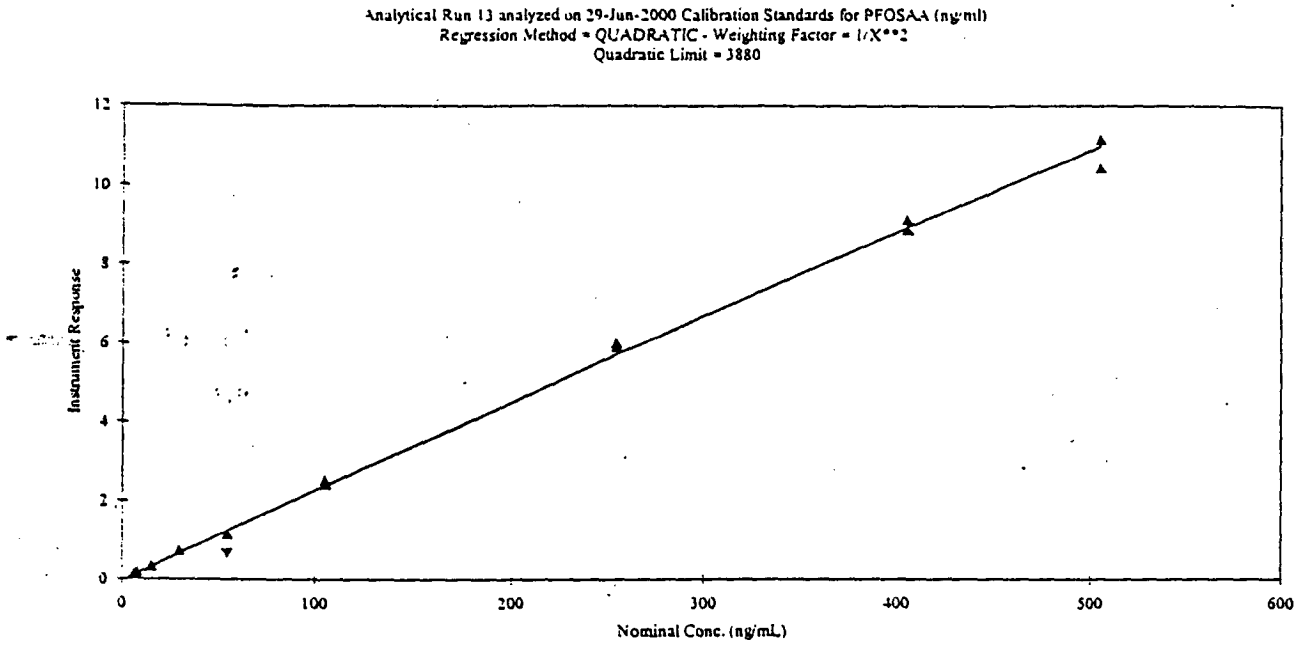


Figure 4. Representative Calibration Curve for POAA

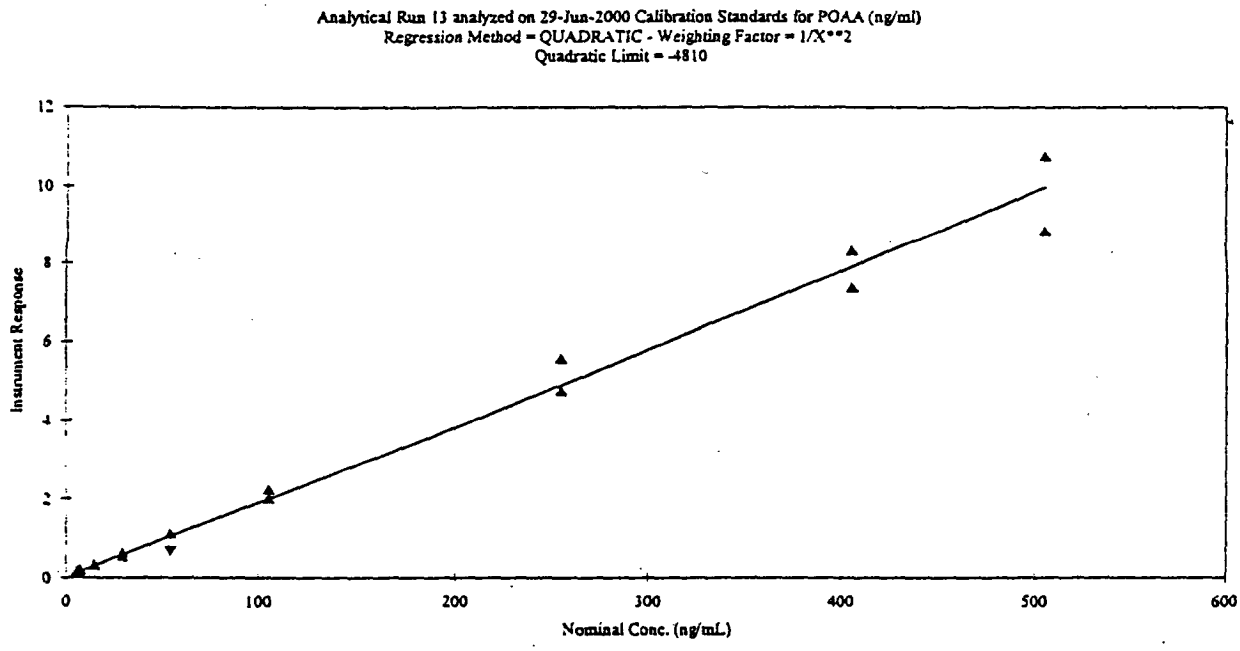


Figure 5. Representative Calibration Curve for PFHS

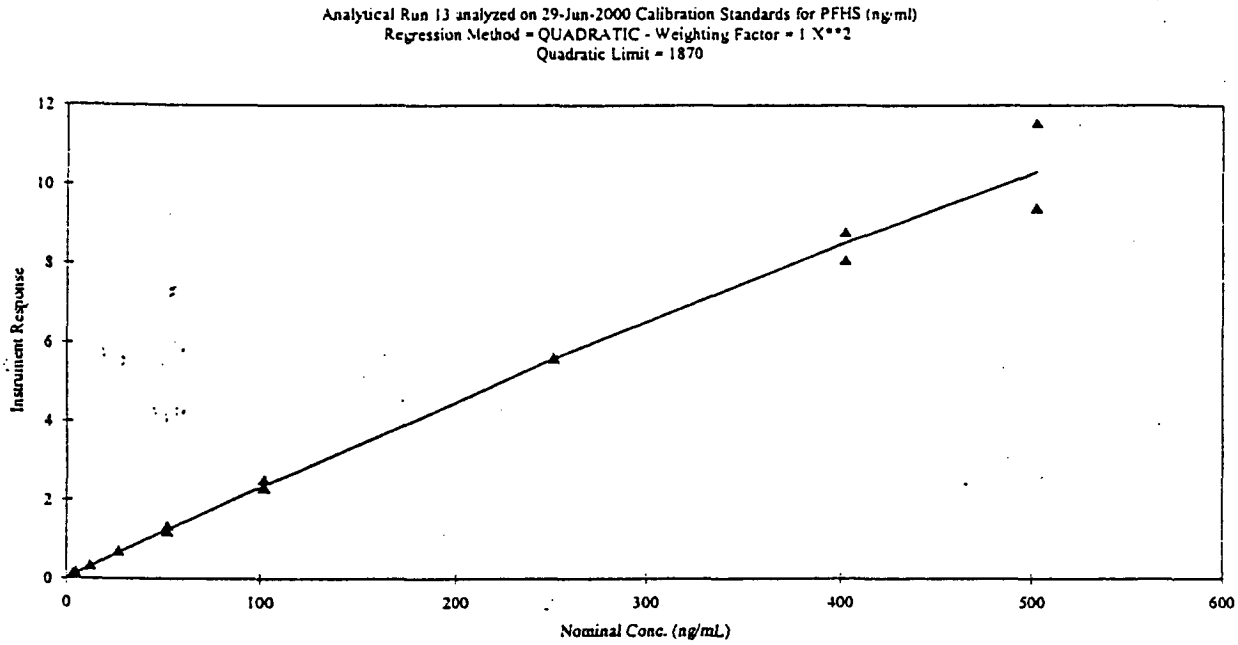


Figure 6. Representative Calibration Curve for M556

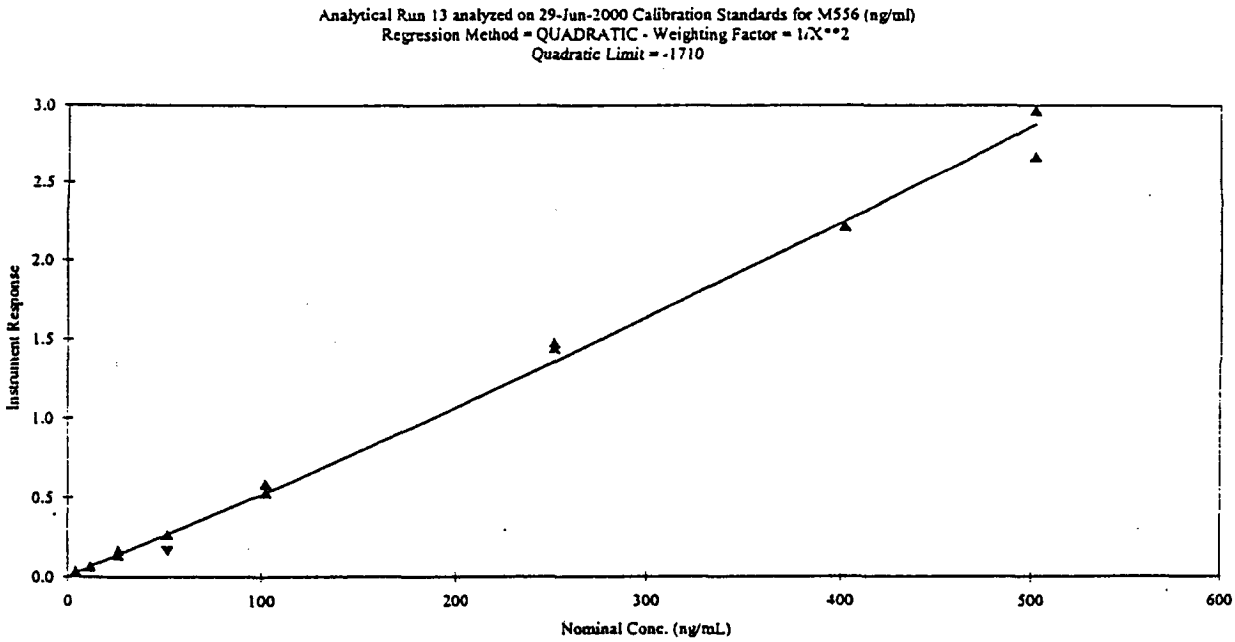


