

UPLC-MS/MS Determination of Organophosphorus Flame Retardants and Ultraviolet Absorbers in River Water and STP Effluent Samples

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Abstract—A sensitive, selective and rapid method for the analysis of organophosphorus flame retardants (OPFRs) and ultraviolet absorbers (UVAs) in river water and STP effluent samples by solid-phase extraction (SPE) with ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was developed. It allows the determination of 8 OPFRs and 6 UVAs. A single SPE consisting on the loading 200 ml of sample adjusted to pH 2 on Oasis HLB 60 mg cartridges and elution with methanol and dichloromethane, permitted obtaining good recoveries of higher than 60% for river water and STP effluent, except for TEEedP and UV-328 in STP effluent. The developed method was applied to the determination of target analytes in various samples, including river water and wastewater. Among the tested emerging pollutants, UV-P alone was found in river water at 72.3 ng/l level. In STP effluent, 5 OPFRs (TnBP, TPP, DnBP, DPhP and DEHP) and one UVA (UV-P) were detected with concentrations up to 135 ng/l.

Keywords: emerging pollutants, UVA, OPFR, UPLC-MS/MS, SPE, water samples

INTRODUCTION

Organophosphorus flame retardants (OPFRs) and ultraviolet absorbers (UVAs) are regarded as emerging environmental contaminants and ubiquitously distributed in the aqueous environment as a consequence of human activities and continuous discharge of wastewaters. OPFRs have been used in flame retardants and plasticizers in many products, such as furnitures, textiles, cables, building materials, insulation materials, toys, paints, and electronic appliances, and a variety of plastic products (EFRA, 2006). OPFRs account for approximately 15% of the total amount of flame retardants employed, and their worldwide usage is rapidly increasing. These compounds have been reported as contaminants of indoor environments, of sewage treatment plants (STPs), and of river waters with concentrations ranging from ng/l to several $\mu\text{g/l}$ (Carlsson *et al.*, 1997; Andresen *et al.*, 2004; Bacaloni *et al.*, 2007).

Another group of chemicals, UVAs have been used in order to protect human skin against skin cancer and sunburn caused by direct exposure to the harmful UV radiation from sunlight. These compounds are increasingly used in personal care products, such as sunscreens, cosmetics, lotions, shampoos, hair sprays, lipsticks, etc. (METI, 2006). Japanese government has already banned the production, use, and import of the UV absorber, UV-320 since 2007, because of its persistent, bioaccumulative and toxic properties (METI, 2006). These compounds have been detected in the environment, depending on the type of sample, geographical location, etc. (METI, 2006; Nakata *et al.*, 2009).

Although some concern has arisen on their potential effects, their possible effects on the environment is still quite unknown. In addition, the development of analytical methods for their determination in environmental samples is a matter that still needs much effort.

In recent years, there is a growing need to develop reliable analytical methods, which enable a rapid, sensitive and selective determination of these emerging pollutants in environmental samples, at trace levels. In addition, multi-residue analytical methodologies are becoming the required tools to provide reliable and wider knowledge about the occurrence, partition and ultimate fate of these pollutants in the environment.

In this paper, we described a new method developed for the determination of two compounds (OPFRs and UVAs) in water samples by solid-phase extraction (SPE) with ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). This method represents an attractive alternative to the existing methods due to its simplicity, selectivity and short turnaround time. The method was applied to the analysis of river water and STP effluent.

MATERIALS AND METHOD

Chemicals

TnBP, TEEpP, TPP, DPhP, UV-P, UV-320, UV-326, UV-328 and UV-329 were purchased from Wako (Osaka, Japan). TCP, DnBP, and UV-327 were purchased from Sigma-Aldrich (Ehime, Japan). DBzP, DEHP and TPpP were purchased from Tokyo (Tokyo, Japan).

Individual stock solutions of each compound were prepared in acetonitrile at 1 mg/ml. Standard solutions were freshly prepared for every 3 months and stored at -20°C in amber vials. Working standard mixtures of 1 mg/l were prepared in methanol (MeOH) by dilution from the stocks and stored at -20°C in the dark. These mixed standards were used for fortification in recovery experiments and for preparation of calibration standards.

Sample preparation

Two samples, river water and STP effluent, were collected in amber glass bottles, previously washed with acetone, methanol and ultrapure (Milli-Q) water. Water from Isite River and STP effluent samples were collected at a depth of 0.5

m in October 2009 from Matsuyama, Japan and kept at 4°C. To avoid possible sample alteration, the storage time was no longer than 2 days. Before processing water samples, suspended particulate matter was removed by filtration with a 47 mm diameter GF/F glass fiber filter (Whatman, Maidstone, UK) to avoid plugging of the SPE cartridge.

