

# Development of a comprehensive analysis method using LC-MS for organic components in wastes and related environmental samples

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## Abstract

A new comprehensive analysis method has been developed for organic components in illegal dumping wastes, based on liquid chromatography-mass spectrometry (LC-MS). This study consists of two subjects: one is identification for not-specified substances and another quantitative analysis for specified substances. The 160 objective chemical substances were selected from a viewpoint of the distribution coefficient between water and octanol. Their mass spectra and retention times on LC-MS using an ODS column were stored in the data base for identification of unknown mass spectra. As quantitative analysis, analytical methods have been developed for several representative chemicals known as important hazardous compounds. This paper describes the analytical method for melamine as a typical example.

*Keywords: landfills, illegal dumping waste, hazardous wastes, LC-MS, database, melamine.*



## 1 Introduction

It has been reported that 200,000-400,000 metric tons of waste is illegally discarded a year in Japan, besides 70 percent of these waste being unable to be restored. In general, little is known about the contained chemicals therein and the environmental impact of such uncharacterized waste. A new comprehensive analytical method is developing to clarify organic compounds contained in illegal dumping wastes. In particular, validity of LC-MS is important, since validity of gas chromatography-mass spectrometry (GC-MS) has been established in determination of hazardous compounds such as phthalate esters and alkyl phenols in the leachates from waste landfill sites in Japan [1-3]. However, any information is almost not available regarding high molecular weight compounds, high polarity compounds and non-volatile compounds. In this investigation, a promising method using LC-MS has been applied to many kinds of chemical substances with various characteristics. The 160 objective chemical substances were selected from the substances designated by the Japanese PRTR (Pollutant Release and Transfer Register) law. Also, two kinds of fundamental data, their mass spectra and retention times on LC-MS using ODS column were measured to construct the data base for the analysis. As was previously reported, each of various LC-MS instruments models provided different mass spectra. A reliable LC-MS method, therefore, has been developed to determine tetrabromobisphenol A, hexabromocyclododecane, amitrole, and melamine in aqueous samples.

## 2 Current state of illegal dumping of industrial waste in Japan

According to the Ministry of the Environment, the number of the illegal dumping incidents, the quantity, the kinds and the scales are summarized as follows [4].

### 2.1 Illegal dumping incidents and its total amount

The number of illegal dumping incidents exceeded 1,000 cases a year from 1998 onwards; however, it decreased down to 934 in 2002. The quantity of the wastes reached around 400 thousand metric tons from 1997 to 2000 annually, and then greatly decreased to 240 thousand metric tons in 2001. Nevertheless, it increased again up to 320 thousand metric tons in 2002. Judging from the fact that the total discharge amounts of industrial wastes in 2002 reached as large as about 400 million metric tons, the ratio of the illegal dumping among the total discharge amounts from industrial circles accounts for 0.06%. As shown in Figure 1, majority of the illegal dumping wastes is waste plastics and construction wastes such as waste woods. It was due to the decrease of these two major constituents that the total volume of the waste in 2001 was sharply reduced.



## 2.2 Desirable analytical method

Investigation on the current state of illegal dumping revealed that the quantity of wasted oil and hazardous wastes, for which appropriate countermeasures are urgently demanded, increased despite of overall decrease in the total amount. Moreover, it also disclosed urgent needs of the effective screening method conducive to our judgement whether the targeted waste should be promptly removed or not because of its toxic features. Considering these situation, development of a more comprehensive analytical method is indispensable to determine hazardous chemical substances in the illegal dumping wastes.

