SUPPORTING INFORMATION

Manuscript Title	Branched Perfluorooctane Sulfonate Isomer Quantification and Characterization in Blood Serum by HPLC/ESI-MS(/MS)
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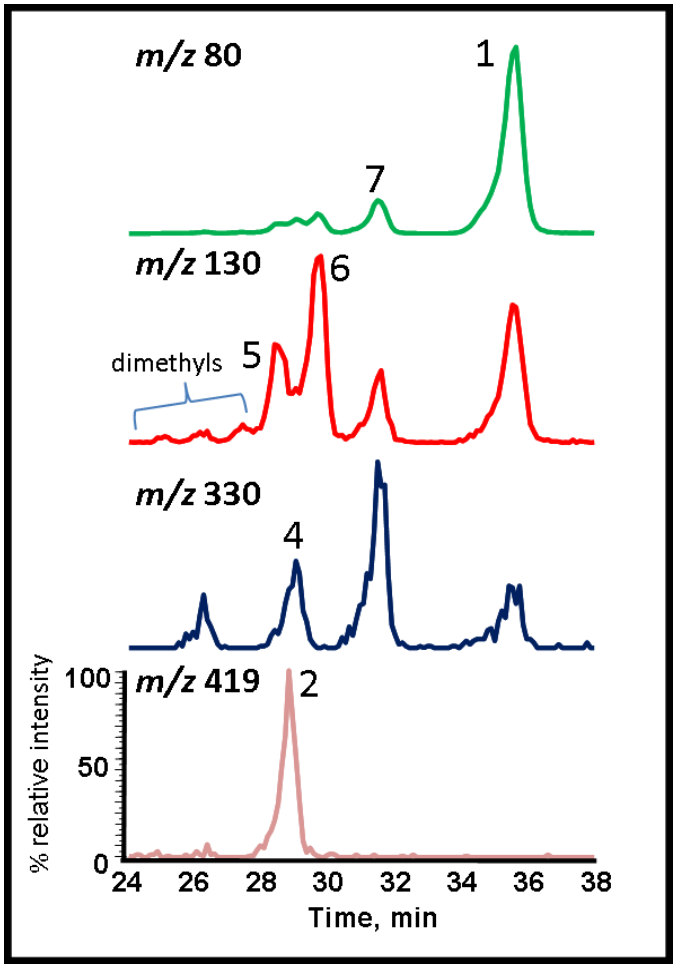

Figure S1. HPLC-MS/MS chromatograms of the brPFOSK standard produced using the Benskin et al method (*19*) showing the product ions used for isomer-specific quantification. Numbers denote the isomer quantified in each transition (Figure 1).

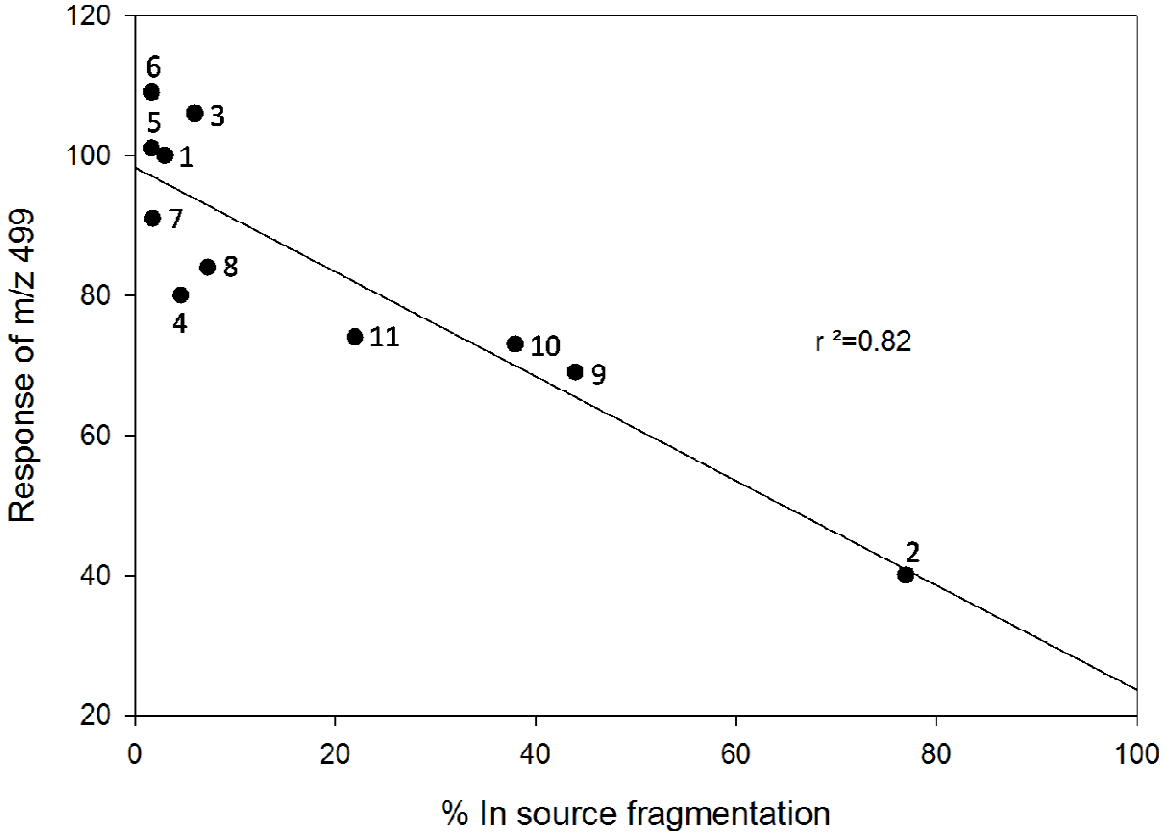

Figure S2. The response of each isomer in selected ion monitoring mode (m/z 499) was inversely associated with the amount of in source fragmentation to detectable fragments. Isomers are identified by number (structures are shown in Figure 1).

