Optimized Design of Dioxin Analysis for Water Sample

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Abstract

The analytical methods for dioxins in water sample from wastewater to tap water were reviewed. For extraction method, liquid-liquid extraction (LLE) has been widely used, however, this process needs too much time and man power. New approach including solid phase extraction (SPE) is now applicable to large volume of water sample with high extraction efficiency. Column clean up in classical analytical methods were very complex and time consuming procedures during decade. Modifications were tried to decrease solvent and reagents volume. Moreover, use of column connection method has been demonstrated in the environmental matrices. Instrumental configurations also have been improved, in which GC/MS/MS with large volume injection approach can analyze picogram levels. Absolute sensitivities of HRMS increased compared to old versions of double focusing sector type mass spectrometers. Based on these analytical evolutions during last 10 years, we tried to optimize the analytical method for dioxins in water sample from sample extraction to instrumental analysis.

keywords : Dioxin, PCDD/F, Analysis, Toxicity equivalency factor (TEF), Toxic equivalent (TEQ), Tap water

INTRODUCTION

Dioxins are an unwanted by-product of incineration, uncontrolled burning, and certain industrial processes. Dioxins are released into the air from combustion processes such as waste incineration and from burning fuels (Addink et al., 1998; Ogura et al., 2001). The term dioxin refers to a large family of compounds that includes 17 toxic compounds of particular interest because it is thought that these compounds have similar mechanisms of toxicity (Van den Berg et al., 1998). Dioxins occur as complex mixtures of these 17 family member compounds. Because dioxins are hydrophobic compounds which have extremely low partition coefficients, for example, octanol/water partition coefficients (log Kow) range from 6.64 to 8.78, this means that most dioxins are expected to present in particle-adsorbed form in water environment (Japanese Industrial Standards Committee, 1999a), therefore these compounds will easily be removed during coagulation, precipitation, and sand filtration processes in the regular water treatment. Lately, however, dioxin occurrence in ambient water or drinking water treatment process and the laboratory-scaled formation using precursor materials were issued (Kim et al., 2001; Magara et al., 1999).

Risk assessment of tolerable daily intake (TDI) study demonstrated that the relative dioxin intake from drinking water is negligible (Environmental Agency and Ministry of Health and Welfare of Japan, 1999), so drinking water itself is not a major source of dioxin exposure for humans. A more usual source is our food supply, for example, food products from animals (Tsutsumi et al., 2001). However, the presence of dioxin in drinking water is sensitive issue because not only the analytical method but also quality management in drinking water treatment is very difficult (Tysklind et al., 1993). Several recognized and accredited analytical methods for dioxins have been published in developed countries for reliability and assurance of dioxin data. Those methods were mainly for high concentration of dioxins from stack gas of solid waste incinerator, or contaminated environment media. Detection limits for these methods are much higher than water concentration demonstrated (Ministry of Health and Welfare of Japan, 1999). Although US EPA method 1613 (US EPA Office of Water Regulations and Standards, 1990), JIS K 0312 (Japanese Industrial Standards Committee, 1999a) or methods for raw and tap water (Ministry of Health and Welfare of Japan, 1999) were published for water analysis which may support providing accurate data, these procedures are difficult, time consuming, and still do not include that new analytical techniques have been improved from automated sample extraction method to increased sensitivity of GC/MS during the last decade. These approaches should be added and combined for "fast and easy" analysis for low level of dioxins.

This report provides reviews and optimized design of

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dioxin analysis for low concentration of water sample from river to tap. Topics on the new techniques including sample volume estimation for low concentration, extraction, evaporation, column chromatography, GC/MS determination were discussed.

SAMPLING AND EXTRACTION METHODS

The classic extraction method for hydrophobic chemical such as chlorinated hydrocarbons in water sample was liquid-liquid extraction (LLE). This method is based on the chemical partitioning between organic solvent and water, for example the representative is Kow. The coefficients represent hydrophobicity of chemicals. The log Kow of dioxin increases depending on the number of chlorine (Kuramochi et al., 2002), for example log Kow of TCDD and OCDD is 6.64 and 8.60, this indicates 100-1000 times more hydrophobicity than that of tetrachloroethylene (TCE), respectively. Therefore, dioxin homologue is expected to be present in organic carbon-adsorbed form in water environment. This means that extremely low concentrations from ng/L to pg/L in ambient water is expected considering their low solubility to water. However, EPA method 8290 (US EPA Office of Water Regulations and Standards, 1994) suggests that the proper sample volume is 1 L with high detection limits. Before the field survey, optimal-sample volume should be estimated with instrumental sensitivity. As mentioned above, dioxin concentration in ambient water is expected to be very low, large volume sample should be considered. Sample volume can be estimated using parameters as following equation 1 (Japanese Industrial Standards Committee, 1999a). Simulated results are shown in Table 1.

Equation 1.

$$\text{Sample volume (L)} = \frac{\text{Detection limits of analytical method (pg)}}{\frac{\text{Final sample volume (μL)}}{\text{GC injection volume (μL)}} \times \frac{\text{Sample volume (mL) *}}{\text{Sample volume (mL) **}} \times \frac{1}{\text{Detection limits of sample (pg/L)}}}$$

* after extraction

** used for analysis

Moreover, improved sensitivity of high-resolution mass spectrometer during several years should be considered prior to the sample volume calculation. The JIS K 0312 or EPA method suggest absolute detection limits of HRMS, for example, from 0.1 pg for TCDD to 0.5 pg for OCDD, however, newly HRMS provides signal-to-nose (S/N) more than 100 with 0.1 pg TCDD injection with splitless mode. For calculations, therefore, factors are combined and optimized for each scales under the various sample preparation and injection methods. Calculated sample volume ranged 16.7~66.7 L per sample. Consequently, considering transport and sample preparation time, 20 L sample is the most recommendable volume. LLE methods is used widely due to high extraction efficiency, but it can often be applied below 2 L sample volume for auto-shaking extraction. Method of dioxin for raw and tap water of Ministry of Health and Welfare of Japan suggesting that sample volume for raw and tap water should be 200 L, 2000 L, respectively (Ministry of Health and Welfare of Japan, 1999). This method needs specific sampling facility with polyurethane foam (PUF) which provides fast rate sampling and low detection limits. However, this method has disadvantages, such as high cost, and electricity supply in field.

