

Research article

Development and Application of a New LC/MS/MS Method for Analysis of Phthalic Acids in Environmental Water and Sediment

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Received: 04-07-2016

Accepted: 06-07-2016

Published: 06-16-2016

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Abstract

Phthalic acids have been known to form from various plastics, which contain alkyl phthalic esters as a plasticizer, after they were disposed into the environment. The limit of detection (LOD) in a newly developed LC/MS/MS method for phthalic acids analysis was 48 ng/L for phthalic acid, 22 ng/L for terephthalic acid and 16 ng/L for isophthalic acid in water samples. The limit of quantitation (LOQ) was 140 µg/kg for phthalic acid, 66 µg/kg for terephthalic acid and 48 µg/kg for isophthalic acid in water samples. These values are comparable to those of conventional gas chromatographic methods. The 12 environmental water samples collected from the moat of the Imperial Palace in Tokyo, Japan, contained phthalic acid at levels ranging from 75 ± 0.86 ng/L to 830 ± 9.6 ng/L. Isophthalic acid was found in only 2 samples and terephthalic acid in only 3 samples. A newly developed method demonstrated that equivalent results to traditional methods could be obtained without the need for tedious derivatization steps in sample preparation, thus providing a significantly simpler procedure than with the most commonly used gas chromatographic method.

Keywords: Phthalates; Phthalic Acid; LC/MS/MS; Environmental Samples

Introduction

Large amounts of phthalic acid esters (phthalates) have been used as plasticizers in the production of numerous plastic products in many countries since the 1930s. In addition to their use as plasticizers, these esters have also been used as a starting chemical to synthesize various phthalates. Consequently, many cases of phthalate contamination, particularly in the environment, have been reported over the years [1, 2]. Therefore, adverse effects caused by phthalates have received much attention by many researchers and there are many research reports on phthalates associated with toxicities and diseases, including mutagenicity [3], teratogenicity [4],

hepatocarcinogenicity [5], cardiac effects [6], reproductive toxicity [7], neurotoxicity, effects to the immune system, breast cancer [8], fetal testicular dysgenesis syndrome [9], and asthma [10], as well as suspected endocrine disruptors [11].

It is proposed that many chemicals, such as phthalic acids, must formed from phthalates after they are deposited in the environment through plastic wastes [12]. The degradation of phthalates by various processes, including microorganisms, enzymes, photo-Fenton, and ozonation [13]. One study demonstrated that di-(2-ethylhexyl) phthalate degrade into phthalic acid and benzoic acid [14]. Also, phthalic acid showed

mutagenicity, developmental toxicity, and reproductive toxicity, which are consistent with the toxic effects caused by phthalate [15]. All these reports suggest that phthalic acids are hazardous environmental contaminants.

There have been many studies of phthalate analysis in various samples, including waste water [13], foods [16], and biological matrices [17]. However, the exact fate of these phthalates in the environment is not yet well understood. We have previously reported that significant quantities of phthalates in water collected near a landfill were removed by the ashes from an incinerator used for waste materials [18]. As a result, phthalates could not be detected in water samples collected from the landfill, which was used to dispose of ashes from a domestic incinerator [19]. It is proposed that phthalates were not detected because they were hydrolyzed into phthalic acids under basic conditions. A large amount of aqueous solution containing slaked lime (calcium hydroxide) had been sprayed on the ashes from the domestic incinerator. Consequently, basic conditions, which were caused by the residual calcium hydroxide, would hydrolyze phthalates into phthalic acids and alkyl alcohols in the environment. There are two isomers of phthalic acid (1,2-benzenedicarboxylic acid). One is isophthalic acid (1,3-benzenedicarboxylic acid), and the other is terephthalic acid (1,4-benzenedicarboxylic acid).

Before LC/MS became available, many methods for phthalic acids analysis using GC had been developed [20]. However, phthalic acids had to be derivatized into more volatile compounds via tedious derivatizing processes to ready them for GC. In the present study, we developed an LC/MS/MS method to determine the presence of phthalic acids without derivatization and subsequently analyzed them in samples from environmental waters and sediments.

Materials and Methods

Twelve water samples were collected from the outer-moat of the Imperial Palace in Tokyo, Japan during the period from January 2008 to September 2009 (dates collected are shown in Table 1). A sediment sample was collected from the Tone Canal, in Chiba Prefecture, Japan on March 4, 2008. The sampling spot is surrounded by various facilities, including business offices, residence houses, schools, and universities. It is expected that large amounts of plastic products in the domestic wastes are washed into the landfill by heavy rains and then are deposited in the sampling spot.