Figure 7. Representative Calibration Curve for M570

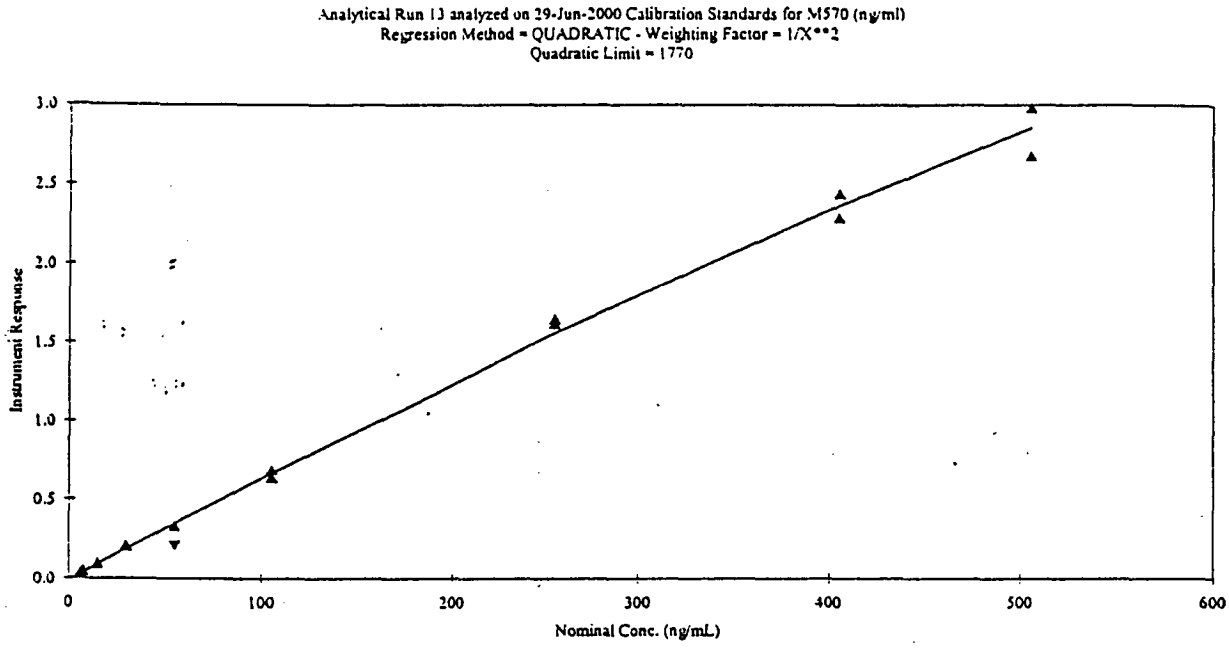


Figure 8. Standard (57.1 ng/mL) Chromatogram for PFOS

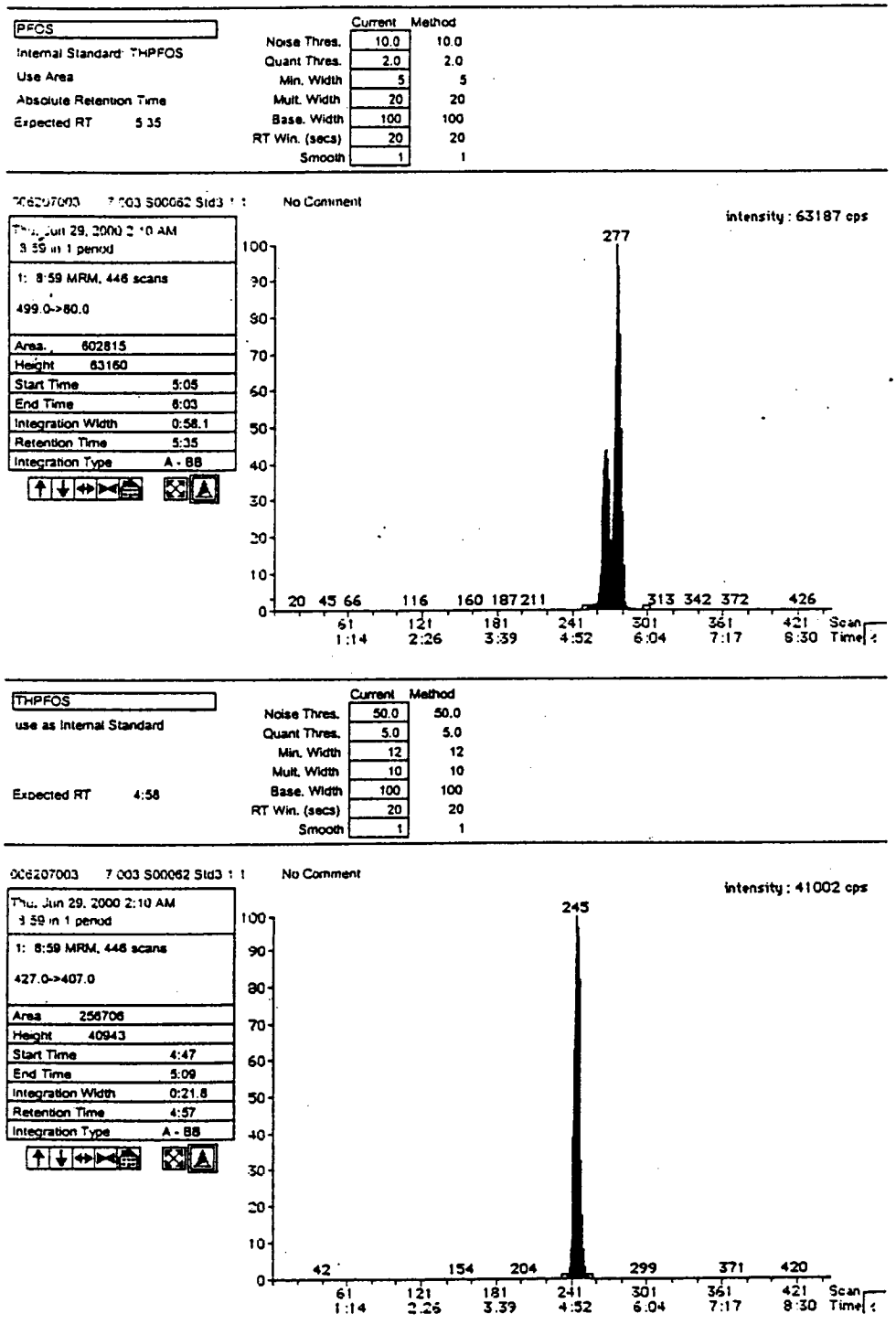


Figure 9. Standard (10.1 ng/mL) Chromatogram for PFOSA

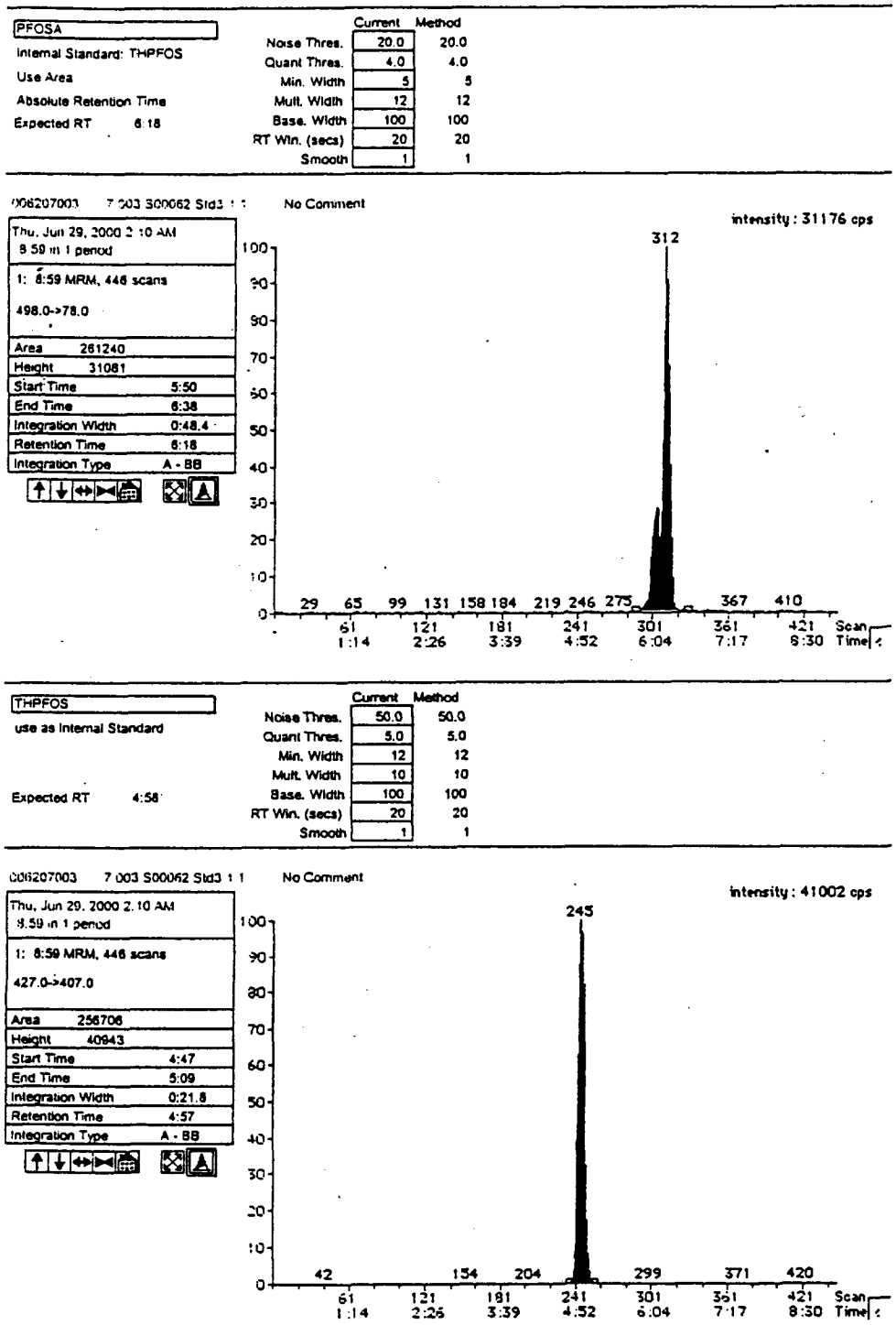


Figure 10. Standard (15.0 ng/mL) Chromatogram for PFOSAA