To extract 20 L sample, extraction methods such as LLE method have disadvantages like time and solvent volume. For example, 20 times LLE with 1 L-sized funnel is necessary. By using SPE, dioxins in ambient water can effectively be partitioned to the solid phase absorbent such as C18 disk. Large size Empore disk (90 mm, 3 M, USA)

Table 1. Factors used and estimated sample volume

Factors for calculation	A	B	C	D	E	F	unit
Detection limits of analytical method	0.04	0.04	0.04	0.04	0.05	0.05	pg
Final sample volume in vial	500	500	500	500	500	500	μL
GC injection volume	100	100	100	50	50	50	μL
Sample volume after soxhlet extraction	50	50	50	50	50	50	mL
Sample volume used for analysis	40	25	25	25	25	25	mL
Detection limits of sample	0.015	0.02	0.01	0.015	0.02	0.015	pg
	16.7	20	40	53.3	50	66.7	L

is well demonstrated for tap water (Otaka et al., 2004). After conditioning, water sample is introduced to a disk type solid phase containing a suitable adsorbent to which the analytes adsorb. After washing the soild phase, the extraction of the analytes is done with Soxhlet extractor. This method has advantages for time- and labor-saving with high extraction efficiency. Use of semi-automated disk-type adsorption system is recommendable for large volume extraction (Choi et al., 2004). Structural concept of semi-automated SPE system is illustrated in Fig. 1.

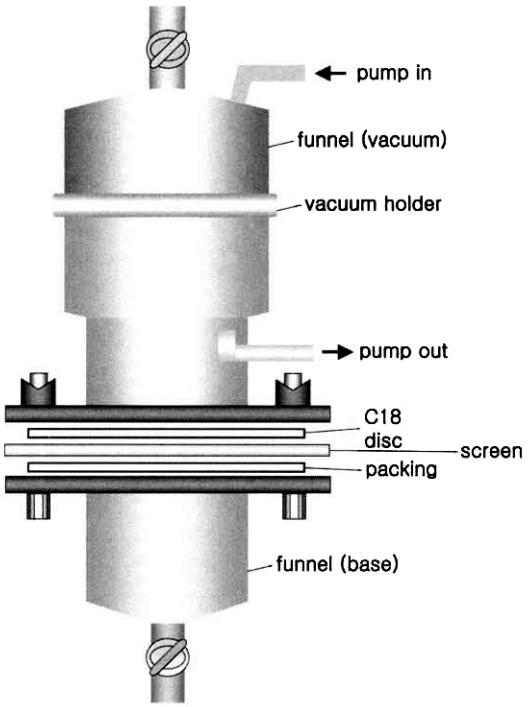

Fig. 1. Solid phase extraction system using C18 disk.

Dioxins adsorbed in the C18 disk are extracted directly in Dean-stark type Soxhlet with toluene. Soxhlet extraction, which was developed to determine the fat content in milk, is now the most classical extraction method between solid and liquid phase. Moistures trapped in the disk can be removed during extraction by using this type of extractor. Disadvantages of Soxhlet are the long extraction times (16-24 hr) and the large volumes of solvent required. Or, as a good and fast alternative method, it is recommendable the use of pressurized liquid extraction methods which commonly applied to solid phase samples such as an ash and soils (Richter et al., 1997; Saito et al., 2003).

Accelerated solvent extraction (ASE) system, as one of popular PLE devices, is well demonstrated for environmental samples especially for solid phase samples. ASE is a technique that uses conventional liquid solvents at elevated temperatures (50-200°C) and pressure (1500-2000 psi). In the serum sample monitoring project, my coworker compared two liquid-liquid extraction (LLE) methods and ASE approach (Kitamura et al., 2004). After addition of 13C-labelled dioxins to an extraction cell containing liquid sample homogenized with sodium sulfate, the cell is extracted with acetone or ethanol by ASE. The results of dioxins and dioxin-like PCBs were presented in Table 2. Similar extraction efficiencies between LLE and ASE were obtained from tetra- to octachloro-dioxins and dioxin-like PCBs without significant differences between solvent combinations. This indicates PLE method provide faster and comfortable analysis without reducing extraction efficiencies. Compared to classical LLE, the amount of solvents and time needed were reduced significantly. The other choice is supercritical fluid extraction (SFE) method, which uses a fluid that has the properties of both a liquid and a gas.

COLUMN CHROMATOGRAPHY AND MULTIEVAPORATIONS

Since the concentration of dioxins in water is extremely low, interfering materials must be removed in the clean up procedures prior to GC/MS analysis. General clean up methods for dioxin analysis exist for more than 15 years now. The base of clean up flow are sulfuric acid treatment, activated neutral or base (KOH) or acid (H_2SO_4) silica gel, alumina and active carbon column chromatography (Japanese Industrial Standards Committee, 1999a; US EPA Office of Water Regulations and Standards 1990). But conventional clean up procedures are time consuming and requiring manual sample preparation, this may be possible to a decrease of accuracy and precision. Fast, simple and low-cost methods have been developed and demonstrated in the last decade (Kitamura et al., 2003; Landin et al., 2003; Saito et al., 2003; Todaka et al., 2003). For example, different kinds of silica gels are packed in a column in several layers, making the clean-up even more efficient. These new effective approaches should be added to the advanced clean up methods. Further, for water sample, some omissions or simplification may be possible compared to those of flue gas, soil and animal fats due to the small quantities of interfering organic matrices.

