Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 21, No. 3, 183-190, 2008

The survey of exposure level for PFOS and PFOA in human plasma from several residential areas in Korea

Jaeyeon Chung[1], Hae-Seong Yoon[2], Hee-Young Ryu[2], Jong Uk Won[3], Ki-jung Paeng[4] and Yunje Kim[1,★]

[1]*Environmental Technology Research Center, Korea Institute of Science and Technology, P.O.Box 131, Cheongryang, Seoul, Korea*
[2]*Risk Assessment Research Department, National Institute of Toxicological Research, Seoul*
[3]*Institute for Occupational Health, College of Medicine, Yonsei University, Seoul, Korea*
[4]*Department of Chemistry, Yunsei University, Wonju, Korea*
(Received May 6, 2008; Accepted June 3, 2008)

주거지역별, 연령별 및 성별 인체 혈장중의 PFOS, PFOA 함유량 조사

정재연[1] · 윤해승[2] · 류희영[2] · 원종욱[3] · 팽기정[4] · 김연제[1,★]

[1]한국과학기술연구원, 에너지환경연구본부, 환경기술연구단
[2]국립독성연구소, 위해성 평가팀
[3]연세대학교, 의과대학, 예방의학교실, [4]연세대학교, 화학과
(2008. 5. 6. 접수, 2008. 6. 3. 2008)

Abstract: PFOS (Perfluorooctane sulfonate) and PFOA (Perfluorooctanoic acid) are environmental hormones which belong to potential future persistent organic pollutants (POPs), and it is easy to exposure to human because they are used in a wide variety of consumer products. We studied exposure route and the relativity through determining and monitoring of PFOS and PFOA in Korean plasma of metropolis, small town, rural area and industrial area. And we monitored the concentration of PFOS and PFOA regarding the gender and age. The older age is, the higher concentration of PFOS is. The mean concentration of PFOS and PFOA in men (4.74 ng/mL, 2.20 ng/mL) was higher than that in women (3.53 ng/mL, 1.17 ng/mL). In the comparison of residential areas, the mean concentration of PFOS and PFOA was the lowest in metropolitan plasma (2.47 ng/mL, 0.79 ng/mL) whereas it was the highest in the industrial area (6.57 ng/mL, 2.19 ng/mL).

요 약: PFOS (Perfluorooctane sulfonate)와 PFOA (Perfluorooctanoic acid)는 POPs 물질로 환경호르몬 중의 하나이다. 이 물질들은 포장재, 일회용품 등 널리 사용되어지고 있는 소비상품에 함유되어 있기 때문에 사람에게 쉽게 노출될 수 있다. 본 연구에서는 노출경로에 대해 연구하기 위해 대도시, 중소도시 및 시골의 주거지역과 산업지역에서의 PFOS, PFOA에 대한 인체 혈장 중에 함유된 수준을 조사하였으며, 성별과 연령별도 조사하였다. 고령일수록 PFOS의 농도는 높아졌으며, PFOS와 PFOA의 평균농도는 남

★ Corresponding author
Phone : +82-(0)2-958-5060 Fax : +82+(0)2-958-5805
E-mail : yjkim@kist.re.kr

자(4.74 ng/mL, 2.20 ng/mL)가 여자 (3.53 ng/mL, 1.17 ng/mL)보다 높게 조사되었다. 거주지역을 비교해 보면, PFOS와 PFOA의 평균농도는 대도시 거주자(2.47 ng/mL, 0.79 ng/mL) 의 경우 가장 낮았으며, 산업지역 거주자(6.57 ng/mL, 2.19 ng/mL)의 경우가 가장 높게 조사되었다.