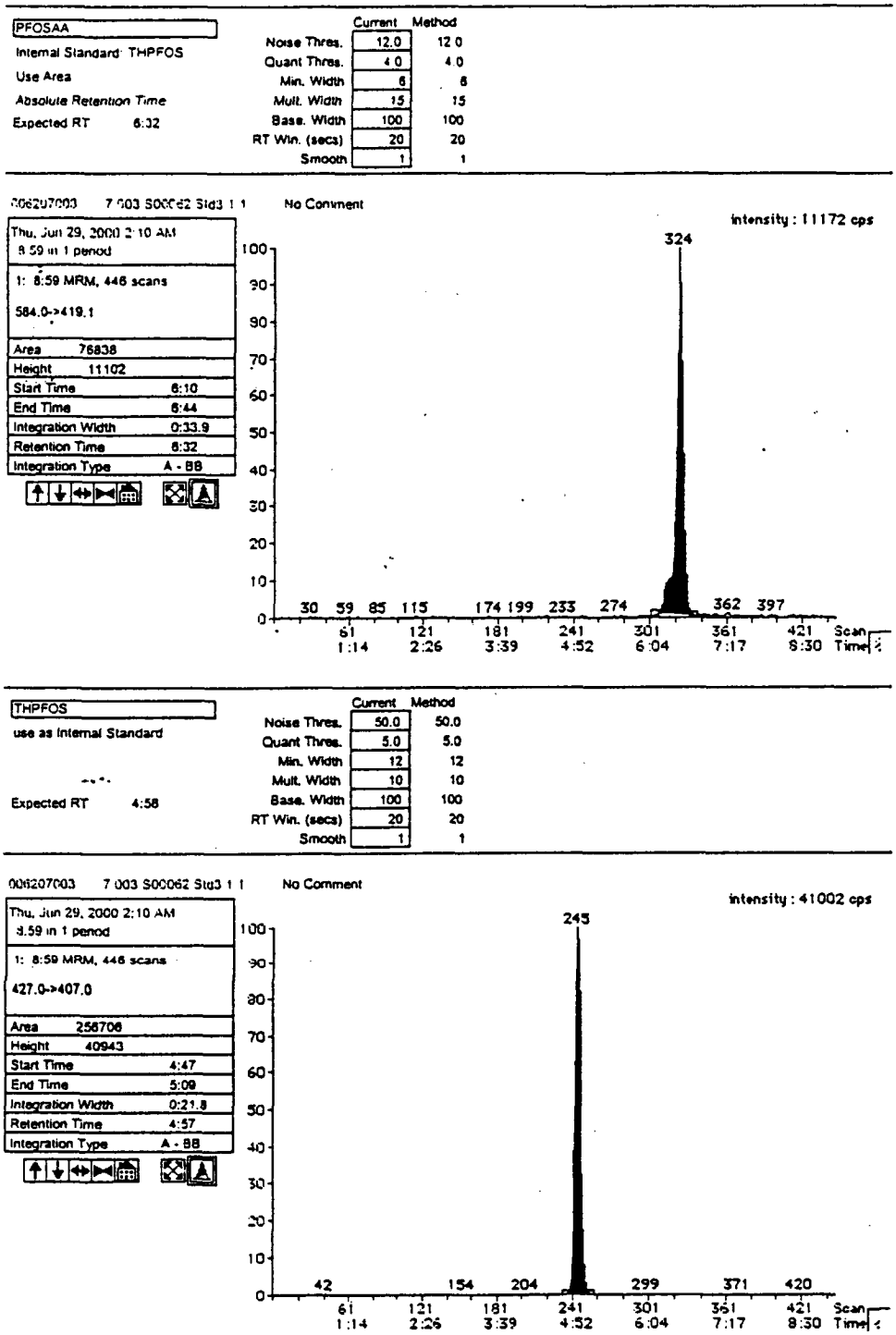


Figure 11. Standard (14.8 ng/mL) Chromatogram for POAA

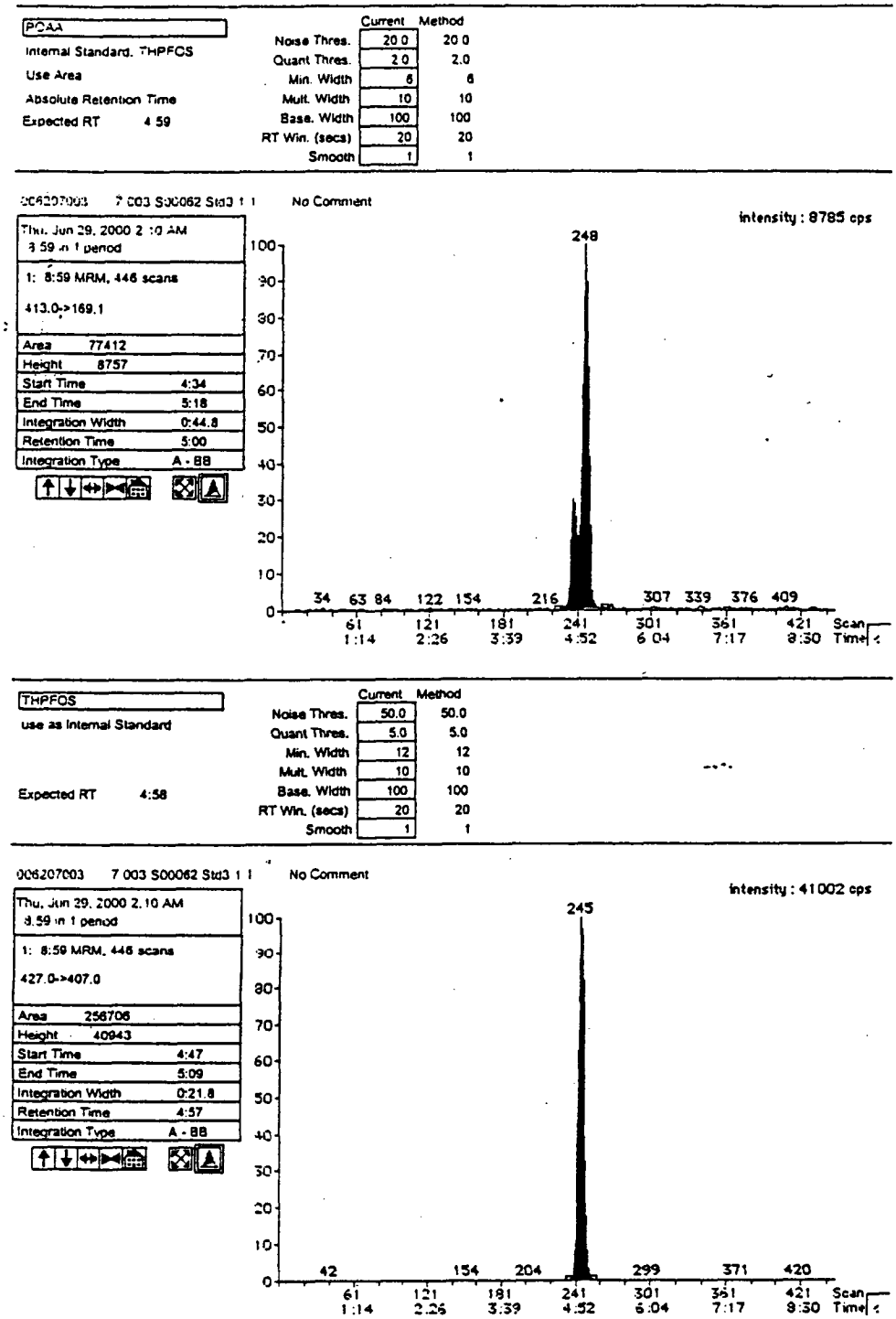


Figure 12. Standard (12.2 ng/mL) Chromatogram for PFHS

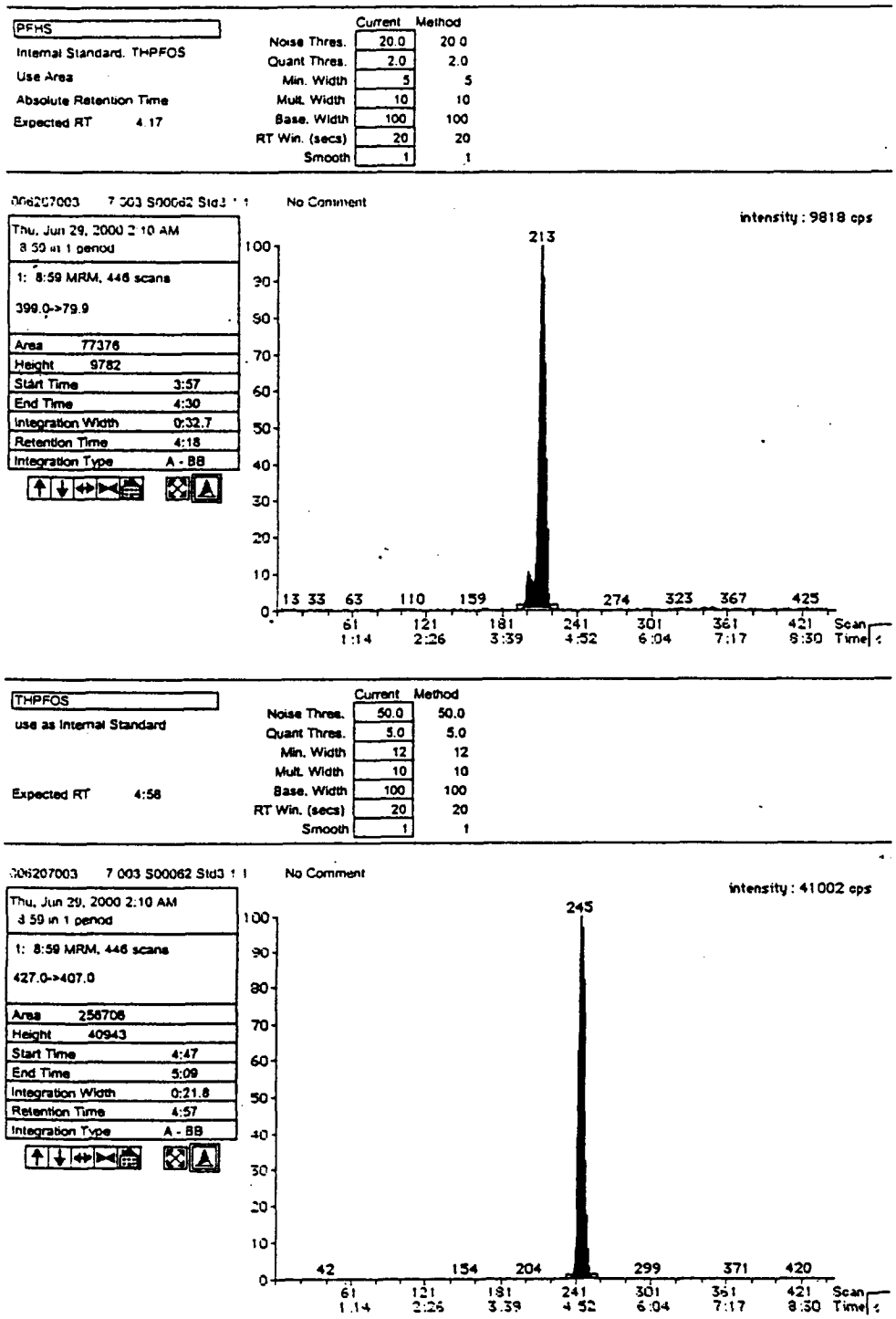


Figure 13. Standard (11.8 ng/mL) Chromatogram for M556

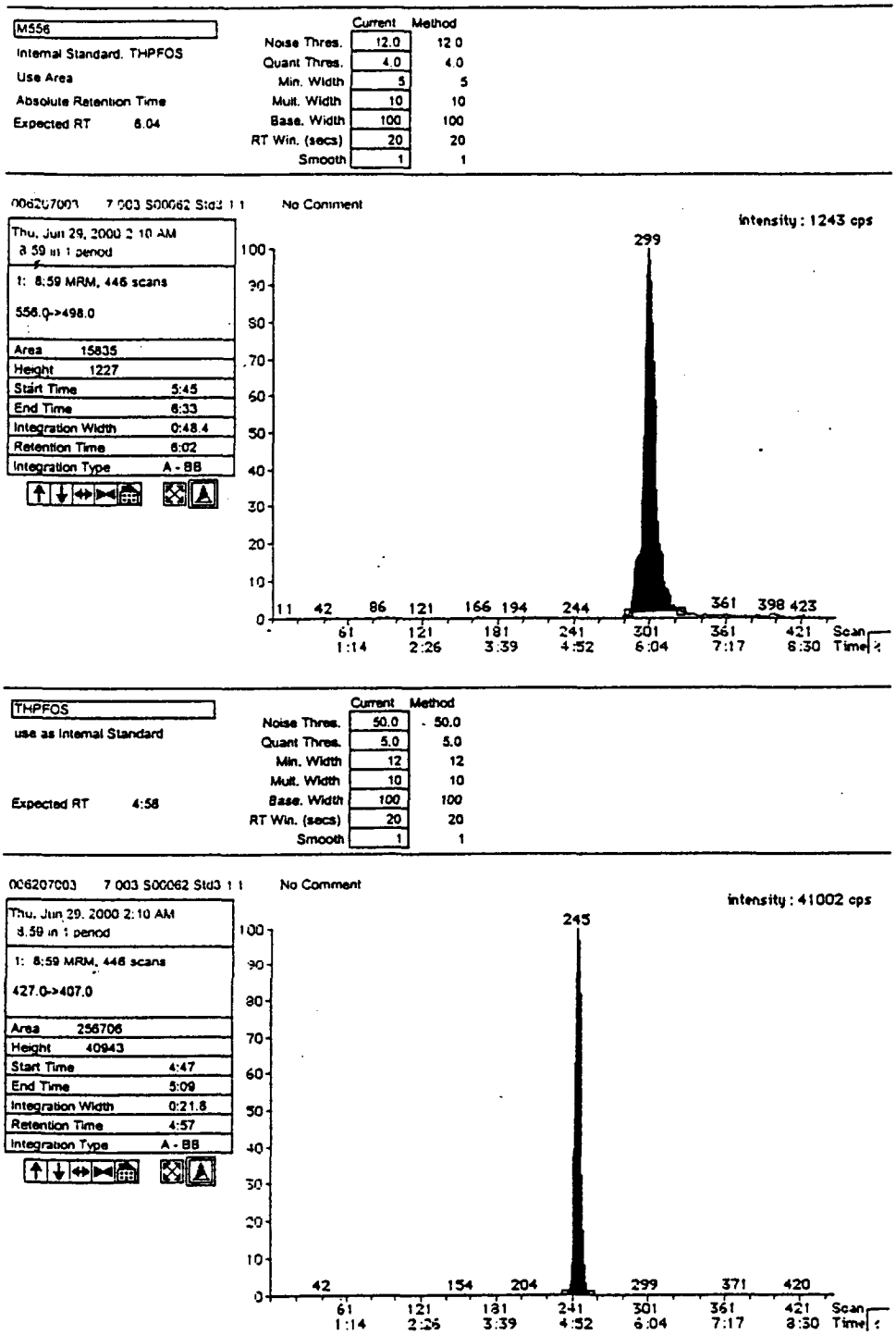
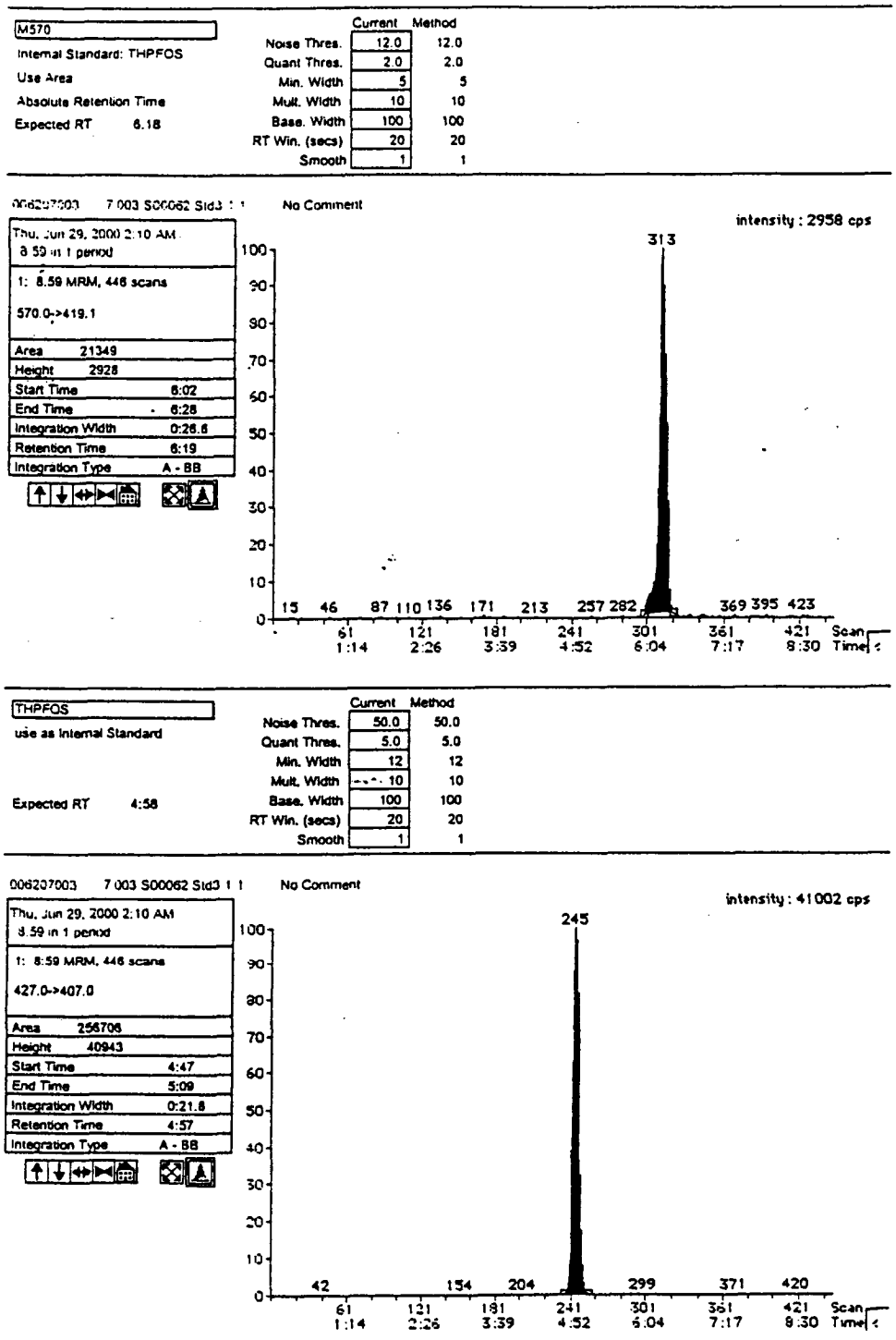


