

# A Comparison of Whole Blood, Plasma, and Serum Evaluations for the Determination of PFOS, PFHS, PFBS and PFOA in Human Subjects.

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## Abstract

The purpose of this investigation was to compare the measurement of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexanesulfonate (PFHS) and perfluorobutanesulfonate (PFBS) in serum, plasma and whole blood samples collected from the same individuals. As part of this comparison, the possible effect of different anticoagulants for whole blood and plasma was investigated. The matrices evaluated included: whole blood collected in heparin (lithium); whole blood collected in ethylenediamine tetraacetic acid (EDTA); plasma samples collected with heparin; plasma samples collected with EDTA; and whole blood samples where no anticoagulation agents were used allowing the blood to clot. The clotted blood was then processed by centrifugation to yield serum samples. The 3M Institutional Review Board approved this study. Blood sample tubes were drawn sequentially from a single venipuncture site from 18 voluntary participants of the 3M Company. These included six fluorochromical production, six laboratory and six administrative employees. Analyses were completed using high-pressure liquid chromatography / tandem mass spectrometry methods. Solid phase extraction methods were used for all analytical sample preparations. Regardless of the anticoagulant used, whole blood specimens contained substantially lower concentrations of all fluorochromicals measured. Values less than the limit of quantitation were not included in the statistical analyses. PFBS was not quantifiable in most samples; therefore, ratios were not possible to determine. Serum to plasma ratio for PFOS, PFHS and PFOA was 1:1. Serum or plasma to whole blood ratio, regardless of the anticoagulant used, approximated 2:1. There were slight differences in this ratio between PFOS and PFHS versus PFOA. Based on washed-cell preparations, lower concentrations of fluorochromicals observed in whole blood corresponded to volume displacement by red blood cells, indicating that the fluorochromicals are not found intracellularly or attached to the red blood cells, but are in fact associated with serum proteins.

## Introduction

Recent interest in human exposure to perfluorinated acids, including perfluorooctanesulfonate (PFOS), perfluorohexanesulfonate (PFHS), and perfluorooctanoate (PFOA) has led to reports of measurements of these substances in either whole blood, plasma, or serum (Olsen et al., 2003; Harada et al., 2004; Kannan et al., 2004; Kulkerny et al., 2004). These fluorochromicals have been found to be highly bound to serum proteins (3M Company, 2003). Comparison of measurements of these compounds in different blood-based matrices have not been rigorously investigated. In addition, the potential effect of different anticoagulants used during collection of blood samples has not been investigated. The purpose of this investigation was to compare the measurement of PFOS, PFOA, PFHS and perfluorobutanesulfonate (PFBS) in serum, plasma and whole blood samples collected from the same individuals. As part of this comparison, the possible effect of different anticoagulants for whole blood and plasma was investigated.

Whole blood is one of the most difficult matrix choices from an analytical standpoint and is not typically used for clinical chemistry measurements. Whole blood has long-term stability issues unless frozen, which results in the lysis of its cellular components. While ethylenediamine tetraacetic acid (EDTA) is a commonly used anticoagulant for hematology studies, EDTA plasma is not the usual specimen of choice for most clinical chemistry and analytical determinations. Heparinized plasma can be used for both clinical chemistry and analytical determinations; however, long-term storage and fibrin clot formation are common problems associated with this matrix. For these reasons, serum is the most common matrix employed for clinical chemistry and trace analyses.

In order to investigate potential differences between measurements of PFOS, PFOA, PFHS and PFBS in whole blood (EDTA and heparin), plasma (EDTA and heparin), and serum, we obtained samples from voluntary participants from 3M Company that represented different potential exposure levels. These included production workers, laboratory researchers, and administrative employees.

## Methods

### Sample Collection

This study was approved by the 3M Company Institutional Review Board. All participants signed an informed consent form. Blood sample tubes were drawn sequentially from a single venipuncture site from 18 voluntary participants of the 3M Company. These included six fluorochromical production workers, six fluorochromical laboratory researchers, and six administrative employees. Whole blood and plasma samples were collected in heparin (lithium) and in EDTA. Whole blood samples were also collected without anticoagulant for serum.

