Determination of Persistent Organochlorine Pollutants in Rat Hair by Gas Chromatography-Mass Spectrometry

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A gas chromatography/mass spectrometric assay method was developed for the determination of persistent organochlorine pollutants (POPs) in hair. For the exact extraction study was used hair of rat exposed with POPs. Sonication of the hair matrix with 3 M HCl solution in methylene chloride of the extraction methods studied was the most efficient and rapid sample preparation method. After sonication of rat hair was achieved clean up with a solid phase extraction procedure using silica gel-florisil. Elution was performed with 8 mL of methylene chloride. The eluate was concentrated to approximately 100 μL and analyzed by gas chromatography-mass spectrometry (GC-MS). Detection limits of POPs were in the concentration range of 0.6-1.2 ng/g in rat hair. Aldrin, dieldrin, p,p-DDT and mirex were dosed rat for 4 weeks at concentration of 0.01 mg/L in drinking water and detected in rat hair at concentration of 2.8, 11.3, 7.9 and 15.6 ng/g, respectively. Aldrin and p,p-DDT were metabolized to dieldrin and p,p-DDE, which were detected in concentration of 9.7 and 2.9 ng/g in rat hair, respectively. The developed method may be valuable to be used to analyze POPs in human hair.

Key Words : Persistent organochlorine pollutants, Hair, Sonication, Gas chromatography/mass spectrometry

Introduction

The chlorinated pesticides named as persistent organic pollutants (POPs) bioaccumulate in the food chain due to their lipophilicity and persistence, and they have become a major issue of research in order to investigate their ubiquitous environmental occurrence, biochemical and toxic effects, human exposure and health risk assessment.[1-4]

Body fluids and tissues were commonly in use to characterize human exposure of POPs.[5-7] This approach results in integrative description of exposure in the past without enabling us to identify distinct body burdens. In recent years, hair analysis has rapidly progressed as a useful method for detecting and monitoring POPs over the long term. Moreover, human hair can be easily collected from people over wide range of age, sex, residential area, eating habits and working environments, and there is no need for special apparatus for sampling of hair.

To date, several analytical methods for the determination of POPs in hair have been described.[8-12] Methods used in sample preparation for analysis of POPs in hair generally involve digestion of the hair matrix and subsequent extraction. Such methods involve a long preparation time or a high temperature condition. Indeed, it may be difficult to extract POPs from hair matrix in a simple and reproducible manner.

Sonication have used for the extraction of pesticides or petroleum hydrocarbons in environmental matrix.[13,14] The use of sonication for the extraction of POPs from hair may fulfill these drawbacks.

This paper describes the development of a sonication preparation method, which allows the rapid quantification of POPs in hair at the low-ng/g level. After extraction, the chosen method for quantitation is generally GC-MS. This method is a good method for the determination of POPs in hair due to its specificity and sensitivity allowing the determination of very low concentration in hair.[8-12]

Experimental Section

Chemicals and reagents. d10-Phenanthrene (internal standard) and d10-pyrene (internal standard) were purchased from Aldrich (USA). Hexachlorbenzene, heptachlor, aldrin, heptachlor epoxide, α-, γ-chlordane, p,p-DDE, dieldrin, p,p-DDD, endrin, o,p-DDD, p,p-DDT and mirex were purchased from Sigma (St. Louis, MO, USA). Analytical grade sodium sulfate (Junsei, Japan) was used as reagent, and *n*-hexane, acetone, methylene chloride and methanol (J.T. Baker, USA) were used as solvents. Silica gel and florisil of chromatography grade (Sigma, USA) were activated by overnight heating at 350 ^{o}C and 170 ^{o}C, respectively, and stored in a desiccator. Water was purified in milli-Q (Millipore Corp., Milford, MA).

Animals and treatment. Six young female Sprague-Dawley rats with a body weight of about 160 g were obtained from Haehanbiolink (Chongju, Korea). They were acclimatized for one month in Macrolone cages (temp. of 25 ^{o}C, humidity of 30-70%, illumination time from 6 a.m. to 6 p.m.) and had free access to tap water and food. Their hair was cut partially before the treatment with POPs and used as

blank. A group of the animals (3 per group) was treated with aldrin and p,p-DDT, and the other with dieldrin and mirex for 4 weeks at concentration of 0.01 mg/L in the drinking water. Content of POPs in the drinking water preparations was confirmed by GC-MS. After 4 weeks of the treatment, the hair was cut, washed with deionised water, dried, cut into pieces of 1-2 mm length, and carefully mixed.