Isomer-specific product ions employed in the method of Benskin et al. (*19*) for analysis of human serum samples were optimized by infusion of br-PFOSK, and ramping of collision energy and declustering potential to produce a maximum response in each product ion. Additional experiments were carried out to quantify the change in MS sensitivity from optimizing isomer-specific product ions using individual isomer standards compared to br-PFOSK. Interestingly, the sensitivity did not increase by more than 12.5 % (Isomer **9**) for any isomer when using individual isomer standards for optimization compared to br-PFOSK (Table S1). This demonstrates that good sensitivity can be obtained when optimizing isomer-specific product ions using br-PFOSK compared to individually with purified isomer standards. This is likely due to the tendency for specific isomers to produce specific product ions,

resulting in the other isomers in the mixture having less influence on collision energy optimization. For example, isomer **2** is the only major isomer with the *m/z* 419 product ion, thus other isomers present in br-PFOSK will not influence CE optimization for this transition, compared to the *m/z* 99 product ion, which is produced by many isomers. However, for labs wishing to obtain the utmost sensitivity in isomer-specific analysis, we recommend optimizing MS parameters for isomer-specific product ions with individual isomer standards, one at a time (rather than mixing them together, which will result in misleading collision energies).

Table S1. Table showing the change in signal strength as a result of optimizing instrument parameters (IP) with brPFOSK compared to individual isomer standards. Instrument parameters 'A' were optimized by infusion using brPFOSK. Instrument parameters 'B' were optimized by infusion using the individual isomer standards. Individual isomer standards were then infused with IP A and then IP B and the signal strengths were compared. DP (declustering potential) and CE (collision energy) were obtained at the point of maximum signal strength. DP maxima were broad, such that IP A and IP B produced the same maximum instrument response for all isomers when CE was held constant. CE maxima were narrow and the differences between IP A and IP B that resulted are given.

		Instrument parameters A		Instrument parameters B		
PFOS isomer	**Product ion**	**DP**	**CE**	**DP**	**CE**	**Observed % increase in sensitivity when using IP B**
1	80	-160	-91	-89	-110	11.1
7	80	-160	-91	-110	-111	11.1
6	130	-145	-60	-140	-56	0.00
5	330	-131	-46	-103	-46	2.86
4	130	-145	-60	-82	-55	4.00
3	219	-164	-45	-77	-46	7.14
2	419	-94	-35	-60	-36	3.70
8	130	-145	-160	-55	-55	4.44
9	130	-145	-60	-95	-55	12.5
10/11	169	-145	-60	-100	-58	5.13

Table S2. Comparison of the results from the 2nd Worldwide Interlaboratory Study Report on PFCs-Human Serum and our quantification of total PFOS using brPFOSK.

	Interlaboratory study		Total PFOS *m/z* 99 (our results)		Total PFOS *m/z* 80 (our results)	
	SERUM A ng/mL	SERUM B ng/mL	SERUM A ng/mL	SERUM B ng/mL	SERUM A ng/mL	SERUM B ng/mL
Average	4.91	22.9	3.7	18.1	2.9	21.1
median	4.73	23.1	3.8	17.7	3.0	20.5
min	3.70	13.2	3.5	16.7	2.5	19.6
max	6.35	31.1	3.9	20.0	3.1	23.3
SD	0.69	4.5	0.2	1.7	0.3	2.0
%RSD	14%	20%				
95% CI			0.5	4.2	0.8	4.9
n	15	15	3	3	3	3

Table S3. Linear PFOS concentrations in Serum A and B using brPFOSK or LPFOS and the *m/z* 499→80 and 499→90 transitions. Resulting values were all indistinguishable.

	Quantified using brPFOSK		Quantified using LPFOS	
SERUM A	Linear PFOS (*m/z* 80)	Linear PFOS (*m/z* 99)	Linear PFOS (*m/z* 80)	Linear PFOS (*m/z* 99)
AVG	**1.8**	**1.8**	**1.8**	**1.8**
STDEV	0.3	0.3	0.3	0.3
95% CI	±0.7	±0.7	±0.7	±0.7
SERUM B				
AVG	**10.7**	**10.3**	**10.6**	**10.7**
STDEV	1.2	1.1	1.2	1.1
95% CI	±3.0	±2.7	±2.9	±2.8

Table S4: Comparison between measured response factors relative to MPFOS ($^{13}C_4$-PFOS) and those calculated using the RRFs of the branched isomers and ^{19}F NMR data for various PFOS standards.

Isomers		% composition based on ^{19}F NMR		
		TCI	brPFOSK	LPFOS
1 (linear)		67.0	78.9	100
2-**11** (branched)		33.0	21.1	0
		RRF		
m/z 499→99	theoretical	87.3	92.2	100
	measured	92.1	102.4	100
m/z 499→80	theoretical	111.5	107.5	100
	measured	113.5	114.5	100