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Simultaneous Determination of 285 Chemicals in Water at ppt Levels by GC-Ion Trap Mass Spectrometry

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Abstract : The authors have developed an analytical method for determining trace amounts of 285 kinds of chemicals in natural waters by GC-ion trap MS. The results of overall recovery tests at 0.1 μg/l showed that the mean recovery was 92.1 % and the mean relative standard deviation was 10.8 %. The mean of the method detection limits was 0.036 μg/l. From the results of analysis of real samples, it was confirmed that this method is useful to elucidate the concentration levels and the fate of chemicals in the aquatic environment.

Keywords : Simultaneous determination, Ion trap MS, Gas chromatography/mass spectrometry, Water sample

1. Introduction

Accompanying the increase of production amounts and kinds of chemicals, the environmental pollution caused by hazardous chemicals is one of the global environmental problems. The Japan Environment Agency has been carrying out environmental surveys on synthetic chemicals since 1974. They had already surveyed 716 kinds of chemicals up until 1993 and had detected 257 chemicals from environmental media[1]. These results indicate that the environment is polluted with a large number of chemicals. To evaluate the adverse effects of chemicals on the ecosystem and human health, therefore, it has been required to monitor a large number of chemicals.

Many simultaneous analytical methods have been developed in Japan[2-4] and the USA[5-7] for monitoring chemicals in environmental media. However, the maximum number of measurable chemicals in one chromatographic analysis is several dozens, because

these methods use GC with a specific detector or GC/MS in a selected ion monitoring mode (SIM). Therefore, several instrumental analyses are needed to determine a large number of chemicals. On the other hand, as ion trap MS is capable of picogram detection limits even in a scanning mode, ion trap MS seems to be the most suitable instrument for simultaneous analysis of a large number of chemicals. Therefore, ion trap MS has become popular in the field of environmental analysis for chemicals[8-12].

We have developed an analytical method for determining trace amounts of 285 kinds of chemicals in water by using the advantages of ion trap MS. Before developing the method, we determined the capabilities of this method. They are 1) measure as many chemicals as possible, 2) measure in a short time, 3) determine at concentrations of ng/l levels, 4) quantify with high accuracy and high precision, and 5) prepare suitable quality control methods. In this report, we described the results of the investigation which was carried out to achieve these purposes. In addition, the results of examinations when this method was applied to real samples are mentioned.

2. Experimental

2.1 Apparatus

The gas chromatograph/mass spectrometer system consists of a Vallian 3400 GC and a Finnigan Mat ITS40 MS.

2.2 Target compounds

The total number of target compounds are 285 summarized in Table I. These target compounds are mainly known to have adverse effects on human health or the biological system. We selected the targets from chemicals regulated by laws relative to environmental protection in Japan or the USA. Additionally, we extracted the targets from chemicals detected in environmental surveys by the Japan Environment Agency[1], and chemicals identified in the environment of Kitakyushu district.

2.3 Analytical procedure

One liter of water sample was collected in a glass bottle. The sample water was transferred into a separatory funnel, and then the mixed surrogate solution and 50 g of sodium chloride were added to the sample. One hundred milliliters of dichloromethane was added into the bottle for rinsing its wall, and then transferred into the separatory funnel. Extraction was carried out with a mechanical shaker for 10 min. The extraction procedure was repeated once with another 50 ml of dichloromethane. After the second extraction, pH of the sample solution was adjusted below 2 using 6 M HCl. The acidified sample was again extracted with another 50 ml of dichloromethane. After the third extraction, pH of the sample solution was adjusted above 11 with 6 M NaOH solution. The alkalized aqueous phase was extracted with another 50 ml of dichloromethane. Each extract was combined and dehydrated by passing through a column packed with anhydrous sodium sulfate (7 ml), and concentrated to a few milliliters with a Kuderna-Danish (KD) concentrator. Hexane (1.0 ml) was added to the concentrate and re-concentrated to 1.0 ml with a micro-snyder column. Prior to GC/MS measurement, the mixed internal standard solution was added to the concentrate. Finally, a one-microliter aliquot of the concentrate was injected with an auto-sampler.