For the investigation with spiked samples a pool was prepared of rat hair before the treatment of POPs. The hair was washed with deionised water and dried, cut into pieces of 1-2 mm length and carefully mixed.

Extraction method 1. Two hundred (200) mg cut hair (length of 1-2 mm) from each sample were accurately weighed and incubated for 12 hr at 40 ºC in 2 mL of 3 M HCl. The solution was extracted 3 times with 2 mL of hexane: methylene chloride (4 : 1). The total extract was evaporated to dryness and spiked with 5 ng of internal standards (d10-phenanthrene and d10-pyrene). The sample was dissolved with 1 mL of *n*-hexane and gradually transferred to a washed and preconditioned (10 mL of *n*-hexane) SPE column.

Extraction method 2. Two hundred (200) mg cut hair (length of 1-2 mm) from each sample were accurately weighed and sonicated in an ultrasonic bath (Branson 5210, Branson Ultrasonic Cleaner, USA) for 3 hr at 40 ºC with 2 mL of 3 M HCl. The solution was extracted 3 times with 2 ml of hexane : methylene chloride (4 : 1). The total extract was evaporated to dryness and spiked with 5 ng of internal standards (d10-phenanthrene and d10-pyrene). The sample was dissolved with 1 mL of *n*-hexane and gradually transferred to a washed and preconditioned (10 mL of *n*-hexane) SPE column.

Extraction method 3. Two hundred (200) mg cut hair (length of 1 mm) from each sample were accurately weighed and sonicated in an ultrasonic bath (Branson 5210, Branson Ultrasonic Cleaner, USA) for 3 hr at 40 ºC with 2 mL of 3 M HCl in methanol. The solution was extracted 3 times with 2 ml of hexane : methylene chloride (4 : 1). The total extract was evaporated to dryness and spiked with 5 ng of internal standards (d10-phenanthrene and d10-pyrene). The sample was dissolved with 1 mL of *n*-hexane and gradually transferred to a washed and preconditioned (10 mL of *n*-hexane) SPE column.

Extraction method 4. Two hundred (200) mg cut hair (length of 1 mm) from each sample were accurately weighed and sonicated in an ultrasonic bath (Branson 5210, Branson Ultrasonic Cleaner, USA) for 3 hr at 40 ºC with 2 mL of 3 M HCl in methylene chloride. The solution was extracted 3 times with 2 ml of hexane : methylene chloride (4 : 1). The total extract was evaporated to dryness and spiked with 5 ng of internal standards (d10-phenanthrene and d10-pyrene). The sample was dissolved with 1 mL of *n*-hexane and gradually transferred to a washed and preconditioned (10 mL of *n*-hexane) SPE column.

Clean up. The SPE column consisted of 2.5 g of silica gel, 2.5 g of florisil and 2.0 g of sodium sulfate. The maximum flow rate of sample through the SPE column was 1 mL/min. The SPE column was washed with 10 mL of *n*-hexane at a flow-rate up to 1 mL/min. Then, POPs were eluted with 8 mL of methylene chloride at a flow-rate of 0.5 mL/min. The eluate was dried with anhydrous sodium sulfate, evaporated to approximately 100 μL and then transferred into a V-shape auto sampler vial. At appropriate times, 2 μL sample of the solution was injected into the GC system.

Table 1. GC-MS operating conditions of POPs

Parameter	Conditions		
Column	HP-5MS (Cross-linked 5% phenylmethylsilicon), 30 m × 0.2 mmI.D. × 0.25 μm F.T		
Carrier	He at 0.9 mL/min		
Oven Temp	10 ºC/min 1 ºC/min 10 ºC/min 80 ºC (1 min) → 180 ºC (11 min) → 190 ºC → 260 ºC (3 min) 300 ºC (5 min)		
Split ratio	10 : 1		
Injector Temp	300 ºC		
Transfer line	280 ºC		
Selected Ion Group	Group	Start time (min)	Selected Ions, m/z
	1	13.0	142, 188, 249, 284
	2	18.0	100, 272, 337
	3	22.0	66, 263, 293
	4	25.0	81, 353, 388
	5	27.5	212, 237, 272, 373, 410
	6	31.8	79, 176, 246, 263, 277, 318
	7	33.3	81, 165, 235, 263, 320, 345
	8	35.0	165, 235, 237, 354
	9	39.5	237, 272

Gas chromatography-mass spectrometry. All mass spectra were obtained with a Agilent 6890/5973 N instrument. The ion source was operated in the electron ionization mode (EI; 70 eV, 230 ºC). Full-scan mass spectra (m/z 40-800) were recorded for the identification of analytes at high concentration. Confirmation of trace chemicals was completed by two MS characteristic ions, the ratio of two MS characteristic ions and GC-retention time matches to the known standard compounds. The ions selected in this study and the operating parameters of GC-MS are shown in Table 1.