Figure 14. Standard (14.6 ng/mL) Chromatogram for M570



APPENDIX A

The following quality control results are from sample analysis study NWBS00-062.

Table A.1. Inter -Assay Precision and Accuracy for PFOSA Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 4.00 ng/mL	Medium QC 150 ng/mL	High QC 400 ng/mL
22-Jul-2000	21	3.95	158	461
		*5.18	153	364
25-Jul-2000	22	4.14	171	394
		3.50	152	379
31-Jul-2000	23	3.68	151	345
		4.35	141	356
01-Aug-2000	24	4.14	150	395
		3.84	144	359
02-Aug-2000	26	4.51	169	378
		3.96	161	406
09-Aug-2000	29	3.70	141	390
		4.09	134	364
12-Aug-2000	31	4.17	148	382
		3.95	148	346
14-Aug-2000	32	3.92	142	319
		3.86	147	364
15-Aug-2000	33	4.98	156	403
		3.81	157	413
16-Aug-2000	36	4.20	144	409
		3.33	126	382
17-Aug-2000	37	4.56	149	384
		3.43	121	470

* >±25% theoretical

Table A.1. Inter -Assay Precision and Accuracy for PFOSA Quality Control Samples (continued)

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 4.00 ng/mL	Medium QC 150 ng/mL	High QC 400 ng/mL
24-Aug-2000	42	4.57	148	471
		3.06	136	425
26-Aug-2000	43	4.61	162	403
		4.02	161	388
28-Aug-2000	44	3.97	142	409
		3.87	147	392
30-Aug-2000	46	3.85	137	381
		3.84	145	474
Mean		4.03	148	394
S.D.		0.458	11.3	37.8
%CV		11.4	7.6	9.6
%Theoretical		100.8	98.7	98.5
%Bias		0.8	-1.3	-1.5
n		30	30	30

Table A.2. Inter -Assay Precision and Accuracy for PFOSAA Quality Control Samples