After Soxhlet extraction and evaporation, concentrated extracts containing particles or organic matter are to be decomposed by adding sulfuric acid. For soil, sediment and biological tissues, sulfuric acid treatment is necessary because these samples containing much organic materials or fats than that of water. However, as the amount of sulfuric acid was increased, the recovery rate decreased (Kemmochi et al., 2003). If SPE system were used, the

Table 2. Comparison of extraction results between LLE and PLE in serum sample

	Liquid-Liquid Extraction		Pressurized Liquid Extraction	
	Ethanol/ Hexane	Aceton/ Hexane	Ethanol/ Hexane	Aceton/ Hexane
PCDDs				
2,3,7,8-TeCDD	1.2	1.2	1.3	1.2
1,2,3,7,8-PeCDD	4.5	4.4	5	5
1,2,3,4,7,8-HxCDD	2.3	2.3	1.8	1.9
1,2,3,6,7,8-HxCDD	19	19	22	22
1,2,3,7,8,9-HxCDD	3.9	4	4.3	4.1
1,2,3,4,6,7,8-HpCDD	14	13	13	14
OCDD	370	390	380	380
PCDFs				
2,3,7,8-TeCDF	1.3	1.3	0.87	0.78
1,2,3,7,8-PeCDF	0.72	0.77	0.59	0.55
2,3,4,7,8-PeCDF	10	10	11	10
1,2,3,4,7,8-HxCDF	3.4	3.6	3.6	3.4
1,2,3,6,7,8-HxCDF	4.3	4.3	4.8	4.8
1,2,3,7,8,9-HxCDF	ND	ND	ND	ND
2,3,4,6,7,8-HxCDF	1.5	1.5	2	2
1,2,3,4,6,7,8-HpCDF	2.8	3	3.6	3.7
1,2,3,4,7,8,9-HpCDF	ND	ND	ND	ND
OCDF	ND	ND	ND	ND
Non-*ortho*-PCBs				
3,4,4',5-(81)	1.8	1.9	2.6	2.4
3,3',4,4'-(77)	18	18	22	21
3,3',4,4',5-(126)	62	62	72	68
3,3',4,4',5,5'-(169)	42	42	45	46
Mono-*ortho*-PCBs				
2',3,4,4',5-(123)	280	280	250	240
2,3',4,4',5-(118)	14000	14000	14000	13000
2,3,4,4',5-(114)	850	830	850	870
2,3,3',4,4'-(105)	2100	2100	2500	2400
2,3',4,4',5,5'-(167)	2300	2300	2100	2000
2,3,3',4,4',5-(156)	4500	4600	5100	4900
2,3,3',4,4',5'-(157)	990	1100	1200	1100
2,3,3',4,4',5,5'-(189)	740	780	590	580
Total TEQ	26	26	29	28

organic particles in water sample is removed in glass-wool filtration just before C18 disk adsorption step. Therefore, use of multi-layered silica gel or single use of 10% silver nitrate silica gel column may substitute to sulfuric acid clean up. Omission of this step will save more than 1-2 hr per sample.

To achieve fast cleanup, combination of silica gel column has been popular options. The analytical methods for flue gas and soil recommend the combined use of silica gel such as SiO_2, H_2SO_4/ SiO_2, KOH/ SiO_2, or silver-nitrate/ SiO_2 etc. for effective removal of interfering matrices (Japanese Industrial Standards Committee, 1999b; US EPA Office of Water Regulations and Standards, 1990). Single use of H_2SO_4/SiO_2 or silver-nitrate/SiO_2 may provide sufficient clean up in water sample. Activated aluminum oxide, alumina column which are used for remove of fatty acids and other interfering compounds including PCB, recommended as the final clean up step for fly ash and sediments etc (Japanese Industrial Standards Committee, 1999a; US EPA Office of Water Regulations and Standards, 1990). This column is often skipped in the recent analysis when applying to small volume sample or sample containing low concentration of PCBs. Furthermore, preparation of alumina column needs more labors than that of

other silica column. Active carbon column is also used in the final clean up step for both remove of interferences and separation of dioxin-like PCBs. Recently, down sizing or reversible type of carbon column are well used. For example, almost same recoveries and peak shape were shown in the use of 0.1-0.3 g for serum and environmental samples (Aozasa et al., 2003; Kemmochi et al., 2002). Instead of carbon column, other inexpensive applications such as blue-chitin is promising clean up reagent in the dioxin analysis (Kitamura et al., 2003). Recently, column coupling approach has been proved to allow fast and high quality analysis of dioxins in the environmental samples (Kemmochi et al., 2002; Kitamura et al., 2004; Suzuki et al., 2003). The basic system is column connection which enables an elution of the first column such as multi-layered silica gel column directly onto the second column like active carbon column due to strong surface adsorption of dioxins on the second column.