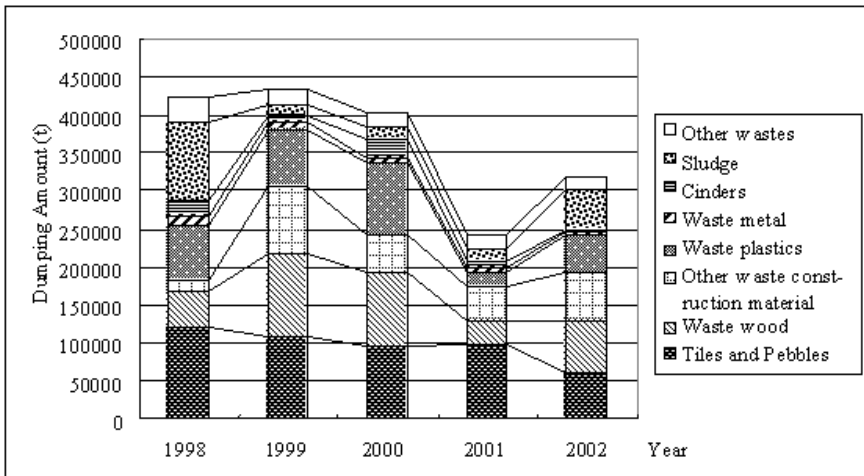


Figure 1: Transition of waste according to type.

## 3 Scheme of examination method, measurement technique, and “comprehensive analytical method” to watch illegal dumping

One of the typical comprehensive analytical methods, SW-846 has been established to characterize the whole of wastes. Based on the relevant law (RCRA: Resource and Recovery Act, 1975), SW-846 has been promulgated as official analytical method for examining "Hazardous waste", consisting of 100 or more individual analytical methods including methods for processing the original samples and their analysis. On the contrary, our attention in this study was mainly focused on development of "Comprehensive analytical method", to get useful data for carrying out appropriate countermeasures against illegal dumping wastes. Upon mapping out the analytical method, we placed our priority on the following three points concretely.





1. It should be composed concisely.
2. The processes from site inspection- classification- sampling- on site analysis- screening analysis at the laboratory through various measurements are described clearly.
3. It can comply with a variety of illegal dumping cases.

Regarding with use of LC-MS, we managed to combine various analytical methods and the unknown mass spectra retrieval systems, whereby we tried to achieve prompt detection of many chemical substances including unknown substances. Figure 2 illustrates the scheme of the proposed "Comprehensive analytical method". Explanatory comments were entered on each process including: site inspection- classification- sampling- on site analysis- screening at the laboratory and various analyses.

Table 1: Analytical conditions for LC-MS data base.

LC-MS	ZMD4000• TSQ7000• API3000• HP1100MSD
Ionization mode	ESI & APCI / positive & negative
Column	SUMIPAX ODS-A (2.0 mm i.d.×150 mm, 5 μm)
Mobile phase	(A) Acetonitrile / (B) H <sub>2</sub> O 1% (A) 3min. – 25min. – 99% (A) 15min. – 2min. – 1% (A) 13min.
Flow rate	0.2 mL / min
Injection volume	5 or 10 μL

#### 4 Construction of the data base for screening

With use of LC-MS, we tried to complete the data base for the purpose of retrieving various chemical substances which were contained in the leachate from waste landfill sites, the soil, and illegal dumping wastes. In this connection, the data base for GC-MS has been used commonly because of its wider applicability. On the other hand, though some data bases for LC-MS are available from each LC-MS manufacturer, their applicability is too limited to be used as the data base to cover the whole areas. One of the significant drawbacks is that mass spectra obtained by LC-MS are dependent on instruments or measurement conditions. This fact seems to decrease the value of the data base. Accordingly, so as to overcome these drawbacks, we commenced investigation on the data base which allows us to make successful retrieval based on both the mass spectrum and the retention time. The data base for LC-MS is quite different from the data base for GC-MS from a point of view that the number of peaks in mass spectrum by LC-MS is extremely a little compared with that by GC-MS. We examined several kinds of LC-MS instruments using the same analytical conditions as long as possible. The targeted compounds were mainly selected from the PRTR substances, including some additional compounds which are considered to be inappropriate to GC-MS analysis. As the result, about 160 compounds were finally selected. Table 1 shows the instruments and analytical conditions which were used to make the data base. The MS condition



was set to a generally recommended value of each instrument. We selected ODS column for the test because it has been used widely, where each column has the same manufacturing lot number from the same factory.