Key words : PFOS (Perfluorooctane sulfonate), PFOA (Perfluorooctanoic acid), monitoring, LC/ESI/TOF MS, SPE

1. Introduction

PFOS (Perfluorooctane sulfonate) and PFOA (Perfluorooctanoic acid) are environmental hormones which belong to potential future persistent organic pollutants (POPs).[1] PFOS is identified to be the end-stage metabolite of many PFCs (perfluorocarbons). The strong carbon-fluorine (C-F) covalent bonds in PFCs account for the thermal and chemical stability of these compounds, which have been manufactured and used in a variety of industrial application for over 50 years. PFOS were used for fabric, leather, and apparel treatment for protection of food packaging and paper products.[2,3] PFOA is essential processing aid in fluoropolymer industry. Cookware, carpets and textiles are treated with fluoropolymer to provide stain, grease and water repellent.[4,5] PFOS and PFOA have toxicity for humans and wildlife because they have been attributed to resistance to degradation in ecosystem and bioconcentration.[5,6] So, PFOS and PFOA are researched their harm and regulated their use all over the world. Kurunthachalam Kannan et al. have investigated the concentrations of PFOS and PFOA in human blood from several countries including Korea. The concentration of PFOS was the highest in the samples collected from U.S and Poland; moderate in Korea, Belgium, Malaysia, Brazil, Italy and Colombia; and the lowest in India. Several serum samples from Korea contained relatively higher concentrations of PFOA than PFOS. The average concentration of PFOA in men was the highest in the other country.[7] This suggests the presence of specific sources of exposure to PFOA in Korea.

Kouji Harada *et al*. studied the influence of age, sex and geographical factor on levels of PFOS and PFOA in human serum from Japan. The PFOS and PFOA concentration in male was higher than female in 20-50 year-old age group of Kyoto city dwellers. In contrast, female over 51 years old, all of whom were menopausal, had significantly higher concentrations than females in the 20-50 year-old age group who were actively menstrual. There were no difference in the serum levels of PFOA and PFOS between males and postmenopausal females. PFOS and PFOA serum levels in male serums are signifi−cantly higher Kinki dwellers than other residents. This was also found to be the case for PFOA levels in females. There were significant geographical differences in PFOA and PFOS concentrations for both male serum and females in Kyoto. Participants belonging to the Kinki district, i.e., Koto, Osaka and Nishinomiya, exhibited significantly high PFOA levels in males. Females in the Kinki district also had higher PFOA levels, followed by those from Chugoku-Shikoku and Okinawa. Serum PFOS levels in the Kinki district were significantly higher than those in the Tohoku and Chubu districts.[8-10]

In the China, the mean concentration of PFOS was the greatest in sample collected from Shenyang, which was well-known for their heavy industries and the worst was in sample from Jintan. No age-related difference in the concentrations of PFOA and PFOS were observed. Gender-related differences were found, with male higher for PFOS.[11]

In the Sri Lanka, the mean PFOS concentration in the urban population was 1.2 and 8 times higher than that of rural conventional tea workers and rural organic tea workers.

In the Washington, median concentration of PFOS was higher in men than in women in 1974 and 1989 whereas no sex-related difference in PFOA was observed. median PFOS level in 1974 was lower in

individuals younger than 40 years of age and median serum concentration for PFOA in 1989 was lower in persons younger.[12]

In this paper, we studied the exposure route and the relativity through monitoring of PFOS and PFOA in Korean plasma which are divided gender, age and residential areas (metropolis, small town, rural area and industrial area).

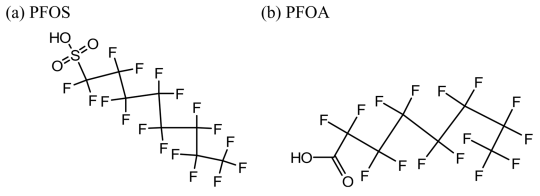
2. Experimental

2.1. Chemicals

PFOS (Heptadecafluorooctanesulfonic acid potassium salt) is purchased from Fluka Chemie AG (Buchs, Switzerland). PFOA (Pentadecafluorooctanoic acid Ammonium salt) and PFHpA (Tridecafluoro heptanoic acid, used as internal standard) are purchased from Sigma-Aldrich Inc. (MO, USA). HPLC grade methanol and acetonitrile (>99.9% purity, Burdick & Jackson, Muskegon, MI, USA), Formic acid (98% purity, MERCK), Ammonium acetate (>97% purity, YAKURI PORE CHEMICALS, Japan), and Tetrabutylammonium hydrogensulfate (97% purity, Sigma-Aldrich) were used for mobile phase and sample pretreatment. Methanol and acetonitrile were HPLC grade from Burdick & Jackson (Muskegon, MI, USA). Methanol and distilled water were used after filtering through a Millipore filter (0.45 μm) and sonication for 20 min.