Sample extraction

SPE of samples was carried out with a Oasis HLB cartridges 60 mg (Waters, Milford, MA, USA). Cartridges were previously conditioned with 5 ml of dichloromethane (DCM), 5 ml of MeOH and 5 ml of Milli-Q water. OPFRs and UVAs were extracted from 200 ml water samples at a flow rate of 10 ml/min, then rinsed the cartridges with 5 ml of Milli-Q water and dried under vacuum for 15 min. Finally, elution of analytes was performed with 2×5 ml of MeOH and 4×5 ml of DCM, and 25 μ l of the IS solution (5 ng/ μ l) were added. The solvent was evaporated to approximately 0.5 ml by a nitrogen stream and made up to a final volume of 1 ml with MeOH. Analyses were performed by injecting 10 μ l of the final extract in to the UPLC-MS/MS system. Quantification of samples was performed using calibration standard in solvent. The relative peak areas were used for quantification purposes.

UPLC-ESI-MS/MS determination

Chromatographic separation was carried out using a Waters Acquit UPLC system coupled to a Quattro Premier triple quadrupole mass spectrometer from Micromass and injection volume was set at 10 μ l. The chromatography was carried out using Milli-Q water (component A) and MeOH (component B) as mobile phase each modified with 0.1% (v/v) formic acid and 10 mM ammonium acetate, respectively. Separation of analytes was carried out in positive mode with 1.8 μ m ZORBAX Extend-C₁₈ 2.1 \times 100 mm column (Agilent) at a flow rate of 0.2 ml/min at 40°C. The operating conditions of the UPLC for positive mode were: 0 min: 20% B; 2.0 min: 20% B; 3.0 min: 95% B; 7.0 min: 95% B; 8.0 min: 100% B; 14.0 min: 100% B. Separation of analytes in negative mode was carried out on a 1.8 μ m ZORBAX Bouns-RP 2.1 \times 100 mm column (Agilent) at a flow rate of 0.2 ml/min at 40°C. The operating conditions of the UPLC for negative mode were: 0 min: 20% B; 1.0 min: 20% B; 2.0 min: 100% B; 6.0 min: 100% B. At the end of the analysis in positive and negative mode, the mobile phase was changed to its initial composition and the column was equilibrated.

RESULTS AND DISCUSSION

MS/MS analysis

To optimize the chromatographic separation, a series of preliminary experiments were performed, using different mobile phases consisting of water and methanol with mobile phase additives, such as ammonium acetate and formic acid. Formic acid and ammonium acetate were found to be the most effective

Table 1. MS/MS optimization conditions for the selected compounds.

Compound	LOD ($\mu\text{g/L}$)	Molecular weight	Precursor (m/z)	Cone voltage (V)	Product ion (m/z)	Collision energy (V)	Retention time (min)
Positive mode							
TnBP	2.3	266.3	267.4	18	99.0	20	4.85
TEEdP	1.3	302.2	303.2	23	173.2	30	4.27
TPP	2.5	326.3	327.2	41	76.8	35	4.67
TCP	1.5	368.4	369.3	45	91.0	45	5.08
UV-P	1.4	225.3	226.4	28	107.0	20	5.55
UV-320	1.5	323.4	324.4	38	56.5	33	9.80
UV-326	2.6	315.8	316.3	30	56.8	26	10.44
UV-327	2.9	357.9	358.3	35	56.8	30	11.23
UV-328	2.4	351.5	352.4	37	70.8	30	10.77
UV-329	2.3	323.4	324.2	38	56.8	27	8.27
Negative mode							
DPhP	2.4	250.2	249.3	37	93.0	30	3.11
DBzP	2.5	278.2	277.2	40	78.8	26	3.17
DnBP	2.2	210.2	209.3	25	78.8	19	3.19
DEHP	2.5	322.4	321.3	38	78.8	26	3.60