Calibration and quantification. Calibration curves for hexachlorbenzene, heptachlor, aldrin, heptachlor epoxide, α-, γ-chlordane, p,p-DDE, dieldrin, p,p-DDD, endrin, o,p-DDD, p,p-DDT and mirex were established by extraction after adding 0.2, 0.5, 1.0, 2.5, 10, 20 and 40 ng of standards and 40 ng of internal standard in 200 mg of hair. d10-Phenanthrene and d10-pyrene were used as the internal standards. The ratio of the peak area of standard to that of internal standard (d10-pyrene) was used in the quantification of the compound.

Spiked samples were prepared by rat hair (200 mg) spiked with 10-40 μL of POPs standard solutions at a concentration of 10-1000 ng/mL and with 20 μL of the solution containing internal standards at a concentration of 2000 ng/mL. The same amount of internal standard solution was added to samples before the extraction.

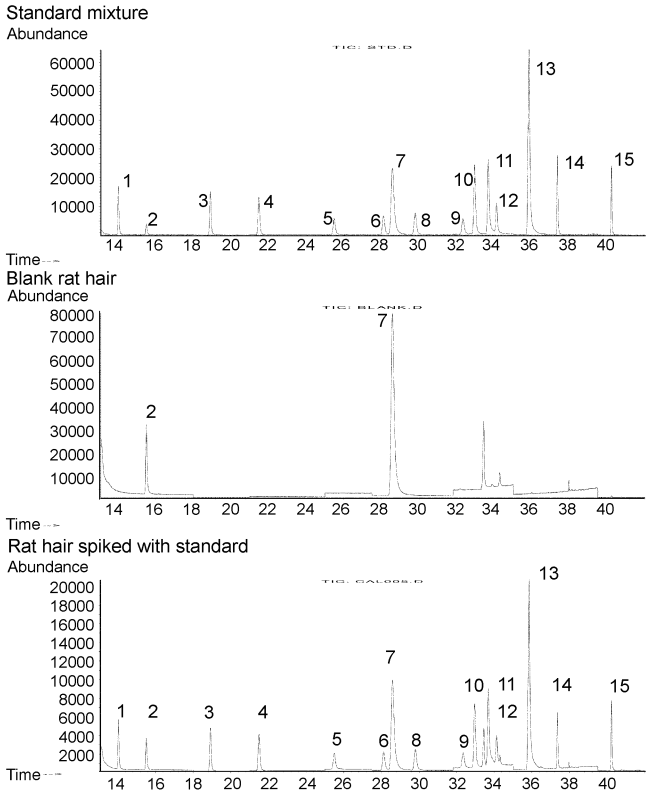

Figure 1. Chromatogram of standard mixture (20 ng/mL) and extract of sample spiked with POPs and ISTD on concentration of 20 ng/g. (1: hexachlorobenzene, 2: heptachlor, 3: aldrin, 4: heptachlor epoxide, 5: r-chloradane, 6: a-chloradane, 7: p,p-DDE, 8: dieldrin, 9: p,p-DDD, 10: endrin, 11: o,p-DDD, 12: o,p-DDT, 13: p,p-DDT, 14: mirex)

Results and Discussion

Chromatogram and identification. The chromatograms of standards and the extract from samples spiked as concentration of 10 ng/g are shown in Figure 1. Separation of the target compounds and internal standard from the background was very good. Residual POPs in rat hair after dose with drinking water were investigated and five POPs (aldrin, dieldrin, p,p-DDT, p,p-DDE and mirex) were identified in rat hair. Aldrin and p,p-DDT were metabolized to diedrin and p,p-DDE in rat hair.