2.2. Instruments and equipment

The LC/ESI/TOF MS system consisting of an HP 1100 series binary pump system HPLC (Agilent, Palo Alto, CA, USA) with a Unique TOF MS equipped with electrospray ionization (St. Joseph, MI, USA). The column for LC was a Shiseido UG120V C18 column, 2.0 mm i.d.×150 mm, particle size 5 μm (Tokyo, Japan).



Structures of PFOS and PFOA

A Rapid Trace SPE (Caliper inc., Hopkinton, MA, USA) was used for extraction of sample. The cartridge was an AccuBondII ODS-C18 Cartridges (200 mg, 3 mL) and was perchased form Agilent. A Turbovap® LV evaporator supplied by Zymark Corporation (Hopkinton, MA, USA) was used to evaporate the extracted organic solvents to dryness.

2.3. Volunteer sample

Plasma samples were collected from the Medical Center of Yonsei University by volunteer donations. The demographic factors were age, gender and sampling regions. Details regarding donor's area of residence, age and gender are provided in *Table* 1. Samples were stored in polypropylene containers. All of the samples were kept at -20^{o}C until analysis.

2.4. Sample preparation

Blank plasma, spiked plasma and sample plasmas were prepared and processed using the same procedure. All standard solutions were allowed to reach room temperature before they were spiked into the plasma. The 5 mL of plasma sample to be spiked

Table 1. Details of Plasma Samples Analyzed

		Metropolis	Small town	Rural area	Industrial area	Sum
Age	20-29	7	2	8	3	20
	30-39	8	12	28	0	48
	40-49	4	21	24	10	59
	50	6	28	15	7	56
Gender	Man	7	16	22	17	62
	Woman	18	47	53	3	121

Table 2. Gradient time table of mobile phases for LC/MS

Time (min)	A (%)	B (%)
0	50	50
2	50	50
5	15	85
8	1	99
15	1	99
20	50	50
30	50	50

with the PFHpA 200 μL (10 μg/mL) as the internal standard, was adjusted to pH 10 by 100 mg of potassium carbonate, and was added 10 mL of acetonitrile for deproteinization. After centrifuged at 840 g for 10 min, upper liquid was got and 3 mL of 50% formic acid was added to prevent to the cartridge from clogging up during the SPE process. After centrifuged at 840 g for 10 min, supernatant were extracted by SPE process.

The cartridges in SPE instrument were conditioned with 2 mL of methanol, distilled water and 0.5 M TBA. The pretreatment samples were loaded at a flow rate of 0.5 mL min^{-1}, after then the cartridges were rinsed with 2 mL of distilled water. The analytes were eluted with 2 mL of methanol, and evaporated to dryness at 40°C under a gentle