### Washed Cells Sample Preparation

Cellular components were isolated from whole blood (EDTA and heparin) by centrifugation to remove plasma followed by four separate washes with commercially prepared 0.9% sodium chloride (Injection quality USP, Abbott Laboratories®). Each wash consisted of an addition of 4 mL of saline, gentle rotation on a mixer for twenty minutes followed by centrifugation at 1500 x g in a Beckman centrifuge.

## Methods (continued)

### Analytical

The extraction was completed using solid phase extraction (SPE). All extractions were based on 100 µL of sample matrix and utilized Waters Oasis® hydrophilic-lipophilic balance (HLB) 3 mL columns. Initially, a fixed amount of PFOS labeled with two <sup>18</sup>O (synthesized by Research Triangle Institute for 3M) in the sulfonate group (internal standard) was added to sample tubes for determination of PFOS, PFHS, and PFBS. For PFOA, a dual <sup>13</sup>C-labeled PFOA (provided by DuPont) was used as the internal standard. Appropriate amounts of analyte were then spiked into the tubes labeled for the standard curve. Commercially purchased whole blood (Lampire Biological) was used for whole blood standard curve matrix. Newborn calf serum (Invitrogen Corp.) was purchased from Gibco for serum and plasma standard curves.

Serum and plasma samples were added to labeled tubes, followed by the addition of 1 mL of 1.0 N formic acid and 100 µL of saturated ammonium sulfate. Tubes were vortexed prior to initiating SPE. Whole blood samples were added to labeled tubes followed by the addition of 1 mL of DI water and vortexed, resulting in the lysis of red blood cells. An additional 100 µL of 1.0 N KOH was then added, and tubes were vortexed prior to loading on conditioned SPE tubes.

### SPE Column Conditioning:

Prior to use, the SPE columns were rinsed with 1 mL of methanol followed by a rinse of 1 mL of acetonitrile. The SPE columns were then flushed with 1.0 mL Milli-Q® deionized (DI) water. All process steps were carried out using low vacuum setting (5-7 in. Hg) to keep the columns from being exposed to excess air and to increase residence time "on-column" when actually loading and/or eluting the samples.

### SPE Column Loading:

Samples were pipetted into the labeled SPE tubes on the vacuum manifold. The original specimen tubes were then rinsed with 1.0 mL of DI water, vortexed and the resulting mixture loaded onto the SPE tubes. The original sample tubes were then rinsed a second time with 1.0 mL of 15% acetonitrile (to minimize absorptive loss onto sample tube), vortexed and the resulting mixture loaded onto the SPE tubes.

### SPE Column Wash (Rinse step):

Interfering substances were removed by using water and an organic wash. The wash step was a single 2 mL rinse of DI water followed by a single 2 mL rinse of 15% acetonitrile. All tubes were then centrifuged for 5 minutes at 2500 x g for 5 minutes to remove residual rinse solvents and any residual water.

### SPE Column Elution:

Elution was achieved in four separate pipetting steps:

- Step 1) 0.5 mL 0.1 N ammonium acetate added to all tubes, and vacuum applied;
- Step 2) 0.5 mL of acetonitrile added to all tubes, and vacuum applied;
- Step 3) 0.5 mL of acetonitrile added to all tubes, and vacuum applied;
- Step 4) 0.5 mL of DI water added to all tubes, and vacuum applied.

All tubes were mixed by vortexing. An aliquot of the mixture was removed from each tube and placed into a labeled polypropylene micro vial, snap capped with a polycarbonate vial seal and placed on the HPLC auto-sampler for analysis. A 10 µL aliquot was injected into the LC-MS/MS instrument system for analysis.