## 5 Development of a determination method for phase III (for detailed state grasp) -development of method for melamine by LC-MS-

The determination methods play a very important role in investigation of the polluted area, together with examining the progress of the remediation work. The determination method for melamine, 2,4,6-triamino-1,3,5-triazine, is shown in Figure 3 as an example. This is mainly used as a raw material for the melamine resin while 70% or more of resin is consumed for the adhesive, and the rest is employed for the cloth, paints, and the resin finishing of paper. Its real consumption quantity reached 111,778 metric ton in 1998 in Japan, whereas the local production amounted to 111,169 metric ton with the import quantity reaching 609 metric ton. The reason why melamine was chosen for the target compound is that melamine is highly soluble in water (0.324 g/100 mL) and is not able to be analyzed by GC-MS at all because of non-volatility and unstableness against heating, indicating that only LC-MS determine melamine without derivatization. As regards the analytical method already known for this compound, trimethylsilyl (TMS)-derivatization GC-MS method has been reported [5] and its detection limit is 0.08 $\mu$ g/L. In the present study, lower or at least comparable detection limit was set as a goal.

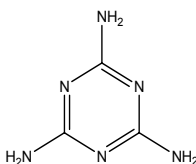


Figure 3: Structural formula of melamine.

### 5.1 Analytical condition of LC-MS

The scan-mode measurement by the infusion method was carried out using 10mg/L standard solution. As the result, protonated molecule ion  $[M+H]^+$  :  $m/z$ 127 was observed in the positive mode on both ESI and APCI. In contrast, however, any specific ion was not detected in the negative mode. Subsequently, each LC-MS parameter was adjusted within tolerance limits in order to find the optimum conditions for determination. In the course of changing the fragmentor voltage from 20 to 200 V, it was found that peak intensity became the maximum at 140V both with ESI and APCI. ESI ion source showed unfavourable stability

despite of higher peak intensity. In case of, APCI, peak intensity was relatively lower than that of ESI, but the working curve was linear; furthermore, stability of the injection repeatability was excellent. Based on these observations, APCI was used in the subsequent studies. Finally, we have established the LC-MS method and its details are shown in Table 2. ODS column could not be used for melamine because its solubility in water was very high. Accordingly, we examined various types of the HPLC column including gel permeation function, the ion exchange function, and the normal phase etc.; however, none was found suitable for melamine except for chiral column using cyclodextrin as its functional group. The well-stabilized retention time was obtained without any pH adjustments. IDL (Instrumental Detection Limit) was calculated to be  $0.016\mu\text{g/L}$  from the standard deviation of the seven replicate measurements using a standard solution.

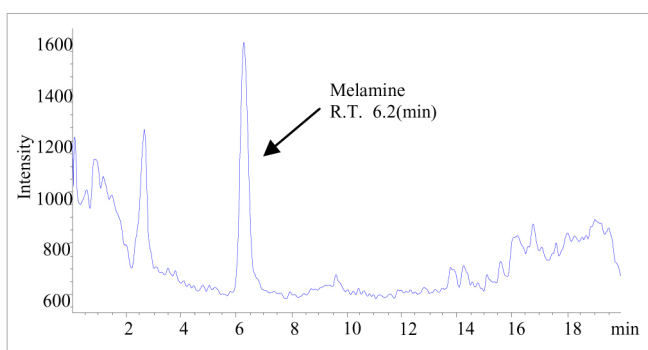


Figure 4: Chromatogram of standard solution (corresponding to  $0.08\mu\text{g/L}$  in sample).

Table 2: Analytical condition for determination of melamine.