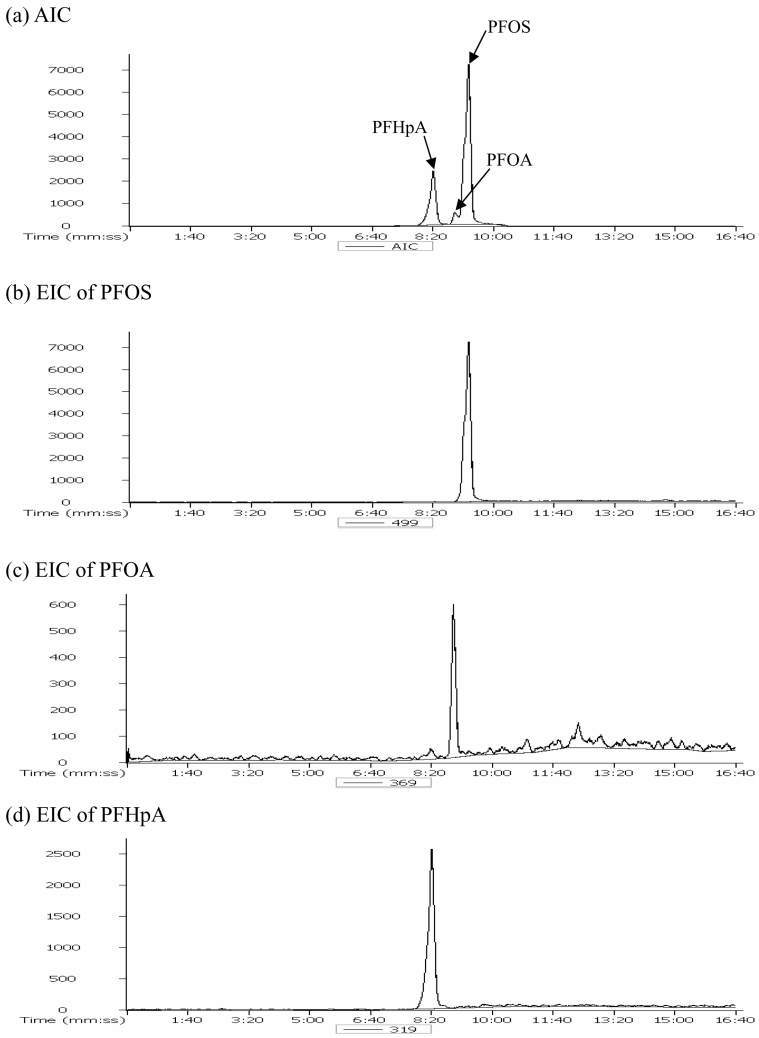


Fig. 1. Adjusted ion chromatogram and extracted chromatograms of PFOS, PFOA and ISTD from standard solution.

stream of nitrogen gas. The residues were dissolved in 50 μL of methanol, and 20 μL of this solution was injected into the LC/ESI/TOF/MS.

2.5. Analysis of PFOS and PFOA by LC/ESI/TOF/MS

The LC/ESI/TOF/MS was operated with electrospray ionization voltage −3.3 kV, interface temperature 120°C, desolvation temperature 300°C, desolvation gas flow 7000 cc/min with nitrogen gas and detected the negative ions. The scan rate was 3.13 spectra/sec. The HPLC column maintained at 40°C oven temperature and flow rate was 0.3 mL/min. The mobile phase A was 2 mM ammonium acetate in water and the mobile phase B was 2 mM ammonium acetate in methanol. The gradient time table is described in *Table* 2.

3. Results

3.1. Determination of PFOS and PFOA by LC/ESI/TOF-MS

The molecular masses of PFOS, PFOA and PFHpA (ISTD) are 500 Da., 414 Da. and 364 Da., respectively. Their structures are shown in scheme 1. The standard and Plasma sample extracted were analyzed by LC/ESI/TOF-MS. $[M-H]^-$ and $[M-COOH]^-$ ions were mainly produced in ESI negative mode in the PFOS, PFOA and PFHpA.

Fig. 1(a) shows the adjusted ion chromatogram (AIC) of PFOS, PFOA and PFHpA from standard solutions. The retention times of PFOS, PFOA and PFHpA are 9.19 min, 8.56 min and 8.21 min, respectively. *Fig.* 1(b)~(d) shows the extracted ion chro-