The recovery efficiency of phthalic acids was examined in three sample matrices: 4 μg each of three phthalic acids was spiked into 200 mL of distilled water and 400 mL of moat water and 5 μg of three phthalic acids was spiked into 5 g of river sediment. Distilled water mixed with a 0.5 mL 1M HCl solution and moat water mixed with a 1 mL HCl solution were eluted through a SEP-PAK column and then eluted with 3 mL of methanol. The sample solutions were condensed to 0.6 mL and subsequently diluted with distilled water to 4 mL in volume in order to de-

termine the quantity of each phthalic acid present by using LC/MS/MS. A SEP-PAK column was conditioned with 10 mL each of methanol and water in series prior to use.

Sample No.	Date collected	Phthalic acid	Terephthalic acid	Isophthalic acid
1	01/15/2008	380 \pm 4.4	ND*	32 \pm 0.13
2	02/26/2008	500 \pm 5.7	ND	ND
3	03/25/2008	120 \pm 1.4	ND	ND
4	05/27/2008	560 \pm 6.4	ND	ND
5	07/29/2008	830 \pm 9.6	120 \pm 0.28	52 \pm 0.21
6	08/26/2008	140 \pm 1.6	ND	ND
7	12/18/2008	400 \pm 4.6	ND	ND
8	03/24/2009	200 \pm 2.3	ND	ND
9	04/27/2009	120 \pm 1.4	ND	ND
10	05/26/2009	75 \pm 0.86	ND	ND
11	08/25/2009	240 \pm 2.8	37 \pm 0.09	ND
12	09/29/2009	550 \pm 6.3	90 \pm 0.21	ND

Table 1. Amount of phthalic acids (ng/L) found in samples from the moat

The sediment sample (5 g) was mixed with 10 mL of 1 M HCl and 100 mL of methanol, and then sonicated for 20 min. After the sample solution was filtered under reduced pressure, the filtrate was condensed to approximately 10 mL in volume with a rotary evaporator under reduced pressure. The sample was diluted with 200 mL of distilled water and then 0.5 mL of 1 M HCl was added. The sample was treated with a SEP-PAK column as described above for the water samples, and the resultant sample (1 mL) was diluted to 5 mL with distilled water for analysis of phthalic acids with LC/MS/MS.

The water samples and sediment samples were analyzed for phthalic, isophthalic, and terephthalic acids by LC/MS/MS. The analytical procedures were exactly the same as those used for the recovery efficiency study.

A Prominence HPLC (Shimadzu, Kyoto, Japan) interfaced to an AB SCIEX Model Q-trap MS/MS System (Framingham, MA, USA) was used to analyze the phthalic acids. A 2.1 mm i.d. x 150 mm Inertsil WP300 C18 column (5 μm) was used for HPLC. Mobile phase A was 0.1% formic acid solution, and mobile phase B was methanol. The gradient mode was initially set at A/B ratio of 90/10 from 0 to 20 min and then linearly increased to 10/90. The flow rate was 0.2 mL/min. The column temperature was 40 $^{\circ}\text{C}$ and the injection amount was 10 μL . Ion mode was ESI negative. For the mass spectrometer: First (Q1) and third (Q3) mass numbers were set at $m/z = 164.60$ and $m/z = 121.10$, respectively. Ion spray voltage and cone voltage were 4500 V and 25 V, respectively. Ion source temperature was 600 $^{\circ}\text{C}$.

Results and Discussion

Table 2 shows the results of the recovery tests. The values are mean \pm SD ($n = 5$). In the water samples, the limit of de-

tection (LOD) in a newly developed LC/MS/MS method for phthalic acids analysis was 48 ng/L for phthalic acid, 22 ng/L for terephthalic acid and 16 ng/L for isophthalic acid. The limit of quantitation (LOQ) was 140 ng/kg for phthalic acid, 66 ng/kg for terephthalic acid and 48 ng/kg for isophthalic acid in the present study. These values were consistent with previous reports [21]. In the sediment samples, LOD was 4.8 µg/kg for phthalic acid, 6.6 µg/kg for terephthalic acid and 4.8 µg/kg for isophthalic acid. LOQ was 14 µg/kg for phthalic acid, 2.2 µg/kg for terephthalic acid and 1.6 µg/kg for isophthalic acid.