Details of the sample loading and elution are as follows; the coupled column is conditioned with hexane and after loading the sample on the top of the multi-layered silica gel column, the connected column is eluted with hexane. After the first elution, the columns are disconnected and the active carbon column is eluted with dichloromethane/hexane for dioxin-like PCBs and then eluted with toluene for dioxins. Optimized column coupling for water clean up is designed and illustrated in Fig. 2. Combined solid cartridge type column chromatography with elution pump is one of rising approaches (Kawano et al., 2003; Matsukami et al., 2003). Because elution rates by the conventional opened-type glass column at atmospheric pressure is dependent on the staff's skill, use of elution pump by liquid chromatograph will provide semi-automation and precise elution rates with small variation. The elution pump devices were well demonstrated for fly ash, flue gas, ambient air and soils (Abad et al., 2000). For sample evaporation, choice of multi evaporator will save analytical time, but disadvantages of some multi evaporator are nitrogen gas supply and slow purge for toluene having high boiling point. On the base of these new clean up systems, the optimized scheme of sample preparation from sample adsorption to column chromatography for water sample is summarized in Fig. 3.

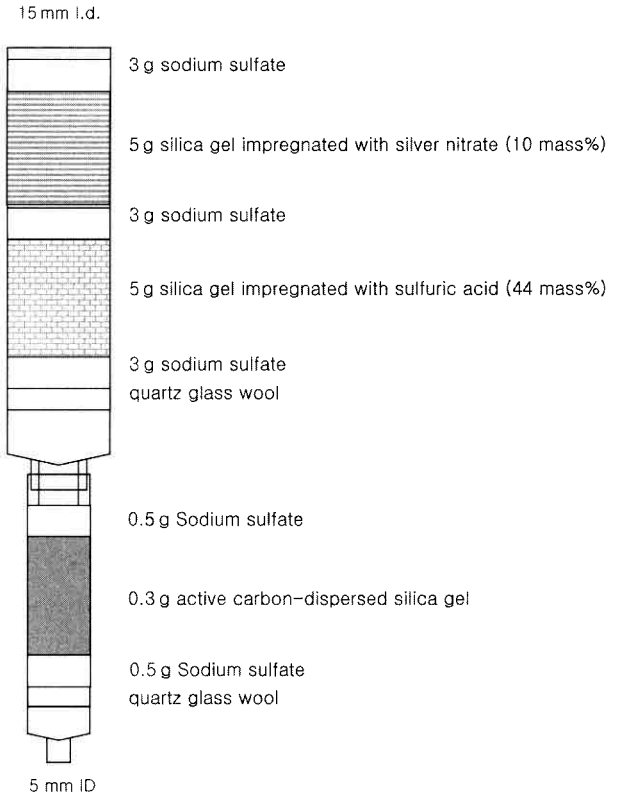

Fig. 2. Optimal column coupling for water sample.

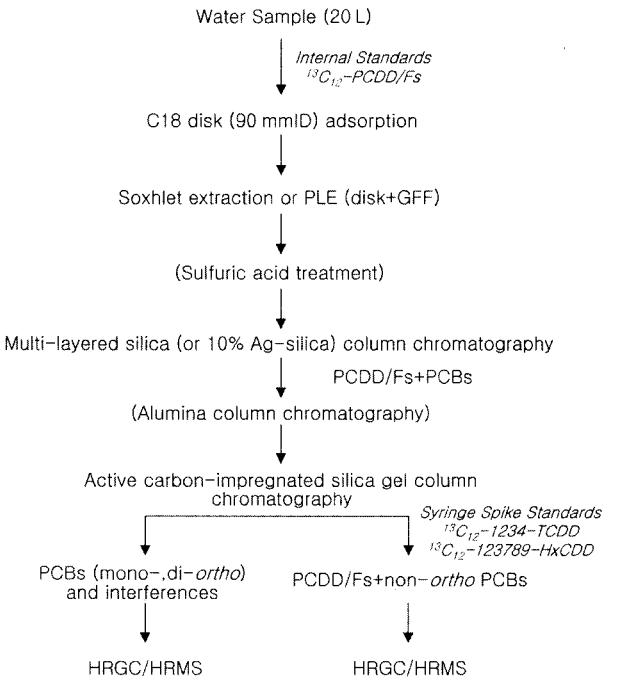

Fig. 3. Proposed analytical method from extraction to clean up for water sample.

INJECTION TECHNIQUES

In order to improve the sensitivity of GC/MS analysis, there are two options. First option is to increase the sample volume which may increase clean up time and reagents. Second option is to increase injection volume which may increase interfering matrices or often have limits for injection volume. These have been limitations for increasing the sensitivity. Splitless injection has been used as the most representative injection method in GC/MS for dioxin analysis. During the last few years, large volume injection

method (LV) is widely applied for the trace analytes, specifically to improve sensitivity of dioxins. Comparisons of LV and splitless method are shown in Table 3. The simulation is based on the spike volume, last volume, and injection volume etc. The merits of LV method can be focused as following 1) reduce the interfering matrices to the injector, 2) increase the sensitivity, 3) save the use of expensive labeled compounds, 4) duplication or triplication of measurement etc. The most popular injectors for LV injections are the programmable temperature vaporization (PTV) injector, the cold on-column (COC) injector and AT-column (de Koning et al., 2004).

PTV-LV system is one of the representative methods of LV approaches. PTV is divided into four phases including injection, vaporization, transfer and cleaning. Based on the descriptions of PTV mechanism by Eppe et al. (2004), the operation flow for dioxins are summarized as following; During injection, the split valve is open and the sample is introduced into the cold liner with glass wool set at a certain temperature below the boiling point of the sample solvent. No significant losses of dioxins can be observed because their high boiling points during this phase. During the evaporation step, the PTV temperature is raised with fast rate in order to eliminate the solvent. The solvent is vented through the split valve at an optimized split flow. The third step consists in transferring the components to the analytical column. The split valve is closed and the temperature rapidly raised to 270-300°C in splitless mode for 1-1.5 min. After transfer of the components, the split valve is opened again with 50-100 mL/min and the liner is kept at 270-300°C during the GC run for cleaning. One of important point for LV injection is choice of the solvents for sample delivery. Solvents with high boiling points should be transferred to low boiling points such as hexane.