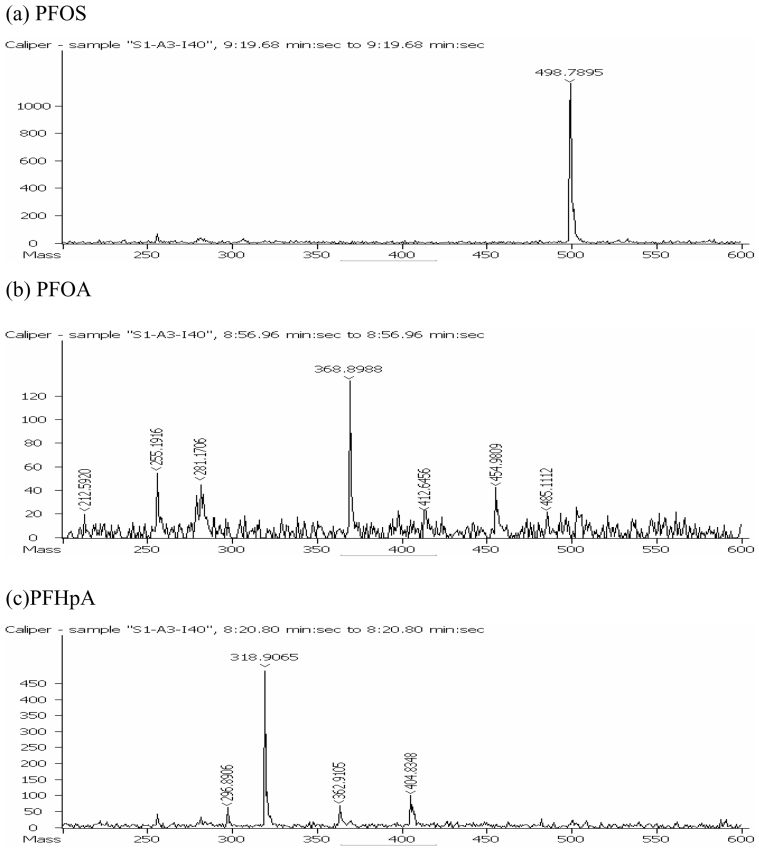


Fig. 2. Mass spectrum of PFOS, PFOA and PFHpA (ISTD) from standard solution.

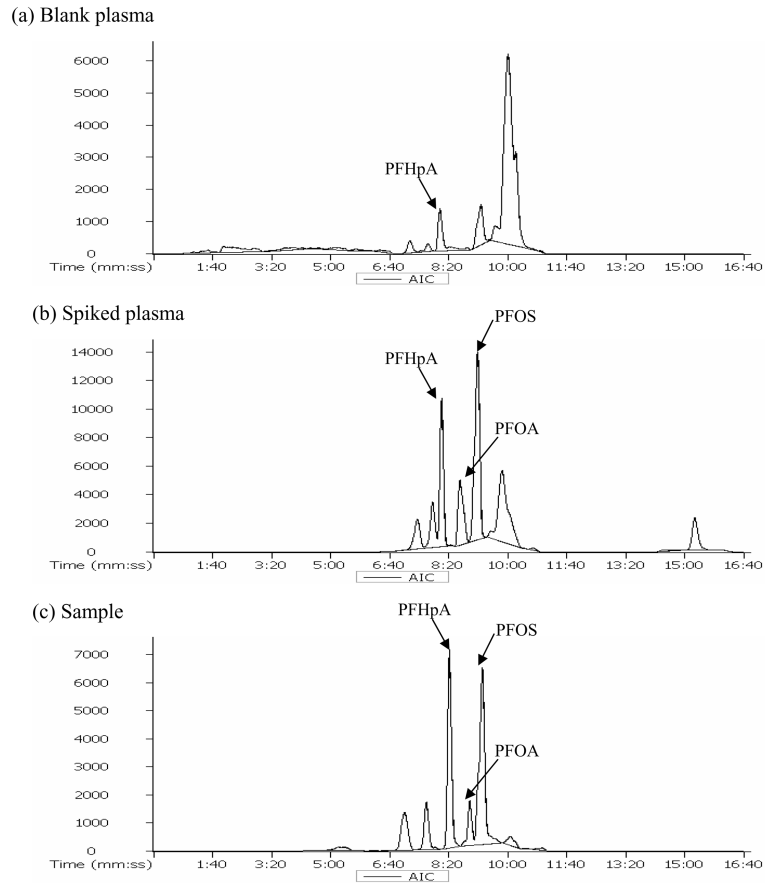


Fig. 3. Adjusted ion chromatograms of PFOS and PFOA in blank plasma, spiked plasma and sample by LC/ESI/TOF/MS.