Extraction and clean up. In order to examine whether sonication method can be used as an extraction method of POPs from hair and to select optimal extraction solvent for the sonication method, we compared four extraction procedures. Rat hair was investigated for the target compounds, which were naturally present from incorporation after dose with drinking water. The pooled rat hair samples were weighed, and incubated with 3 M HCl or sonicated with three different extraction solvents. Incubation method found as the best method by Covaci[10] was selected as reference method (Method 1) and three sonication methods using different extraction solvents were evaluated as peak area ratios of target compounds to internal standard.

As a result, it was found that sonication of the hair matrix

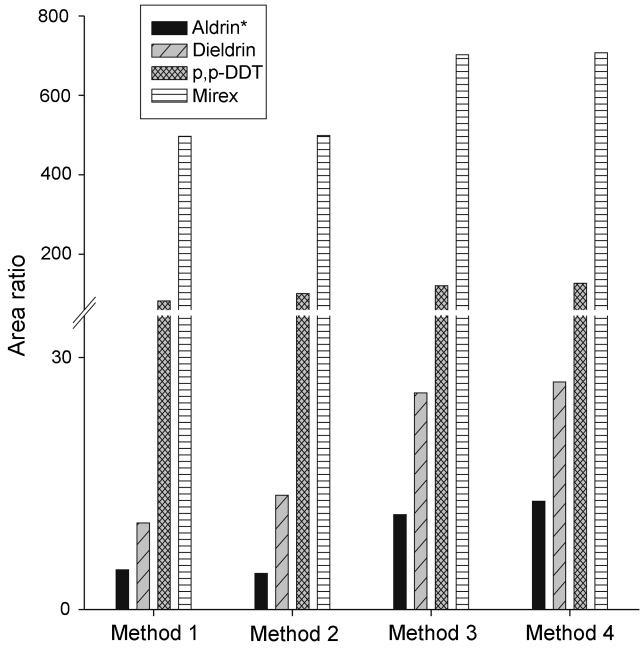

Figure 2. Comparison of extraction yields according to extraction methods used.

with 3 M HCl solution in methylene chloride was the most efficient preparation method among the four methods (Figure 2). HCl and methylene chloride were miscible in the sonicator and efficient as the solvent for the extraction of POPs from hair.

In order to examine the optimum sonication time, the extraction yield according to extraction time was studied. Maximum extraction yield can be achieved in 3 hr at 40 °C when 3 M HCl in methylene chloride was used as solvent (Figure 3).

Good recoveries and purification were achieved with a solid phase extraction procedure using the silica gel-florisil and the elution solvent of methylene chloride. Elution

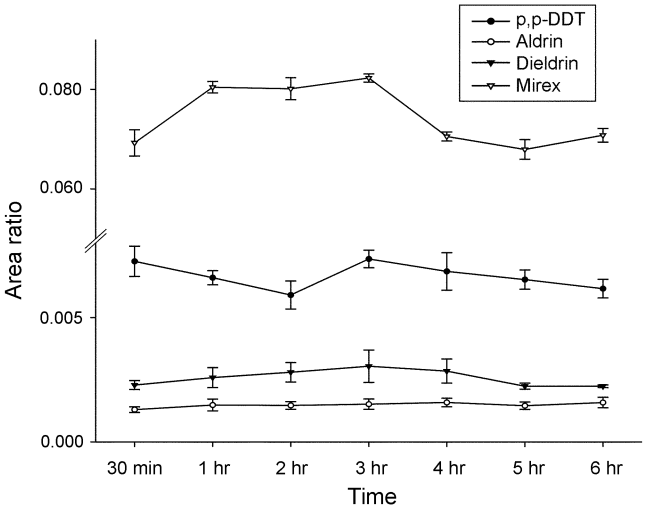

Figure 3. Comparison of extraction yields according to extraction time.