2.4 Gas Chromatography/mass spectrometric analysis

Table II shows the GC/MS conditions. A pre-column was used to achieve the retention gap by which compounds having low boiling points showed good shapes. Concentrations of the targets were calculated by the internal standard method. In this study, 5 kinds of deuterated chemicals were used as internal standards.

Table I Summary of the target chemicals

Compound	Number	Compound	Number	Halogenated	Number
Compounds consisting of CH	88	Aliphatic compounds	25	No	21
				Yes	4
		Benzenes	16	No	3
				Yes	13
		Polycyclic compounds	43	No	41
				Yes	2
		Others	4	No	3
				Yes	1
Compounds consisting of CHO	52	Ethers	8	No	3
				Yes	5
		Ketones	5	No	5
		Phenols	22	No	12
				Yes	10
		Phthalates	9	No	9
		Others	8	No	5
				Yes	3
Compounds consisting of CHN (O)	60	Aromatic amines	36	No	21
				Yes	15
		Quinoline	1	No	1
		Nitro compounds	19	No	14
				Yes	5
		Nitrosoamines	3	No	3
		Others	1	No	1
Compounds consisting of CHS (NO)	7			No	7
Compounds consisting of CHP (NOS)	6	Phosphoric esters	6	No	4
				Yes	2
Pesticides	72	Insecticides	36		
		Herbicides	20		
		Fungicides	16		
Surrogate Compounds	15				
Total	300				

The number of halogenated compounds except for pesticides is 60.

Table II GC/MS conditions for determination of the targets chemicals

Column	J&W DB-5 ms (5% phenyl-95% methylsilicone) fused silica capillary column, 30 m X 0.25 mm i.d., 0.25 mm film; pre-column: Supelco deactivated fused silica tubing, 1 m X 0.25 mm i.d.
Temperature	
Column	temperature programmed: 2 min at 50 C, 8 C/min to 300 C, 8 min at 300C
Injector	250 C
Transfer line	280 C
Ion source	230 C
Injection method	splitless, 1 min for purge-off time
Carrier gas	He
Linear velocity	30 cm/s
Ionization method	EI
Scan range	45 amu to 600 amu
Scan rate	0.6 s/scan
Mass defect	50 mmass/100 amu

3. Results and Discussion

3.1 Measurement using ion trap GC/MS

The GC/MS-SIM method is generally used for trace analysis of chemicals because of its high sensitivity. On the other hand, because mass chromatography by using an ion trap is as highly sensitive as the SIM method, we measured the chemicals by mass chromatography. Quantitative ions in mass chromatography must be specific and in large abundance to obtain high sensitivity and high selectivity. Therefore, it is very important to select quantitative ions. Especially, when some chemicals

have the same retention time, each quantitative ion of overlapping peaks must be different from each other and must not be involved in ions of other overlapping chemicals. In this study, selecting suitable quantitative ions was difficult, because many chemicals were overlapped on a chromatograph. For example, peaks of 6 compounds (isofenphos, chlorfenvinphos, methyl dymron, heptachlor epoxide, oxychlordene and phenothiazine) appeared within only 3 seconds. After measuring retention times and the mass spectra of these chemicals, we decided on the quantitative ions which met the above conditions.

GC/MS-SIM method utilizes a few reference ions to confirm chemicals. The reliability of this confirmation is not too high, because identification of chemicals is performed by comparing the abundance of a few ions. Therefore, the possibility of miss identification increases when some peaks have the same retention time: this case sometimes occurs in analysis of the environmental samples because of the complexity. On the other hand, as the ion trap offers a mass spectrum, qualification is performed by matching both retention time and mass spectrum of a sample and the standard registered in a calibration curve. The probability of miss identification by using a ion trap is much lower than that by the SIM method. Therefore, at present the ion trap is one of the most suitable instruments for simultaneous determination of a large number of chemicals. The ITS40 had the ability to automatically identify chemicals in samples, however we sometimes had to identify manually, because it could not identify correctly when chemicals in samples were at ng/l levels.