Table 3. PFOS and PFOA concentration in plasma (ng/mL)

		PFOA		PFOS	
		Mean	Range	Mean	Range
Age	20-29	1.49	N.D ~ 3.81	3.10	0.97 ~ 7.18
	30-39	1.48	N.D ~ 5.28	3.08	1.23 ~ 7.21
	40-49	1.66	N.D ~ 8.05	3.96	1.14 ~ 10.39
	> 50	1.41	N.D ~ 4.76	4.96	1.64 ~ 11.80
Gender	Man	2.20	N.D. ~ 8.05	4.74	1.48. ~ 10.71
	Woman	1.17	N.D. ~ 3.76	3.53	0.97. ~ 11.80
Residential	Metropolis	0.79	N.D. ~ 2.28	2.47	0.97 ~ 8.71
Area	Small town	1.53	N.D. ~ 8.05	3.97	1.14 ~ 11.80
	Rural area	1.59		3.70	
	Industrial area	2.19	N.D. ~ 3.87	6.57	3.39 ~ 10.71

N.D : not detected

matograms (EICs) of the $[M-H]^-$ ion of PFOS (m/z 499), the $[M-COOH]^-$ ion of PFOA (m/z 369) and the $[M-COOH]^-$ ion of PFHpA (m/z 319) for standard solution.

The MS spectrum of PFOS, PFOA and PFHpA standard include m/z 499, 369 and 319, as shown in

Fig. 2. The peak at m/z 413 is $[M-H]^-$ in *Fig.* 2(b) and m/z 363 is $[M-H]^-$ in *Fig.* 2(c).

Fig. 3 shows the adjusted ion chromatograms in blank plasma, spiked plasma and sample plasma. The LC peak for the sample appeared at almost the same retention time as for the standard solution and spiked plasma.

3.2. Recovery and Reproducibility

The recovery and reproducibility were determined by five plasma samples spiked standard (PFOS and PFOA) and ISTD (PFHpA). The recovery results for PFOS (10 ng/mL) and PFOA (30 ng/mL) were 97.2% and 78.9%, respectively. Reproducibility results for PFOS (10 ng/mL) and PFOA (30 ng/mL) were 3.3% and 10.0%, respectively.

3.3. Calibration curve

The three calibration standards were prepared by spiking plasma with PFOS and PFOA to final added concentrations of 0.5, 3, 5 ng/mL in plasma, respectively. The correlation coefficient (r^2) of PFOS was 0.9979 and PFOA was 0.9986. (*Fig.* 3)

The limit of quantification (LOQ) of PFOS was 0.4 ng/mL and that of PFOA was 0.6 ng/mL.

3.4. The results of monitoring

The results for PFOA and PFOS levels monitoring in human plasma were shown to age, gender and residual area (*Table* 3).

The PFOA level was 1.66±1.174 ng/mL in the 40-49 years old age and PFOS level was 4.96± 2.469 ng/mL in over 50 years old age. This group was the highest than another age. The PFOS was detected in all samples and the PFOS levels were higher than PFOA. The mean level in their twenties was higher than thirties but they have a little difference level.

The PFOA level (2.20±1.200 ng/mL) and PFOS level (4.74±2.241 ng/mL) in male serum were higher than the PFOA level (1.17±0.902 ng/mL) and PFOS level (3.53±1.999 ng/mL) in female. The PFOA level in men was 2-fold higher than women.

In the case of residual area, industrial area determined PFOA (2.19±0.764 ng/mL) and PFOS (6.57± 1.613 ng/mL) was higher than metropolis, small town and rural area. The PFOA level in small town and rural area was 2 times higher than metropolis and The PFOS level was the same as PFOA level. The order of PFOA level is industrial area > rural area > small town > metropolis and PFOS is industrial area > small town > rural area > metropolis. We suggest that reasons for the lowest level in metropolitan are high recognition about environmental materials, or low exposure to PFOS and PFOA due to the lowest mean age. The reason for the highest level in industrial area is there was more exposed to PFOS and PFOA than other regions. The PFOS and PFOA level in male was higher than female because 20~50 year-old age in female group was actively menstrual as Kouji Harada et al. suggestion. Therefore, we expect the differences of PFOS and PFOA level were more influenced by consumed recognition pattern of consumers and gender. In the case of industrial area, the exposure measures for these