Compound	Spiked in		
	Distilled water	Moat water	Sediment
Phthalic acid	72 ± 0.83	65 ± 0.75	98 ± 1.1
Terephthalic acid	58 ± 0.13	70 ± 0.16	52 ± 0.12
Isophthalic acid	66 ± 0.27	86 ± 0.35	58 ± 0.24

Table 2. Percent recovery of phthalic acids from three matrices

One molar HCl solution (1 mL) was added to sample solutions (400 mL) prior to SEP-PAK column application because phthalic acids are not absorbed on a solid phase under non-acid conditions [22, 23]. The recovery efficiency ranged from 52 ± 0.12% (terephthalic acid from sediment) to 98 ± 1.1% (phthalic acid from sediment). The results indicate that the recovery efficiencies of the samples were generally satisfactory and it was possible to evaluate data from environmental samples.

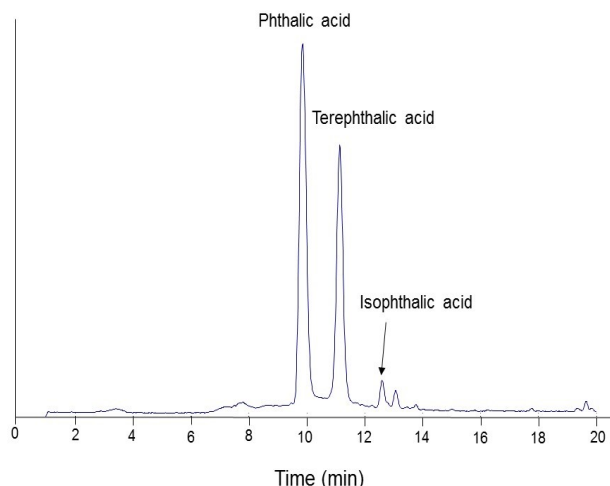


Figure 1. A typical HPLC of a moat water sample collected on July 29, 2008

Under the HPLC conditions described above, retention time of phthalic acid was 9.7 min, terephthalic acid was 10.9 min, and isophthalic acid was 12.5 min.

Table 1 shows the analytical results of samples obtained from the moat waters along with the date collected. The values are mean ± SD (n = 3). Phthalic acid was found in all samples at levels ranging from 75 ± 0.86 ng/L (Sample No. 10) to 830 ±

9.6 ng/L (Sample No. 5). Terephthalic acid was detected in only three samples (Sample No. 5, 11, and 12) and isophthalic acid was found in only two samples (Sample No. 1 and 5). Only one of the 12 samples contained all three phthalic acid isomers (Sample No. 5). The sediment sample collected on March 4, 2008 contained 440 ± 5.1 µg/kg (dry weight) of phthalic acid, 160 ± 0.37 µg/kg of terephthalic acid, and 16 ± 0.066 ng/kg isophthalic acid. These results indicate that the LC/MS/MS method developed in the present study is satisfactory for the analysis of environmental phthalic acids.

Prior to the development of LC/MS, gas chromatography had been used for phthalic acids analysis as mentioned above. However, sample preparation procedures are much more tedious than with the LC/MS method. For example, samples collected from sea and surface waters and sediments were cleaned up with a silicagel or a Sep-pak column and then phthalic acids were derivatized with pentafluorobenzyl bromide (PFBB). The derivatized samples were analyzed for phthalic acids by a GC equipped with an electron capture detector [22]. The recovery efficiency of phthalic acids by this method was 89–101% from water samples and 83–86 % from sediment samples. LOQ was 20 µg/L for phthalic acids and isophthalic acid, and 50 µg/L for terephthalic acid in water samples and 100 µg/kg for phthalic and isophthalic acids and 250 µg/kg for terephthalic acid in sediment samples. The same article reported that the amount of phthalic acid ranged from 45 µg/L to 540 µg/L and from 14 µg/L to 170 µg/L in the environmental water and sediment samples, respectively. A recent article reported that aerosol samples collected from the Pearl River Delta region in China were successfully analyzed for phthalic acids using diazomethane derivatives and GC/MS [24].

PFBB has been most commonly used as a derivatizing agent for phthalic acids analysis by GC. The major drawback of this method is that PFBB is a strong lacrimator and so requires special handling to protect the researcher. In addition, a relatively expensive crown ether has to be used as a solvent for this derivatization reaction. Therefore, a new method for phthalic acids analysis using LC/MS has been a pressing need. In the present study, phthalic acids in environmental waters were satisfactorily analyzed directly using a newly developed LC/MS method. The results were comparable to those of previously reported gas chromatographic methods. Moreover, the preparation processes were significantly simplified.

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