3.2 Countermeasures for contamination

Prevention of contamination is important to obtain reliable data as well as low detection limits. In the case of ultra trace analysis of chemicals, preventing contamination is very difficult, because there are many causes of contamination originating from reagents, solvents, glassware, instruments and the atmosphere. In this study, we paid special attention to and took several countermeasures against these kinds of contamination. When we performed recovery tests, we used organic free water and glassware which had previously been washed with dichloromethane. However, small amounts of phthalates and n-alkanes were detected in blank samples. In addition, the recoveries of 7 replicates were much different from each other. As a result, the calculated method detection limits (MDL) were larger than those of other analytes which were not affected by contamination. As these compounds are ubiquitous compounds in the environment, the cause of the contamination was presumed to be from the atmosphere and septa of auto-sampler vials of GC. Since it seemed impossible to prevent the contamination perfectly, we had to attentively evaluate their data obtained from real samples.

3.3 Recoveries of chemicals by liquid-liquid extraction methods

Liquid-liquid extraction used in simultaneous analysis methods in the USA (US EPA Methods 1625[5] and 8270[6] and Standard Methods[7]) is carried out at pH >11 for base and neutrals, and then carried out at pH <2 for acidic chemicals. However, there are two major problems in this extraction procedure. The first one is emulsions, when seawater is extracted under basic conditions, a lot of emulsions generate. The second one is decomposition of some chemicals. In this study, as many pesticides and organochlorines were interested analytes, some of them were supposed to decompose under the basic conditions. Therefore, we examined suitable extraction procedures. We spiked all the targets into 1 liter of each neutral, acidic (pH <2) and basic (pH >11) water to give 2 μg/l for each target, and then extracted twice with 100 ml and 50 ml of dichloromethane. After adjusting the pH of acidic and basic solutions to pH >11 and pH <2, respectively, extraction was repeated twice with another 100 ml and 50 ml of dichloromethane. Each extract was concentrated and injected into the GC/MS to obtain recovery data. The average recoveries of each extraction procedure are listed in *Table III*.

Table III Recoveries (%) of liquid-liquid extraction and KD concentration

Recovery by Extraction			Recovery by KD
Neutral	Acid-Base	Base-Acid	Concentration
89	85	81	106

Base-acid extraction resulted in the lowest recoveries, because some chemicals were decomposed at the first base extraction step. Although the recoveries of some phenols and some amines under the neutral condition were slightly lower than those under other conditions, the best recovery was obtained under the neutral condition. From these results, it was confirmed that liquid-liquid extraction with dichloromethane under the neutral condition had sufficient ability to simultaneously determine most of the chemicals. In this study, as we took into consideration the base and acidic compounds, we used the extraction procedure as described in the Analytical Procedure. However, if we omit both extraction steps under base and acid conditions to reduce volume of dichloromethane for extraction, the omission will affect the recovery little. In this case, the pH of a sample has to be adjusted to pH = 7.0 with 2M potassium dihydrogenphosphate-sodium hydroxide buffer solution.

3.4 Losses of the chemicals during KD concentration

In the case of the analysis of semi-volatile chemicals, KD concentrators or rotary evaporators are usually used to concentrate extracts. Although the KD concentration takes longer than the rotary evaporation, losses of chemicals due to evaporation are smaller than by the rotary evaporation. As the targets included chemicals having low boiling points, such as styrene, we examined their losses during concentration using a KD concentrator. After adding all the chemicals to 150 ml of dichloromethane, the solution was concentrated to 1 ml with a KD concentrator and a micro-snyder column. The results in *Table III* show that most of the analytes, including volatile compounds, are satisfactorily recovered by the KD concentration method.