Instrument	1100LCMSD SL (Agilent)	
HPLC	Column: SUMICHIRAL OA-7000 5 $\mu\text{m}$ 2mm i.d. 15cm Mobile phase: 50mM Ammonium Acetate /Acetonitrile=20 / 80 Flow: 0.2mL/min Temp: 40°C Inject: 10 $\mu\text{L}$	
MS	Ionization: APCI / Positive Fragmentor: 140V Vaporizer: 320°C Neb. Pres.: 40psig Corona(Positive) current: 6.0 $\mu\text{A}$	Mode: SIM m/z: 127 Gas Temp.: 320°C Drying Gas flow: 8.0L/min VCap(Positive): 3000V

## 5.2 Pretreatment

Target compounds are extracted with a solid phase cartridge. C18 solid phase cartridge (Sep-pak C18) and carbon cartridge (Sep-pak AC-2) are conditioned with methanol 10mL and subsequent pure water 20mL. C18 cartridge is



installed in front of AC-2 cartridge, and sample 250mL is loaded at a ratio of 10mL/min. After loading the sample, C18 cartridge is removed. The target compound adsorbed on AC-2 is eluted with 10mL of 28% ammonium solution / chloroform / methanol (1/1/3). Elution was performed with reverse direction flow (a back flash method). The elute is concentrated under N<sub>2</sub> purge, and is made up to 1mL by addition of acetonitrile; the resultant solution is used as the test solution for LC-MS. The aqueous solution added with standard, whose concentration was 0.2µg/L, was analyzed 7 times according to this pretreatment diagram. MDL (Method Detection Limit) was calculated from the standard deviation of the result of seven replicates. Calculated MDL, average recovery ratio and RSD was 0.020µg/L, 93.4% and 2.5%, respectively. The target compound was not detected at the operational blank.

Table 3: Analysis of the samples related to the waste.

Site/Sample(Sampling date)	Concentration(µg/L)
Operational blank	< 0.02
Y / leacheate(25.Feb.2003)	0.6 *recovery84.9%
U / leacheate (26.Feb.2003)	21
U / Soil No.8(26.Feb.2003)	3.6
U / Soil No.14(26.Feb.2003)	3.1
Y / sediment(25.Feb.2003)	0.9
U / sediment(26.Feb.2003)	3.5

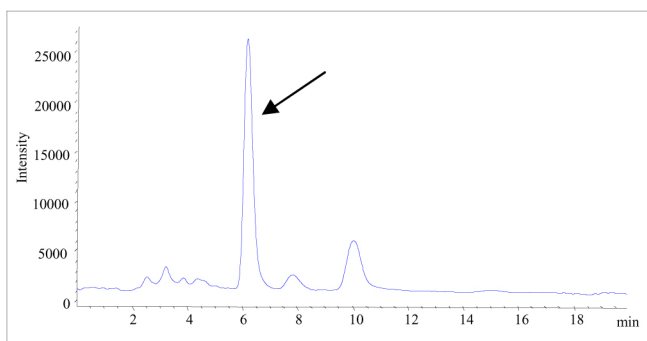


Figure 5: Chromatogram of U/leacheate (26.Feb.2003 Sampling).

### 5.3 Analysis of the samples related to the waste

We applied this analytical method to the samples related to the waste that had been collected by sampling in Japan in 2003. The samples were leacheate, sediment, and soil. For the soil and the sediment, their extracts were prepared with purified water and then subjected to analysis. 25mL sample was used for analysis because the sample volume was extremely small. In such cases, the





detection limit was assumed to be 0.2 $\mu$ g/L. Table 3 shows the results of analysis while the example of chromatogram of a leachate is shown in Figure 5. The recovery ratio in standard addition technique was as high as 84.9%, suggesting that the influence from the background would appear negligible. As the results of determination of the samples related to the waste, melamine was detected from all the samples.

## 6 Conclusion

From investigating current state of illegal disposal, we examined the scheme of the proposed "Comprehensive analytical method". Details are described on each process consisting of site inspection- classification- sampling- on site analysis- screening at the laboratory- various determination analyses. The data base system with generality constructed for identification in this study, where mass spectra and retention times of 160 target compounds obtained by LC-MS were stored, was examined regarding with reliability. A quantitative analytical method using LC-MS has been developed to determine melamine. Calculated MDL was 0.020 $\mu$ g/L, with average recovery ratio and RSD being 93.4%, and 2.5%, respectively. This analytical method was practically applied to the samples related to the wastes that had been collected in 2003 in Japan. The recovery ratio of spiked samples accounted for 84.9%. Analysis of the actual samples related to the wastes revealed that melamine was detected from all the samples.

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