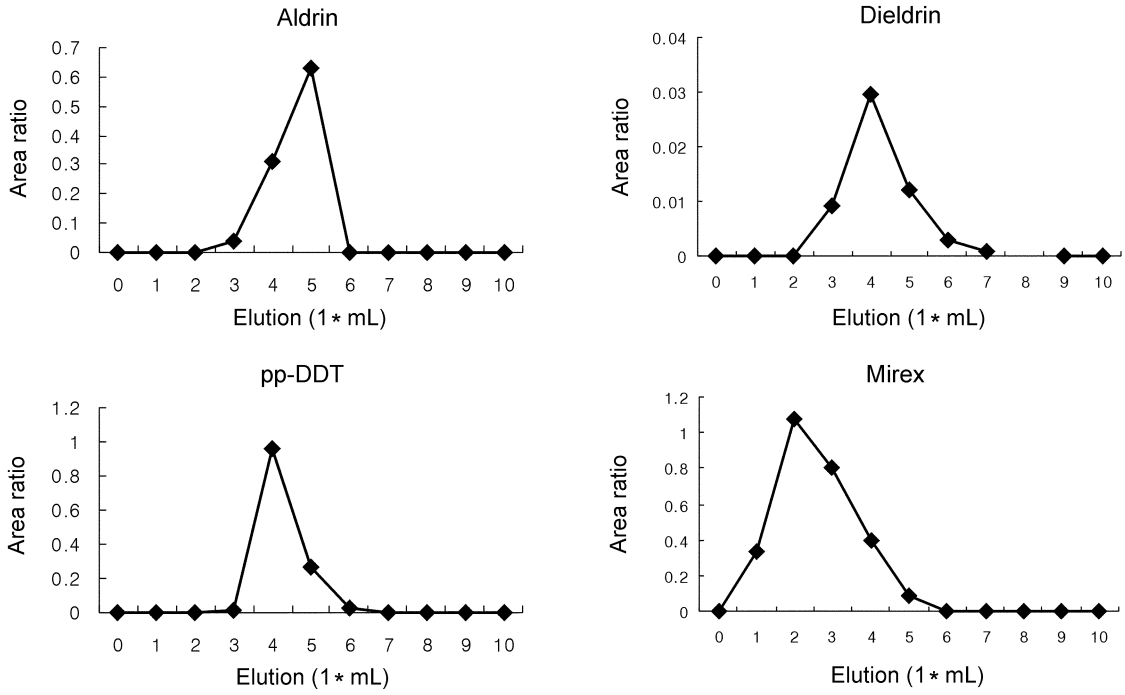

Figure 4. Elution patterns of aldrin, dieldrin, p,p-DDT and mirex through the silica gel-florisil column.

Table 2. Linearlity and detection limits of POPs

Compounds	Range (ng/g)	Calibration curve	γ	MDL (ng/g)
Hexachlorbenzene	1-200	y = 76.511x + 10.919	0.996	0.6
Heptachlor	1-200	y = 239.09x – 15.675	0.994	1.1
Aldrin	1-200	y = 305.99x + 33.67	0.996	1.1
Heptachlor epoxide	1-200	y = 216.9x + 33.55	0.992	1.2
α-Chlordane	1-200	y = 427.63x + 78.439	0.993	0.6
γ-Chlordane	1-200	y = 651.75x – 54.165	0.996	1.0
p,p-DDE	1-200	y = 401.32x + 82.528	0.993	1.1
Dieldrin	1-200	y = 710.01x – 44.438	0.994	0.8
p,p-DDD	1-200	y = 422.96x – 71.498	0.995	0.9
Endrin	1-200	y = 673.82x – 49.849	0.994	0.9
o,p-DDD	1-200	y = 929.09x – 62.638	0.996	1.2
p,p-DDT	1-200	y = 190.43x – 24.795	0.991	0.6
Mirex	1-200	y = 342.09x + 85.968	0.996	0.9

patterns of POPs through the adsorbents are shown in Figure 4. Most of POPs were eluted with 8 mL of methylene chloride at a flow-rate of 0.5 mL/min.

Linearity and detection limits. Standard curves were made by computing regression lines of peak area ratios of hexachlorbenzene, heptachlor, aldrin, heptachlor epoxide, α-, γ-chlordane, p,p-DDE, dieldrin, p,p-DDD, endrin, o,p-DDD, p,p-DDT and mirex to internal standard. Correlation coefficients using a least-squares fit demonstrated linear relationships with being greater than 0.991 (Table 2).

Detection limits were defined by a minimum signal-to-noise ratio of 3 and coefficients of variation for replicate determinations (n = 5) of 15% or less of the extract of sample and calculated in Table 2 based upon an assayed sample of 200 mg.

Precision and accuracy. The range and standard deviation values for the precision and accuracy are given in Table 3. For five independent determinations at 10.0 and 50.0 ng/g, the coefficient of variation was less than 10%.