3.5 Overall recovery tests

In order to examine the accuracy and the precision of the present method, 7 replicates of the recovery tests were carried out. After spiking 0.1 μg of the targets and the surrogates into 1 liter of organic free water, all procedures were performed according to the Analytical Procedure. *Fig. 1* shows the distribution of overall recoveries. The mean recovery was 92.1 %. The recoveries of 248 compounds which corresponded to 87% of the analytes ranged from 80 to 120%. Polar compounds such as dimethylsulfon, 1,3-benzenediol and phenols showed lower recoveries.

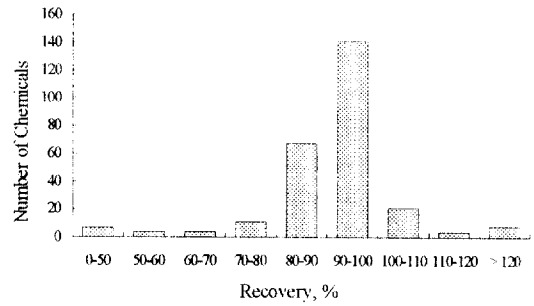

Fig. 1 Distribution of Recovery

Fig. 2 shows the distribution of relative standard deviation (RSD). The mean RSD was 10.8 %. The analytes having less than 10% of RSD were 81% of all the analytes. These results confirm that the present method can determine most of the analytes having a wide range of physicochemical properties with high accuracy and high precision.

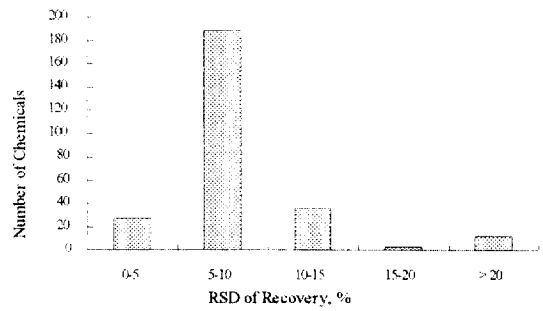

Fig. 2 Distribution of Relative Standard Deviation of Recovery

3.6 Detection Limit

In this study, we obtained two types of detection limits, MDL[13] and IDL. MDL was calculated from results of the overall recovery tests by using the formula:

$$MDL = St(n\text{-}1,\ 1\text{-}\alpha = 0.99)$$

where S = standard deviation of replicate analyses, μg/l; n = number of replicates; $t(n\text{-}1,\ 1\text{-}\alpha = 0.99)$ = Student's t value for the 99% confidence level with $n\text{-}1$ degrees of freedom.

IDL is the concentrations corresponding to S/N = 10. As the mean of IDL is 0.008 μg/l, the present method has enough capability to carry out environment surveys and study the fate of chemicals. On the other hand, MDL is higher reliability in qualification and quantification in exchange for its larger detection limit (mean of MDL = 0.036 μg/l). IDL is suitable for field surveillance because of its high sensitivity. MDL can be used as a regulatory analysis because of its reliability.

3.7 Quality Control

We adopted 3 quality control techniques in the present method; utilizing surrogate compounds, measuring GC/MS performance check standards and maintenance of calibration curves by using a response factor (RF). Surrogate compounds are used to monitor overall method performance. Even if one of their recoveries is insufficient in routine analysis, an analyst has to analyze the sample again. In the present method, because extraction and concentration procedures seemed to the cause of errors, we selected 15 surrogates similar to the targets which consisted of various kinds of chemicals having a wide range of volatility and polarity.

We decided on 14 chemicals to check GC/MS performance. Prior to a GC/MS analysis, GC/MS performance has to be examined by measuring the standards. If the data obtained are not accepted by a criteria, the GC/MS system has to be re-adjusted. By carrying out this check, GC/MS can always maintain constant performance.