We used hexane for PTV injection in the previous study of human exposure and the injection volume was reached to 100 μL with no loss of target components (Choi et al., 2002). Other researchers achieved an alternative solvent cut LV system (SCLV) with multiple injections, which has injection capacity up to 15 μL(Masuzaki et al., 2000; Matsumura et al., 2000). After injection, the solvent is vented through a heart-cut valve. Dioxins are cryogenically focused in a cold trap into a narrow band at the head of the analytical column. This system was applied to human blood analysis with improved S/N ratio, congener separation, and data comparability. But, due to the cold trap uses a carbon dioxide gas as coolant, CO_2 supply in a routine analysis is one of demerits in the system.

INSTRUMENTAL ANALYSIS USING GC/HRMS AND TOXICITY ASSESSMENT

Recently, the absolute sensitivities of quadrupole and ion trap type mass spectrometer with GC are apparently increased. Several approaches using these system were applied successfully to fly ash, soil and sediments. Most accredited and formal analytical methods however, designate the use of GC with magnetic sector type high resolution mass spectrometer (GC/HRMS) with selected ion monitoring (SIM) method for identification and quantification of dioxins. For HRMS, electron impact (EI) ionization and the lock mass method are basic operating conditions. Tuning, mass and electric calibration should be carried out until all performance criteria for the measurements are completely met. Each dioxin congeners should be identified by comparing the GC retention time and ion abundance ratio of two selected parent ions with those of their corresponding internal standards. The parent ion abundance ratio between the target congener and its corresponding internal standards should be within the designated limits. Non-2,3,7,8-substituted dioxins are also quantified using an average of the relative response factors from all of the labeled 2,3,7,8-substituted congeners with the same numbers of chlorine atoms. The details of GC/HRMS for identification and quantification of dioxins

Table 3. Comparisons of LV method and splitless from spiking to injection

Injection Method	13C-internal spike (pg)	Last vol. (uL)	Unit conc. (pg/uL)	Injection vol. (uL)	Quantity injected (pg)
Splitless	100	100	1	2	2
	100	50	2	2	4
	100	50	2	1	2
	50	100	0.5	2	1
Large volume	50	500	0.1	50	5
	25	500	0.05	100	5
	10	500	0.02	100	2
	5	500	0.01	100	1
	0.25	500	0.0005	100	0.05

Table 4. HRGC/HRMS conditions and QA/QC

Gas Chromatograph	
Injector	Splitless or on-column type max temp. 250~280℃
Capillary Column	ID 0.25~0.32 mm, length 25~60 m, fused silica capillary column more than two types of capillary column
Carrier Gas	Pure helium (>99.9999%)
Oven Temperature	Temperature control range: 50~350℃
Mass Spectrometer	
Mass Type	Double focusing magnetic sector mass spectrometer
Resolution	Resolution>10,000 (10% vally, 12,000 is necessary in case the use of $^{13}C_{12}$-OCDF as internal standard)
Ion Detection Method	SIM-EI mode
Ion Source Chamber Temp.	250~300℃
Ionization Current	0.5~1 mA
Ionization Voltage	30~70 V
Accelerating Voltage	5~10 kV
Ion Detection Assuarance	Ions monitored are more than two lock mass method grouping the channel is suitable
Calibration Curve	> 3 times injection of 5 steps of calibration standards, RRF
Peak Detection	S/N>3、designation for signal and noise
Identification	Relative isotope ratios within 15% the same retention time with internal standards
Quantification	Isotope dilution method subtract the blank value for calculation significant digits of the concentration should be rounded 2 designation for detection limits detection limits of sample gas, originated from the MDL, should below the 1/30 of criteria
TEQ	WHO-TEQ (pg-TEQ/L)
Others	Duplication of measurement (if possible, within 10% frequency)

including QA/QC are summarized in Table 4.

For assessment of toxic equivalents (TEQs), internationally agreed toxicity equivalency factors (TEFs) for 17 congeners for 2,3,7,8-substituted PCDD/Fs should be used. For example, new TEF model was suggested and widely used by the WHO (Van den Berg et al., 1998). Still, TEF model used in Korea is international TEF which published more than 15 years ago. For international comparison and submission of TEQ data including inventory, reassessment of TEF adoption should be considered in near future.

ADVANCED CONFIGURATIONS OF GC/MS

For the congener specific analysis of fly ash or soils, which have more than 100 dioxin congeners, multi use of capillary column is recommended(Japanese Industrial Standards Committee, 1999a, 1999b; US EPA Office of Water Regulations and Standards, 1994). For example, polar column such as SP-2331, RTX-2330 and CP-Sil 88 is well used for tetra- to hexa-chlorinated dioxins. Non-polar column like DB-5ms, RTX-5 and CP-Sil 8CBms series is selected for hepta- and octa- chlorinated dioxins or dioxin-like PCBs. Therefore, column change is necessary for the complete analysis. Some interface of commercial mass spectrometer offer specific interface for dual GC installation. If there were interface for dual GC connection, the system will perform fast measurement, for example, more than 10 hour will be saved for analysis for 3 batches. Dual column installed LV system in one GC was also demonstrated for fast analysis and high sensitivity (Ezaki et al., 2001).