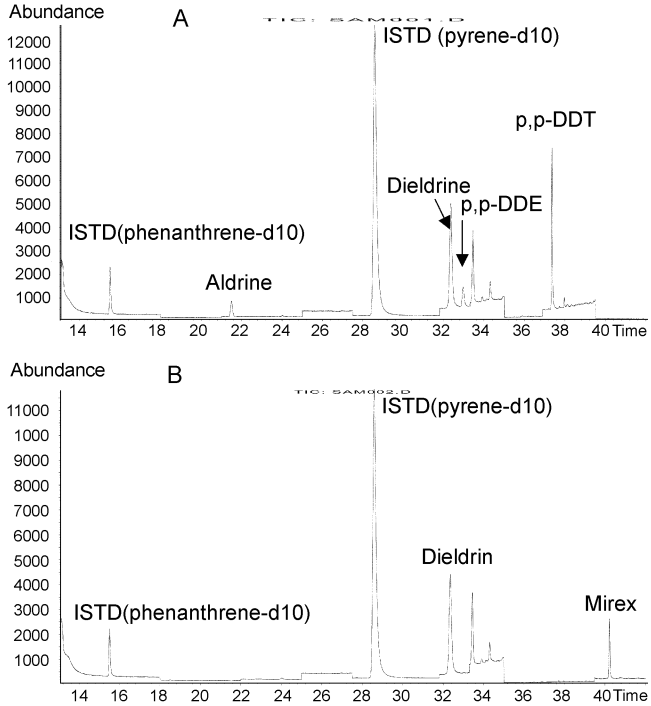

Figure 5. The chromatograms of the extract from rat hair dosed with POPs. (A: rat hair sample dosed with aldrin and p,p-DDT, B: rat hair sample dosed with dieldrin and mirex)

Application. The concentrations of the target compounds in rat hair were quantified. The chromatograms of the extract from rat samples are shown in Figure 5. Aldrin, p,p-DDT,

Table 3. Precision and accuracy of POPs

Compound	Spiked Conc. (ng/g)	Measured Conc. (ng/g)	Mean ± SD (RSD%)
Hexachlorbenzene	10	9.4, 12.4, 11.6, 8.4, 11.0	10.5 ± 1.6 (15.5%)
	50	44.2, 47.4, 39.0, 48.0, 41.7	44.1 ± 3.8 (8.6%)
Heptachlor	10	9.7, 13.4, 11.9, 10.7, 9.8	11.1 ± 1.6 (14.2%)
	50	41.5, 43.6 40.0, 46.6, 51.2	44.6 ± 4.4 (10.0%)
Aldrin	10	11.8, 10.9, 10.4, 11.4, 12.6	11.4 ± 0.8 (7.4%)
	50	54.5, 45.3, 42.9, 52.8, 51.1	49.3 ± 5.0 (10.1%)
Heptachlor epoxide	10	10.7, 11.3, 13.2, 13.4, 11.1	11.9 ± 1.3 (10.6%)
	50	48.3, 49.0, 44.7, 48.5, 44.2	46.9 ± 2.3 (4.9%)
α-Chlordane	10	12.9, 11.9, 12.6, 13.9, 11.0	12.5 ± 1.1 (8.5%)
	50	45.1, 47.2, 50.1, 43.8, 45.0	46.2 ± 2.5 (5.4%)
γ-Chlordane	10	9.1, 9.5, 7.8, 9.2, 7.7	8.7 ± 0.8 (9.8%)
	50	48.9, 46.8, 49.1, 51.1, 39.5	47.1 ± 4.5 (9.5%)
p,p-DDE	10	11.9, 12.4, 10.3, 13.1, 9.7	11.5 ± 1.5 (12.7%)
	50	45.1, 47.2, 50.1, 43.8, 45.0	46.2 ± 2.5 (5.4%)
Dieldrin	10	12.5, 13.2, 11.7, 13.1, 12.0	12.5 ± 0.7 (5.3%)
	50	49.7, 48.6, 54.5, 49.5, 55.7	51.6 ± 3.2 (6.3%)
p,p-DDD	10	12.3, 12.6, 11.3, 13.5, 10.8	12.1 ± 1.1 (8.8%)
	50	56.7, 45.6, 48.3, 51.0, 54.5	51.2 ± 4.5 (8.8%)
Endrin	10	13.5, 14.2, 11.6, 12.5, 12.1	12.8 ± 1.1 (8.3%)
	50	50.8, 42.8, 53.2, 47.0, 47.3	48.2 ± 4.0 (8.2%)
o,p-DDD	10	11.7, 12.3, 11.0, 12.1, 13.3	12.1 ± 0.8 (6.9%)
	50	51.5, 52.2, 48.4, 44.7, 49.5	49.3 ± 3.0 (6.0%)
p,p-DDT	10	12.3, 13.6, 11.6, 11.0, 10.5	11.8 ± 1.2 (10.2%)
	50	49.1, 45.1, 49.9, 43.4, 54.2	48.4 ± 4.2 (8.7%)
Mirex	10	11.7, 12.1, 13.0, 14.0, 13.4	12.9 ± 1.0 (7.4%)
	50	50.1, 45.8, 50.9, 45.0, 56.0	49.6 ± 4.4 (8.9%)