Because the present method determine 300 analytes, it takes a very long time to make calibration curves for all the analytes. Therefore, maintenance of the calibration curves has to be efficiently carried out. To achieve easy maintenance of calibration curves, stability of the sensitivity of the GC/MS is a dominant concern. If the sensitivity is not stable, we have to make calibration curves during every analysis. The sensitivity of the GC/MS can be also checked by the GC/MS performance check. Secondly, validation of calibration curves has to be performed in a short time. RF is useful in the validation of calibration curves. However, RF is able to be applied only to the linear calibration range. RF is calculated by the following equation:

$$RF = (As \times Cis)/(Ais \times Cs)$$

where As is a peak area at a quantification ion for an analyte, Ais is a peak area at m/z for an internal standard and Cis is a concentration of the internal standard. Cs is a concentration of analyte. Prior to analysis of real samples, RF is obtained by measuring the middle concentration of the standard in the calibration curve. If the RF is much different from the average RF of the calibration curve, the GC/MS system has to be re-adjusted and/or recalibration has to be done. Fortunately, since the response factors were stable for 1 year, it was not necessary to make recalibration. Therefore, we could carry out both adjusting of the GC/MS conditions and confirming the calibration curves within 3 hours. From our experience, we have confirmed that these methods are suitable for simultaneous analysis of a large number of chemicals.

3.8 Analysis of real samples

We have been using the present method for evaluating the quality of environmental water and tap water. In this report we introduce the results of survey on underground water in Kitakyushu City. We examined 50 wells in winter of 1995. *Table IV* shows the chemicals whose detected frequency were above 20 %. Highest compound was benzothiazole. This compound is used as a vulcanization-accelerator and has been found in seawater

and river water with a high frequency. Other chemicals detected frequently, n-alkanes, polycyclic aromatic hydrocarbons, dichlorobenzene and phthalic acid esters, are known as ubiquitous compounds. Some pesticides, β-HCH, chlordane and isoprothiolane were detected, though these were detected only a couple of wells and their concentrations were less than 1 μg/l.

From the results of the analysis of real samples, we confirmed that the present method has the 6 following advantages over conventional methods. First, the measurement of a large number of chemicals can be performed in a short time. By using this advantage we can easily and comprehensively evaluate environmental pollution due to chemicals. Second is the high identification ability to match both a retention time and a mass spectrum of a sample and a standard chemical. Third is high sensitivity; the present method can measure at ng/l levels. The levels are sufficient to not only carry out an environmental survey on chemicals, but also to study the fate of chemicals detected. Fourth is the high accuracy and precision obtained by using the quality control system. Fifth is the identification of unknown compounds by using a mass spectrum. This is very useful to examine a cause of contingent pollution by chemicals. Mass spectra also provide the sixth advantage; we will be able to determine chemicals, which are not involved in the target list now, by using analytical data. If environmental pollution is occurred by new chemicals in the future, we will be able to determine their concentrations in past samples by using analytical data stored in a computer.

Table IV Chemicals Detected Frequently in Well Water in Kitakyushu City and Range of the Concentrations

Unit : μg/l

Compound	Detected Frequency	Range of Detection
Benzothiazol	96	0.005 - 0.26
BHT	44	0.005 - 0.14
n-C17H36	44	0.010 - 0.091
Squalane	42	0.010 - 0.11
Benz(a)anthracene	40	0.005 - 0.035
n-C16H34	38	0.010 - 0.048
p-Dichlorobenzene	36	0.006 - 0.038
n-C18H38	32	0.010 - 0.039
n-C14H30	32	0.011 - 0.035
Benzo(a)pyrene	32	0.005 - 0.014
Di-n-octyl phthalate	30	0.006 - 0.108
Tris(2-chloroethyl)phosphate	30	0.005 - 0.072
n-C15H32	30	0.010 - 0.032
Chrysene	30	0.005 - 0.022
Triphenylene	30	0.005 - 0.022
Diethyl phthalate	28	0.005 - 0.096
Diheptyl phthalate	26	0.22 - 11
Anthracene	26	0.005 - 0.006

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