Comprehensive two-dimensional gas chromatography (GC × GC) with HRMS has emerged as a powerful separation technique with advanced sensitivity. According to the

results in GC × GC/HRMS, much higher peak intensities can be obtained than in conventional GC, because each successful small fraction eluting from the conventional-size first dimension column is subjected, in real time, to a second, orthogonal separation, on a relative short second dimension column with different separation characteristics (Korytar et al., 2003). Recently, time-of-flight mass spectrometry (TOFMS) with GC is also emerged system. TOFMS was successfully applied to ash, sediment, vegetation, and fish samples with low detection limits of TCDD (Focant et al., 2004). Fast GC system coupled with HRMS was attempted to quantify dioxins and planar PCBs in less than 10 minutes. This application is the fastest measurement for dioxins compared to published fast method (Patterson et al., 2003; Patterson et al., 1996). In this system, GC × GC modulation with HRMS approach demonstrated the high sensitivity for TCDD of S/N = 14 for 0.35 femtogram. In near future, these improved configurations including GC × GC separations would be connected to the simple sample clean up, so totally advanced system will be emerged.

DETECTION LIMITS AND LOW CONCENTRATION SCHEM OF CALIBRATION STANDARDS FOR AMBIENT WATER ANALYSIS

There are major two definitions of detection limits (DLs) in the quality assurance, namely DLs for instruments and sample (or analytical method). Detection limit of instrument means that the absolute quantity whose signal to noise (SN) ratio is 3 on the chromatogram of GC/MS or, the minimum quantity that can be distinguished from blank values; 3x the standard deviation of low concentrations of standards such as 10-50 fg. Detection limit of sample can be calculated as follows; compare the relative peak area of the sample to that of a reference standard, and then calculate the absolute quantity of sample's peak whose SN ratio is 3 on the chromatogram of GC/MS. Finally, the concentration is divided by the sample volume used. If thetarget peak is not present on the GC chromatogram, the peak area is estimated from the relative peak area of a reference standard. Then, the detection limit of the peak is calculated using the relative concentration of reference standard and the sample volume used. The detection limits of sample should meet the target detection limits regulated in the criteria. Detection limits of instrument for ambient water and effluent water of JIS K 0312 are as following; 0.1 pg for tetra and penta-CDD/Fs, 0.2 pg for hexa and hepta-CDD/Fs, 0.5 pg for octa-CDD/F (Japanese Industrial Standards Committee, 1999a). These DLs of instruments can be more lowered than those in published manuals due to the improvement of sensitivity of HRMS during last decade. DLs in Japanese Ministry of Health and Welfare (1999) are one of reference scale for water analysis. Now, HRMS of major manufacturers provide high sensitivity such as SN ratio > 100-400 for 0.1 pg TCDD. Therefore, instrumental DLs in these manuals can be cleared in the major HRMS.

Dioxin analysis is based on isotope dilution mass spectrometry. Internationally recognized, official organizations suggest use of 13C-labeled reference compounds in order to guarantee good quality data (Environment Canada, 1990; Japanese Industrial Standards Committee, 1999a; Ministry of Health and Welfare of Japan, 1999; US EPA Office of Water Regulations and Standards, 1990). Samples are spiked with 13C-labeled internal standards in soluble solvents, extracted and cleaned up using an established method prior to GC/HRMS determination. Therefore, an appropriate quantity of the labeled internal standards should also be added to the water sample. For quantification of dioxins in ambient water, a series of calibration standard solution with five or six different concentrations should be prepared covering the range 0.01-50 pg/injection including zero. Commercial standard have been provided high level calibrations for flue gas, ash, sediments and soils. Although major manufacturers recently started to offer low concentration standards for ambient air and foodstuff, these calibrations should be diluted to match the real concentrations in water. An example of scales for calibration standards for ambient water including tap water is presented in Table 5.

CONCLUSIONS

There are several accredited methods of dioxins from incinerator, thermal process and soils. These methods are based on high level of dioxins and high detection limits of instruments. On the other hand, dioxin issues are moving from sources, treatment technology to environmental samples containing low concentrations. We focused on the analysis of dioxins for ambient water and tap water for human health management.

Standard methods such as USEPA and JIS K 0312 still available for water analysis, but new technical options like advanced sample extraction, column chromatography, instrumental evolutions including injection methods demonstrated should be added in the accredited manuals for low concentration samples. We discussed the analytical procedures for ambient water, which include semi-automated disk type extraction, dean-stark Soxhlet extraction, column coupling,

Table 5. Example of low concentration of calibration standard for water sample

Standards	Concentration (ng/mL)				
	STD1	STD2	STD3	STD4	STD5
2,3,7,8-TeCDD 1,2,3,7,8-PeCDD 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-HpCDD	0.05	0.1	0.2	0.5	1.0
OCDD	0.1	0.2	0.4	1.0	2.0
2,3,7,8-TeCDF 1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF 1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-HxCDF 2,3,4,6,7,8-HxCDF 1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF	0.05	0.1	0.2	0.5	1.0
OCDF	0.1	0.2	0.4	1.0	2.0
$^{13}C_{12}$-2,3,7,8-TeCDD $^{13}C_{12}$-1,2,3,4-TeCDD $^{13}C_{12}$-1.2,3,7,8-PeCDD $^{13}C_{12}$-1,2,3,4,7,8-HxCDD $^{13}C_{12}$-1,2,3,6,7,8-HxCDD $^{13}C_{12}$-1,2,3,7,8,9-HxCDD $^{13}C_{12}$-1,2,3,4,7,8,9-HpCDD	10	10	10	10	10
$^{13}C_{12}$-OCDD	20	20	20	20	20
$^{13}C_{12}$-2,3,7,8-TeCDF $^{13}C_{12}$-1,2,3,7,8-PeCDF $^{13}C_{12}$-2,3,4,7,8-PeCDF $^{13}C_{12}$-1,2,3,4,7,8-HxCDF $^{13}C_{12}$-1,2,3,6,7,8-HxCDF $^{13}C_{12}$-1,2,3,7,8,9-HxCDF $^{13}C_{12}$-2,3,4,6,7,8-HxCDF $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF $^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	10	10	10	10	10
$^{13}C_{12}$-OCDF	20	20	20	20	20

dual GC with PTV injection, low level calibration and TEQ assessment. We hope this approach would be one of promising references of analytical methods for water sample in Korea.