Table 4. Analytical results of POP in rat hair

Dosed Compound	Detected Compound	Concentration (ng/g)
Aldrin	Aldrin	2.8
	Dieldrin	9.7
Dieldrin	Dieldrin	11.3
p,p-DDT	p,p-DDT	7.9
	p,p-DDE	2.9
Mirex	Mirex	15.6

dieldrin, p,p-DDE and mirex were detected in rat hair and their concentrations were in range of 2.8-15.6 ng/g. Aldrin and p,p-DDT were metabolized to dieldrin and p,p-DDE in rat, which were detected in concentration of 9.7 and 2.9 ng/g in rat hair, respectively (Table 4).

Conclusions

Our results indicated that sonication method was very useful for the extraction of POPs from hair. The mixture of HCl and methylene chloride was an efficient solvent for the extraction, due to its permeability to lipid cells in hair. The sonication-SPE/GC-MS-SIM procedure can reduce the analysis time to 1/4, therefore be evaluated as rapid extraction method of POPs from hair.

The peaks have good chromatographic properties and offer very sensitive response for the EI-MS (SIM). Analytical procedure of target compounds with a range of method detection limits of 2.0-4.0 ng/g was established. The detection limits from this study were lower than or similar to those obtained by procedures described before.[5-12]

The developed method may also be valuable in understanding the contamination level of POPs in human hair.

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References

1. Adrian, C.; Carmen, H.; Paul, S. *Sci. Total Environ.* **2001**, *280*, 143.
2. *Analytical Chemistry of PCBs*, 2nd edn.; Erickson, M. D., Ed.; CRC Press: Boca Raton, FL, 1997.
3. Cecilia, S. E.; Erland, B. *J. Chromatogr. A* **2000**, *902*, 227.
4. Karlsson, H.; Muir, D. C. G.; Teixiera, C. F.; Burniston, D. A.; Strachan, W. M. J.; Hecky, R. E.; Mwita, J.; Bootsma, H. A.; Grift, N. P.; Kidd, K. A.; Rosenberg, B. *Environ. Sci. Technol*. **2000**, *34*(21), 4490.
5. Pauwels, A.; Covaci, A.; Delbeke, L.; Punjabi, U.; Schepens, P. *Chemosphere* **1999**, *39*(14), 2433.

6. Covaci, A.; Pauels, A.; Schepens, P. *Intern. J. Environ. Anal. Chem*. **2000**, *76*(3), 167.
7. Pauwels, A.; Covaci, A.; Weyler, J.; Delbeke, L.; Dhont, M.; De Sutter, P.; D'Hooghe, T.; Schepens, P. *Arch. Environ. Contam. Toxicol*. **2000**, *39*(2), 265.
8. Covaci, A.; Schepens, P. *Chromatographia* **2001**, *53*, 366.
9. Covaci, A.; Tutudaki, M.; Aristidis, M. T.; Schepens, P. *Chemosphere* **2002**, *46*, 413.
10. Dauberschmidt, C.; Wennig, R. *J. Anal. Toxicol*. **1998**, *22*, 610.
11. Nakao, T.; Aozasa, O.; Ohta, S.; Miyata, H. *Chemosphere* **2002**, *48*, 885.
12. Seifert, B.; Becker, K.; Helm, D.; Krause, C.; Schulz, C.; Seiwert, M. *J. Expos. Anal. Environ. Epidemiol.* **2000**, *10*, 552.
13. Shin, H. S.; Kwon, O. S. *Bull. Korean Chem. Soc.* **2000**, *21*, 1101.
14. Shin, U. S.; Shin, H. S. *Bull. Korean Chem. Soc*. **2003**, *24*, 413.