### LC-MS/MS Conditions:

The instrument used for analysis was the API 4000 mass spectrometer from Applied Biosystems / MDS-Sciex Instrument Corporation. Turbo Ion Spray (pneumatically assisted electrospray ionization source) in negative ion mode. We used a Mac-Mod ACE® C-18, 5 µm, 100 x 2.1 mm i.d. HPLC column with an isocratic flow rate of 0.35 mL/min. Our mobile phase was 51% acetonitrile and 49% 2 mM ammonium acetate. All source parameters were optimized under these conditions according to manufacturer's guidelines. Transition ions were monitored as follows:

PFOS: 499 → 80 amu, PFHS: 399 → 80 amu, PFBS: 299 → 80 amu  
Sulfonates Internal Standard: dual <sup>18</sup>O-labeled PFOS: 503 → 84 amu  
PFOA: 413 → 369 amu

PFOA Internal Standard: dual <sup>13</sup>C-labeled PFOA: 415 → 370 amu

### Data Analysis

All curves were evaluated with a linear regression model, standards were all weighted at 1/x and each curve had an "R" value equal to or greater than 0.9994 (Figure 1). Analyses below the lower limit of quantification (LLQ) were not included in statistical analyses. Mean ratios of serum to plasma, serum to whole blood, and plasma to whole blood were determined, along with 95% confidence intervals.

## Results

Individual results are listed in Table 1. PFBS was not quantifiable in most samples, therefore, we limited statistical analysis to PFOS, PFHS and PFOA.

Presented in Table 2 are the mean values of individual participants when both concentrations were above the LLOQ. For example, in Table 2, the PFHS mean ratio for serum/EDTA plasma equals 1.1. This is based on 16 participants as seen in Table 1. Likewise, in Table 2, the PFHS mean ratio for serum / heparin whole blood is 2.5. This is based on 7 participants as seen in Table 1.

Re-suspended washed cells were treated identically to a whole blood specimen and compared with a standard curve extracted from a spiked whole blood matrix. The concentration of fluorochromicals found in the washed cell matrix was less than the detection limit of the assay (1.0 ng/mL) for every analyte measured.

Regardless of fluorochromical, the serum to plasma ratio was 1:1. Serum or plasma to whole blood ratio, regardless of anticoagulant, approximated 2:1. However, this 2:1 ratio was slightly higher for PFHS and PFOS than PFOA.

Lower concentrations of fluorochromicals observed in whole blood corresponded to volume displacement by red blood cells, indicating that the fluorochromicals are not found intracellularly or attached to the red blood cells, but are in fact associated with serum proteins. The analysis of the washed cells supports this conclusion. No concentration above the MDL of the assays was quantitated in the washed cell extractions as performed.

Table 1. Individual Data for PFOS, PFOA, PFHS and PFBS

PFOA						PFOS					
Sample Number	Serum	EDTA Plasma	Heparinized Plasma	EDTA Whole Blood	Heparinized Whole Blood	Sample Number	Serum	EDTA Plasma	Heparinized Plasma	EDTA Whole Blood	Heparinized Whole Blood
1	250	140	140	350	300	50	50	50	50	50	50
2	521	538	804	452	450	2	87	90	91	46	47
3	1446	1000	1720	925	971	3	200	200	200	200	141
4	331	342	314	153	158	4	119	119	122	56	54
5	1186	1180	1180	680	682	5	146	144	147	76	78
6	676	524	662	296	293	6	110	170	170	70	70
7	<10	<10	<10	<10	<10	7	36	36	36	14	12
8	<10	<10	<10	<10	<10	8	14	12	11	<5	<5
9	<10	<10	<10	<10	<10	9	26	26	24	13	12
10	<10	<10	<10	<10	<10	10	26	26	26	37	38
11	<10	<10	<10	<10	<10	11	227	135	137	58	58
12	<10	<10	<10	<10	<10	12	24	22	24	36	36
13	27	32	29	12	12	13	61	61	66	24	23
14	26	26	26	27	14	42	46	46	46	20	20
15	22	19	16	8	9	15	40	38	38	17	17
16	112	118	113	68	67	16	169	171	170	82	88
17	58	59	57	30	32	17	124	129	119	63	60
18	<5	<5	<5	<5	<5	18	21	21	20	8	7

Table 2. Means of Individual Matched Ratios with Concentrations Greater than LLOQ.