REFERENCES

Abad, E., Saulo, J., Caixach, J. and Rivera, J., Evaluation of a New Automated Cleanup System for the Analysis of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Environmental Samples, *J Chromatogr A.*, **893**, pp. 383-391 (2000).

Addink, R., Govers, H. A. J. and Olie, K., Isomer Distributions of Polychlorinated Dibenzo-p-dioxins/dibenzofurans Formed during de Novo Synthesis on Incinerator Fly Ash, *Environ. Sci. Technol.*, **32**, pp. 1888-1893 (1998).

Aozasa, O., Mochizuki, A., Ohta, S., Nakao, T., Miyata, H. and Nomura, T., Optimization of the Purification Method for Dioxin Analysis in Human Serum and Temporal Changes in Background Dioxin Levels in the General Population, *Chemosphere*, **50**, pp. 1157-1165 (2003).

Choi, J. W., Kim, K. S., Lee, J. H., Kim, S. H. and Baek K. H., Development of Analytical Method of Dioxins for Ambient Water Sample (1), *K Soc. Environ. Anal. Abst.*, pp. 169-171 (2004).

Choi, J. W., Miyabara, Y., Hashimoto, S. and Morita, M., Comparison of PCDD/F and Coplanar PCB Concentrations in Japanese Human Adipose Tissue Collected in 1970-1971, 1994-1996 and 2000, *Chemosphere*, **47**, pp. 591-597 (2002).

de Koning, S., Kurano, M., Janssen, H. G. and Brinkman, U. A. T., AT-column, a Novel Concentrating Technique for

Large-volume Injections in Gas Chromatography, *J Chromatogr A.*, **1023**, pp. 165-174 (2004).

Environmental Agency and Ministry of Health and Welfare of Japan, Report on Tolerable Daily Intake (TDI) of Dioxin and Related Compounds (1999).

Environment Canada, Proposed Reference Method for the Determination of PCDD/PCDF in Pulp and Paper Mill Effluents (1990).

Eppe, G., Focant, J. F., Pirard, C. and De Pauw, E., PTV-LV-GC/MS/MS as Screening and Complementary Method to HRMS for the Monitoring of Dioxin Levels in Food and Feed, *Talanta*, **63**, pp. 1135-1146 (2004).

Ezaki, T., Masuzaki, Y., Matsumura, T., Ohashi, M., Ito, H. and Morita, M., Development of Solvent Cut Large Volume (SCLV) Injection Technique with Dual Column Configuration for Rapid and High Sensitive GC-HRMS Analysis, *Organohalogen Compounds*, **50**, pp. 190-193 (2001).

Focant, J. F., Reiner, E. J., MacPherson, K., Kolic, T. and Sjodin, A., Measurement of PCDDs, PCDFs, and Non-ortho-PCBs by Comprehensive Two-dimensional Gas Chromatography-isotope Dilution Time-of-flight Mass Spectrometry (GCxGC-IDTOFMS), *Talanta*, **63**, pp. 1231-1240 (2004).

Japanese Industrial Standards Committee, Japanese Industrial Standard (JIS K 0311) (1999b).

Japanese Industrial Standards Committee, Japanese Industrial Standard (JIS K 0312) (1999a).

Kawano, K., Yamamoto, I., Sotomaru, K. and Kobayashi, S., Study of Rapid Cleanup for Dioxin with Two-layer Carbon Silicagel and Automatic Cleanup System, *J Environ. Chem. Abst.*, pp. 352-353 (2003).

Kemmochi, Y., Tsutsumi, K., Arikawa, A. and Nakazawa, H., Micropore-free surface-activated Carbon for the Analysis of Polychlorinated dibenzo-p-dioxins-dibenzofurans and Non-ortho-substituted Polychlorinated Biphenyls in Environmental Samples, *J Chromatogr A.*, **977**, pp. 155-161 (2002).

Kim, H. K., Kazui, H., Matsumura, T., Ohno, K., Kamei, T. and Magara, Y., Dioxins in Drinking Water Treatment Process, *Organohalogen Compounds*, **51**, pp. 135-138 (2001).

Kitamura, K., Mochizuki, A., Choi, J. W., Takazawa, Y., Hashimoto, S., Ito, H., Fujimine, Y. and Morita, M., Optimization of a Method for Determining Dioxin in Whole Blood Samples Based on Solvent Extraction and Simplified Cleanup, *Analyst*, **129**, pp. 315-322 (2004).

Kitamura, K., Nagao, M., Choi, J. W., Hashimoto, S., Ito, H. and Morita, M., Effective Pretreatment of Human Serum Samples for Dioxin Analysis by Solid Phase Extraction and Blue-chitin Column Cleanup, *Analyst*, **128**, pp. 986-993 (2003).

Korytar, P., Leonards, P. E. G., Danielsson, C., Haglund, P., de Boer, J. and Brinkman, U. A. T., GC x GC Method Development for Analysis of Seventeen 2,3,7,8-substituted Dioxins, Furans and Twelve Dioxin-like PCBs, *Organohalogen Compounds*, **60**, pp. 355-358 (2003).