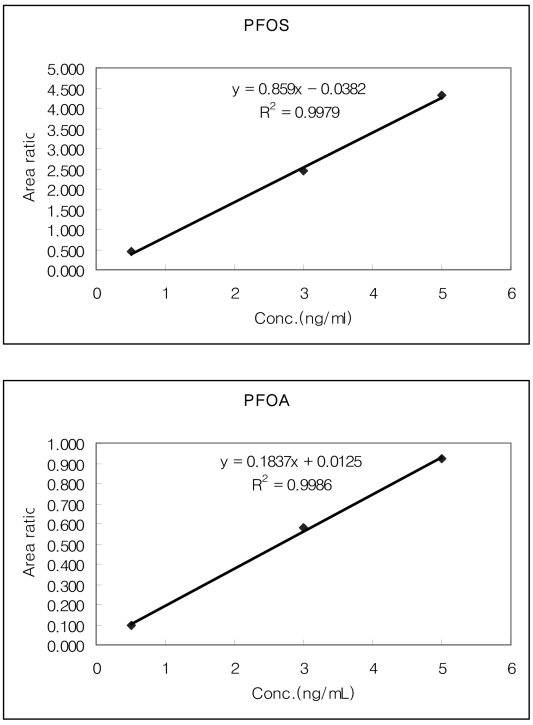


Fig. 4. Calibration of PFOS and PFOA.

compounds were matched common expectation.

4. Conclusion

We determined the concentration of PFOS and PFOA in human plasmas which are divided three groups (age, gender and residual area) by LC/ESI/TOF MS. In all the plasma samples, PFOS was detected and the PFOS level was much higher than the PFOS level. The differences of PFOS and PFOA level were more influenced by consumed recognition pattern of consumers, menstruated female and extent of exposure to PFOS and PFOA i.e. age and industrial circumstance. These patterns are significant so it is necessary to continue monitoring the PFOS and PFOA in human plasma with more samples.

Reference

1. Organisation for Economic Co-operation and Development, ENV/JM/RD(2002)17/FINAL., 2002.
2. K. Anna, B. Bert, J. Ulf, H. Lennart and L. Gunilla, *Anal. Chem.* 77, 864(2005).
3. EPA Public docket AR 226 OPPT fact sheet., 2003.
4. K. Harada, N. Saito, K. Inoue, T. Yoshinaga, T. Watanabe, S. Sasaki, S. Kamiyama and A. Koizumi, *J. Occup. Health* **46**, 141(2004).
5. J. Martin, S. Mabury, K. Solomon and D. Muir, Environ. Toxicol. Chem. **22**, 196(2003).
6. EPA Public docket AR 226-0550., 1999.
7. K. Kannan, S. corsolini, J. Falandysz, G. Fillmann, K. S. Kumar, B. G. Loganathan, M. A. Mohd, J. Olivero, N. V. Wouwe, J. H. Yang and K. M. Aldous, *Environ. Sci. Technol.* **38**, 4489(2004).
8. K. Harada, N. Saito, K. Inoue, T. Yoshinaga, T. Watanabe, S. Sasaki, S. Kamiyama and A. Koizumi, *J. Occup. Health* **46**, 141(2004).
9. K. Harada, K. Inoue, A. Morikawa, T. Yoshinaga, N. Saito and A. Koizumi, *Environmental Research* **99**, 253(2005).
10. K. Harada, A. Koizumi, N. Saito, K. Inoue, T. Yoshinaga, C. Date, S. Fujii, N. Hachiya, I. Hirosawa, S. Koda, Y. Kusaka, K. Murata, K. Omae, S. Shimbo, K. Takenaka, T. Takeshita, H. Yodoriki, Y. Wada, T. Watanabe and M. Ikeda, *Chemosphere* **66**, 293(2007).
11. L. W. Y. Yeung, M. K. So, G. Jiang, S. Taniyasu, N. Yamashita, M. Song, Y. Wu, J. Li, J. P. Giesy, K. S. Guruge and P. L. S. Lam, *Environ. Sci. Tecchnol* **40**, 715(2006).
12. K. S. Guruge, S. Taniyasu, N. Yamashita, S. Wijeratna, K. M. Mohotti, H. R. Seneviratne, K. Kannan, N. Yamanaka and S. Miyazaki, *J. Environ. Moniti.* **7**, 371(2005).
13. G. W. Olsen, H. Y. huang, K. J. Helzlsouer, K. J. Hansen, J. L. Butenhoff and J. H. Mandel, *Environmental Health Perspectives* **113**, 539(2005).