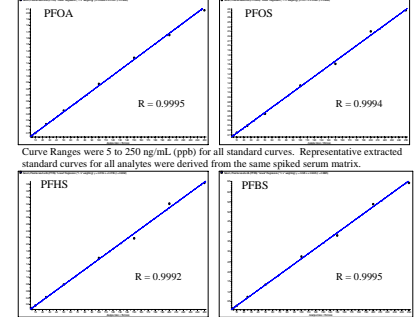
Fluorochromical	Serum/ EDTA PL	Serum/ Heparin PL	Serum/ EDTA WB	Serum/ Heparin WB	EDTA PL/ EDTA WB	EDTA PL/ Heparin WB	Hep PL/ EDTA WB	Hep PL/ Heparin WB
Mean	1.0 <sup>a</sup>	1.1 <sup>a,b</sup>	2.9 <sup>a</sup>	2.9 <sup>a</sup>	2.3	2.3	2.4 <sup>a</sup>	2.3
95% CI	1.0-1.2	1.1-1.2	2.2-2.7	1.8-3.0	2.2-2.5	1.8-2.8	2.2-2.5	1.8-2.9
Median	1.1	1.1	2.4	2.3	2.3	2.3	2.4	2.1
Range	0.9-1.3	0.9-1.3	2.3-2.8	1.8-3.7	2.1-2.5	1.8-3.3	2.1-2.6	1.9-3.6
PFOS	1.0 <sup>a</sup>	1.0 <sup>a</sup>	2.2	2.5	2.3	2.3	2.5 <sup>a</sup>	2.4 <sup>a</sup>
95% CI	1.0-1.1	1.0-1.0	2.1-2.4	2.1-2.5	2.1-2.4	2.1-2.5	2.1-2.5	2.2-2.6
Median	1.0	1.0	2.2	2.2	2.2	2.3	2.3	2.3
Range	0.8-1.2	0.8-1.1	1.8-3.0	1.9-3.0	1.9-2.8	1.9-3.0	1.9-3.3	1.8-2.9
PFOA	1.0 <sup>a</sup>	1.0 <sup>a</sup>	2.1 <sup>a</sup>	2.0 <sup>a</sup>	2.1	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>
95% CI	1.0-1.1	1.0-1.1	1.9-2.2	1.9-2.2	1.9-2.2	1.9-2.2	1.9-2.2	1.8-2.1
Median	1.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0
Range	0.8-1.2	0.9-1.2	1.7-2.8	1.7-2.4	1.7-2.7	1.7-2.7	1.7-2.4	1.7-2.4

a. Significantly (p < .05, student's t test) different than PFHS for this ratio column  
b. Significantly (p < .05, student's t test) different than PFOS for this ratio column  
c. Significantly (p < .05, student's t test) different than PFOA for this ratio column

PL = plasma  
WB = whole blood

## Results (Continued)

Figure 1. Extracted Standard Curves.



## Discussion

Our study has confirmed what other researchers have assumed concerning the relationship between serum/plasma fluorochromical concentrations compared to whole blood concentrations. Whole blood concentrations would be approximately half that of serum/plasma due to the cellular components of whole blood.

We did observe a slightly higher ratio of serum/plasma to whole blood for PFOS and PFBS than PFOA. Whether this represents a true difference between the sulfonates and the carboxylate would require further investigation of the following:

- (1) potential differences in the chemical behavior when using SPE;
- (2) potential differences in binding to specific serum proteins;
- (3) and, potential differences in binding site affinity (selective partitioning).

Our results demonstrate precise correlations can be obtained between serum, plasma and whole blood with the use of appropriately labeled internal standards, SPE and LC-MS/MS. The use of 0.1 M-ammonium acetate was vital to break the weak ion-pair interaction with the Oasis® sorbent during elution. The ammonium acetate is already present in the HPLC mobile phase; therefore, the resulting elution solvent remains compatible with the mobile phase in use. There was no need for rapid HPLC gradient elution, as this may lead to imprecision in quantitation efforts. It is essential to use extracted standard curves from a spiked matrix identical to the matrix being examined. Standard curves and controls must be analyzed with each run and whenever changes are applied to the method.

## Conclusions

Based on 18 participants with various serum concentrations of PFOS, PFHS and PFOA, the serum to plasma ratio was 1:1, and the serum or plasma to whole blood ratio, regardless of anticoagulant, approximated 2:1. The observed ratios were slightly higher for PFOS and PFHS than for PFOA. The observed lower concentrations of fluorochromicals in whole blood samples corresponded to volume displacement by red blood cells. This was confirmed by the lack of fluorochromicals in the washed cell lysate analysis.

## References

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