Kuramochi, H., Ohsako, M., Maeda, K. and Sakai, S., Prediction of Physico-chemical Properties for PCDDs/DFs using the UNIFAC Model with an Alternative Approximation for Group Assignment, *Chemosphere*, **49**, pp. 135-142 (2002).

Landin, P., Llompart, M., Lourido, M., Garcia-Jares, C., Carro, N. and Cela, R., Rapid Determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Water Samples by using Solid-phase Microextraction followed by Gas Chromatography with Tandem Mass Spectrometry, *J AOAC Int.*, **86**, pp. 44-49 (2003).

Magara, Y., Aizawa, T., Ando, M., Morita, M., Ito, H., Seki, Y. and Matsumura, T., Determination of Low Dioxins and PCB's Concentration in Ambient Water using Large Volume In Situ Pre-concentration System, *Organohalogen Compounds*, **40**, pp. 205-210 (1999).

Masuzaki, Y., Matsumura, T., Ezaki, T., Ohashi, M., Ito, H. and Morita, M., High Sensitivity Analysis with Solvent Cut Large Volume (SCLV) Injection Technique, *Organohalogen Compounds*, **50**, pp. 194-197 (2001).

Matsukami, H., Hayashi, A., Takasuga, T., Ohi, E. and Senthilkumar, K., Separation Conditions and Percentage Recovery of Dioxin-like PCBs, PCDDs and PCDFs through Power-prep by Fluid Management System, *Organohalogen Compounds*, **60**, pp. 72-75 (2003).

Matsumura, T., Masuzaki, Y., Ezaki, T., Ohashi, M. and Morita, M., Detection of Low Femto Gram Dioxins-Development of Switching Solvent Cut Large Volume / Multiple Injection - Cryofocus Trap GC-HRMS, *Organohalogen Compounds*, **45**, pp. 25-28 (2000).

Ministry of Health and Welfare of Japan, Analytical Manual for Dioxins in Raw and Tap Water (1999).

Ogura, I., Masunaga, S. and Nakanishi, J., Congener-specific Characterization of PCDDs/PCDFs in Atmospheric Deposition: Comparison of Profiles among Deposition, Source, and Environmental Sink, *Chemosphere*, **45**, pp. 173-183 (2001).

Otaka, H., Shimono, H. and Hashimoto, S., Optimization of Solid-phase Extraction Procedure for Determination of Polychlorinated Dibenzo-p-dioxins, Polychlorinated Dibenzofurans, and Coplanar Polychlorinated Biphenyls in Humic Acid Containing Water, *Anal Bioanal Chem.*, **378**, pp. 1854-1860 (2004).

Patterson, D. G., Barr, J. and Dipietro E. S., Comprehensive Two-dimensional and Fast Single-dimensional GC-HRMS Analysis of Human Serum for PCDDs, PCDFs, cPCBs, Congener PCBs, Persistent and Nonpersistent Pesticides, and PAHs, *Organohalogen Compounds*, **27**, pp. 309-314 (1996).

Patterson, D. G., History and Current Sratus of Fast and Sensitive Analitical Methods for Measuring Compounds of Environmental Concern at the Centers for Disease Control and Prevention, *Organohalogen Compounds*, **60**, pp. 351-354 (2003).

Richter, B. E., Ezzell, J. L., Knowles, D. E., Hoefler, F. A., Mattulat, K. R., Scheutwinkel, M., Waddell, D. S., Gobran, T. and Khurana, V., Extraction of Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans from Environmental Samples using Accelerated Solvent Extraction (ASE), *Chemosphere*, **34**, pp. 975-987 (1997).

Saito, K., Takekuma, M., Ogawa, M., Kobayashi, S., Sugawara, Y., Ishizuka, M., Nakazawa, H. and Matsuki, Y.,

Extraction and Cleanup Methods of Dioxins in House Dust from Two Cities in Japan using Accelerated Solvent Extraction and a Disposable Multi-layer Silica-gel Cartridge, *Chemosphere*, **53**, pp. 137-142 (2003).

Suzuki, T., Murayama, H., Taneoka, Y., Takai, T., Yanaka, K., Shibuya, N., Hashimoto, S. and Ito, H., Simplified Analysis of Dioxins using Disposable Catridge Column Clean-up and GC/ion-storage MS/MS, *J Environ. Chem. Abst.*, pp. 834-835 (2003).

Todaka, T., Hirakawa, H., Tobiihi, K. and Iida, T., New Protocol of Dioxins Snalysis in Human Blood, *Fukuoka Igaku Zasshi*, **94**, pp. 148-157 (2003).

Tsutsumi T., Yanagi, T., Nakamura, M., Kono, Y., Uchibe, H., Iida, T., Hori, T., Nakagawa, R., Tobiishi, K., Matsuda, R., Sasaki, K. and Toyoda, M., Update of Daily Intake of PCDDs, PCDFs, and Dioxin-like PCBs from Food in Japan, *Chemosphere*, **45**, pp. 1129-1137 (2001).

Tysklind, M., Lundgren, K., Eriksson, L., Sjostrom, M. and Rappe, C., Identifying Training Sets of PCDDs and PCDFs for Use in Chemical and Biological Monitoring, *Chemosphere*, **27**, pp. 47-54 (1993).

US EPA Office of Water Regulations and Standards, Method 1613 (revision A). Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS (1990).

US EPA Office of Water Regulations and Standards, Method 8290 (revision 0). Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans by High-resolution Gas Chromatographyh/high-resolution Mass Spectrometry (HRGC/HRMS) (1994).

Van den Berg, M., Birnbaum, L., Bosveld, A. T., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X., Liem, A. K., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F. and Zacharewski, T.,Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and Wildlife, *Environ. Health Perspect*, **106**, pp. 775-92 (1998).