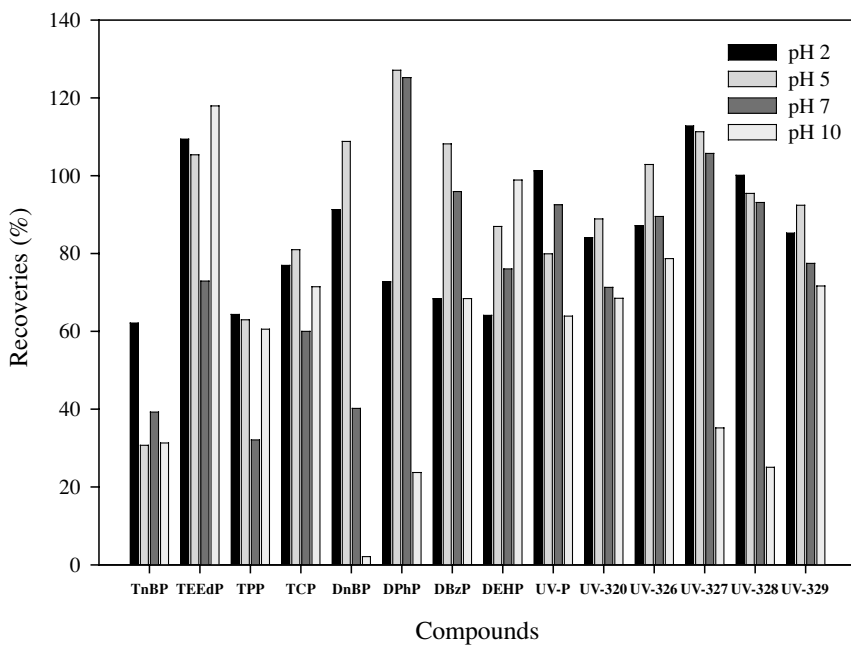


Fig. 1. Recoveries of OPFRs and UVAs at different pH.

Table 2. Recoveries and concentrations of OPFRs and UVAs in river water and STP effluent from Matsuyama, Japan.

	Recovery (%)		River water	STP effluent
	River water	STP effluent	(ng/L)	(ng/L)
TnBP	85.9	91.3	<LOD	61.3
TEEdP	76.8	29.7	<LOD	<LOD
TPP	82.6	69.9	<LOD	25.1
TCP	72.0	82.8	<LOD	<LOD
DnBP	95.5	68.7	<LOD	73.5
DPhP	115	90.0	<LOD	135
DBzP	118	76.8	<LOD	<LOD
DEHP	70.0	84.4	<LOD	29.2
UV-P	101	125	72.30	35.3
UV-320	73.9	60.1	<LOD	<LOD
UV-326	85.8	76.9	<LOD	<LOD
UV-327	84.0	61.1	<LOD	<LOD
UV-328	84.7	50.9	<LOD	<LOD
UV-329	82.7	60.2	<LOD	<LOD

<LOD: Less than limit of detection.

additives for the compounds determined in the positive mode. Although formic acid enhanced the response of some UVAs and neutral OPFRs analytes, ammonium acetate was finally selected because it provides better peak shapes for some UVAs.

MRM parameters (declustering potential, collision energy, etc.) were optimized by infusion experiment at a 10 ml/min flow for each individual compound at 1–500 ng/ml in MeOH/water (1/1). Final operational conditions are summarized in Table 1. The selected precursor ions in ESI positive and ESI negative were either the protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecular ion.

Solid-phase extraction

Optimization of the sample extraction was made with the aim of attaining good recoveries for the wide group of compounds in a single extraction step. To this end, Oasis HLB was the sorbent selected because of its hydrophilic-lipophilic balance, proved versatility and efficiency in the extraction of analytes of a wide range of polarities and acid/base character at different pH values, including neutral pH.

To determine the optimum pH values (pH 2, 5, 7 and 10), 100 ng/ml standard solutions of selected compounds were used. The pH profiles in 200 ml of Milli-Q water samples with 20 ml MeOH and Oasis HLB (60 mg) are shown in Fig. 1. As a result, recoveries for selected compounds were best at acidic pH 2.0. However, when the pH increased, recoveries decreased. This is probably because

the stability at alkaline condition is poor and highly affected at higher pH. For these reasons, pH 2.0 was selected and used in the final method.

To find the optimum extraction solvent, MeOH, DCM and acetone were used. The recoveries of selected compounds in 200 mL of Milli-Q water samples were performed in duplicate. When using 10 mL MeOH after 20 mL DCM good recovery was obtained, 73.7~121% (average 97.0%). Hence, these two were used as the extraction solvents.

Application to real samples

The SPE-UPLC-MS/MS method was applied to the determination of selected compounds in river water and STP effluent samples. Blank values were subtracted and recoveries taken into account for concentration calculations. As presented in Table 2, recoveries were determined at 100 ng/ml concentration level for river water and STP effluent. Recoveries for all compounds except TEEDP and UV-328 in STP effluent were higher than 60%, and were not dependent on the type of aqueous matrix in which they were dissolved. This can be explained by matrix suppression.

UV-P was alone detected in river water, at a concentration of 72.3 ng/l. However, OPFRs and other UVAs were not found in river water. In STP effluent, concentration of 5 OPFRs (TnBP, TPP, DnBP, DPnP and DEHP) and UV-P were quantified in range of 25.1–135 ng/l and 35.3 ng/l, respectively. Although the level of DPnP was relatively higher than other compounds reveals the surveyed area are contaminated by high consumption of DPnP. The rest of the UVAs were not detected in STP effluent. The low UVAs concentrations can be attributed to the little UVAs consumption by the inhabitants of the catchment area. It is noteworthy that, to the best of our knowledge, this is the first data generated on the occurrence of UV-P in the environment in Japan. Though the sample size is meager in this investigation and further extensive study with large number of samples is very much needed to get some clear idea on the contamination status of aquatic environment by OPFRs and UVAs.

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