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 9.00 ng/mL	Medium QC 155 ng/mL	High QC 405 ng/mL
25-Jul-2000	22	8.50	147	359
		7.33	142	431
31-Jul-2000	23	10.2	*115	426
		10.5	170	396
01-Aug-2000	24	10.5	156	416
		10.4	161	444
02-Aug-2000	26	*11.4	177	452
		9.24	171	434
04-Aug-2000	27	*11.3	153	430
		8.37	155	424
09-Aug-2000	29	8.59	152	393
		9.46	152	399
12-Aug-2000	31	10.2	149	395
		10.2	168	455
14-Aug-2000	32	8.46	140	370
		8.89	142	391
15-Aug-2000	33	*10.9	160	439
		9.51	161	449
16-Aug-2000	36	9.40	151	438
		8.70	163	449
17-Aug-2000	37	10.5	158	409
		9.74	157	421

* >±20% theoretical

Table A.2. Inter -Assay Precision and Accuracy for PFOSAA Quality Control Samples (continued)

All concentrations are expressed as ng/mL.

Run Date	Run Number	Low QC 9.00 ng/mL	Medium QC 155 ng/mL	High QC 405 ng/mL
24-Aug-2000	42	9.83	144	461
		8.00	137	475
26-Aug-2000	43	*11.0	169	424
		10.4	167	438
28-Aug-2000	44	9.71	155	436
		10.4	159	447
30-Aug-2000	46	9.30	152	445
		9.65	165	*518
Mean		9.69	155	429
S.D.		1.01	12.5	31.7
%CV		10.4	8.1	7.4
%Theoretical		107.7	100.0	105.9
%Bias		7.7	0.0	5.9
n		30	30	30

* >±20% theoretical

APPENDIX B

Calculation of Persistent Levels of Analytes in Diluted Human Serum Samples

Because there are persistent levels of the analytes in human serum, diluted samples must account for the amount of analyte added from the sample and the amount of analyte added from the human serum matrix. The following section describes equations used to correct for the amount of analyte added from the human serum matrix used to dilute samples.

The total volume (V_T) of a diluted sample is defined as the volume aliquoted from the original sample (V_S) plus the volume of matrix (V_M) added. The dilution factor (DF) is defined as the total volume divided by the volume aliquoted from the original sample.

$$V_T = V_S + V_M$$

$$DF = V_T / V_S$$

Example

V_T = 0.1 mL sample + 0.4 mL control matrix

V_T = 0.5

The amount of analyte A in a diluted sample is equal to the volume aliquoted from the original sample times the concentration of analyte in the sample (C_S) plus the volume of control matrix added times the concentration of analyte in the control matrix (C_M). The concentration of analyte in the sample (C_S) is determined during sample analyses. The concentration of analyte in the control matrix (C_M) is determined experimentally for each lot of control matrix as described in Section 2.1.

Equation 1.

$$\textbf{Total Amount of analyte} = V_S * C_S + V_M * C_M$$

Example

Total Amount of analyte = [0.1 mL sample * 100 ng/mL]+ [0.4 mL control matrix * 10 ng/mL]

Total Amount of analyte = 14 ng

Amount of analyte from sample = 10 ng

Amount of analyte from control matrix = 4 ng

The concentration of the analyte (C_A) in the diluted sample is equal to the sum of the following: 1) the ratio of the volume of sample (V_S) to the total volume of the diluted sample (V_T) times the concentration in the sample (C_S), and 2) the ratio of the volume of control matrix in the diluted sample (V_M) to the total volume of the diluted sample(V_T) times the concentration in the control matrix (C_M). (This is the concentration of analyte in the diluted sample prior to any adjustment for the actual dilution.)

The equation for the above is:

Equation 2.

$$C_A = (V_S/V_T)*C_S + (V_M/V_T)* C_M$$

Example

C_A = [(0.1 mL/0.5 mL) * 100 ng/mL] + [(0.4 mL/0.5 mL) * 10 ng/mL]

C_A = 28 ng/mL

(28 ng/mL = 14 ng total amount calculated above/0.5 mL total volume)

Replacing the volume of control matrix V_M with (V_T-V_S) into this equation produces:

Equation 3.

$$C_A = (V_S/V_T)*C_S + ((V_T-V_S)/V_T)*C_M$$

or

$$C_A = (V_S/V_T)*C_S + (1-V_S/V_T)*C_M$$

Example

C_A = [(0.1 mL/0.5 mL)*100 ng/mL] + [(1-(0.1 mL/0.5 mL))*10 ng/mL]

C_A = 20 ng/mL + 8 ng/mL

C_A = 28 ng/mL

Inserting 1/DF for the term V_S/V_T yields the final equation for C_A:

Equation 4.

$$C_A = (1/DF)*C_S + (1-1/DF)*C_M$$

Example

$$C_A = [(1/5)*100 \text{ ng/mL}] + [(1-(1/5))*10 \text{ ng/mL}]$$

$$C_A = 28 \text{ ng/mL}$$

The concentration determined by the Watson LIMS system (C_W) for any sample is:

Equation 5.

$$C_W = DF * C_A$$

Example

$$C_W = 5 * 28 \text{ ng/mL}$$

$$C_W = 140 \text{ ng/mL}$$

(This is the concentration of analyte after adjusting for the actual dilution.)

Substituting the final equation for C_A (Equation 4.) into the equation for C_W gives:

Equation 6.

$$C_W = DF *[(1/DF)*C_S + (1-1/DF)*C_M]$$

Example

$$C_W = 5 * [((1/5) *100 \text{ ng/mL}) + (((1-(1/5)) * 10 \text{ ng/mL})]$$

$$C_W = 5* [20 \text{ ng/mL} + 8 \text{ ng/mL}]$$

$$C_W = 140 \text{ ng/mL}$$

Solving this last equation for C_S produces the equation for correction due to persistent levels which is used to adjust theoretical calibration and QC concentrations.

Equation 7.

$$C_S = C_W - (DF-1)*C_M$$

Example

$$C_S = 140 \text{ ng/mL} - [(5-1) * 10 \text{ ng/mL}]$$

$$C_S = 140 \text{ ng/mL} - 40 \text{ ng/mL}$$

$$C_S = 100 \text{ ng/mL}$$