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Determination of Pharmaceutical Pollutants in River Environment by the SPE-LC-MS/MS Method: A Mini Review

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Abstract

Recently, new pollution problems due to the presence of pharmaceuticals in the water environment have been reported, and research aimed at understanding the status of this pollution and assessing and dealing with the associated environmental risks is receiving worldwide attention. Because their concentrations in the water environment are quite low (roughly in the range from ng/L to μg/L), a method of highly sensitive and highly selective multicomponent simultaneous analysis is indispensable for detecting and quantifying these products. Here, I summarize the findings of previous research and case study analyses to date of an analytical method that combines solid phase extraction (SPE) and liquid chromatography – tandem mass spectrometry (LC-MS/MS); this is a versatile and reliable method of detecting and quantifying pharmaceutical pollutants in river water and wastewater samples. I also discuss the prospects for environmental analysis techniques.

Keywords: Pharmaceuticals; River environment; Sewage treatment plant; Environmental analysis

Introduction

A newly emerging pollution problem in the water environment—the detection of pharmaceuticals and personal care products (PPCPs) in the waters of rivers, lakes, and marshes and in drinking water sources—has recently begun to receive a large amount of attention. Because PPCPs are comprised of a diverse chemical compounds, in this review we focused on pharmaceuticals that were targeted as environmental water pollutants (pharmaceutical pollutants). Generally their concentrations are low (roughly in the range from ng/L to μg/L) worldwide [1-3]. Even in this low concentration range, however, there have been reports of the endocrine-disrupting chemicals these products contain having serious environmental impacts, such as feminization of males in fishes [4,5]. Pharmaceuticals are designed to have specific physiological effects on targeted areas of the body, so that even at low concentrations, concern is rising about their toxic effects on ecosystems and their impacts on human health via residues in drinking water [1,6]. Detection of pharmaceutical pollutants in the water environment is mainly derived from their biotic and abiotic recalcitrance after discharge into the environment [1,2].

Pharmaceutical pollutants in the wastewater are transferred by sewerage systems to sewage treatment plants. However, because they are highly water soluble to make them easily discharged from the body and retained in the soluble state unattached on the sediments, they tend not to be removed during the traditional sewage treatment process, which is centered on biological treatment. Therefore, collected pharmaceutical pollutants are released into rivers without sufficient treatment [7,8]. Water discharged from sewage treatment plants is a major source of their pollutant loads in river basins [9,10].

The history of pollution of water environments by recalcitrant pharmaceuticals are long and goes back to the 1970s [11,12]. At the time, for example, there was a report of the detection of the lipid-lowering agent clofibric acid [11] in the discharge water from a sewage treatment plant in the United States. The antipyretic analgesic agent salicylic acid, along with clofibric acid, was also detected in the discharge water from a sewage treatment plant in Germany [12]. Neither of these discoveries led to the establishment of research programs. Nevertheless, worldwide interest in this environmental pollution problem increased between the 1990s and 2000s, and an abundance of research targeting a wide range of their components covering more than 100 components of antibacterial, psychotropic, X-ray contrast, antiseptic, antihypertensive, lipid-lowering, and antipyretic analgesic was launched worldwide. The scope of these research projects extended from research aimed at understanding the status of pharmaceuticals pollution [2,3] and their behavior in river environments [13,14] to research aimed at understanding the behavior and removal of pharmaceutical pollutants at sewage treatment plants [15, 16], developing water treatment technologies to effectively remove [17,18] or performing ecotoxicity impact assessments [19,20]. Figure 1 summarizes the concentration of each pharmaceutical pollutant detected in treated water discharged from sewage treatment plants into rivers [9,21,22].

The increase in worldwide interest in the problem of pharmaceuticals-related water pollution is due partially to an increase in social interest in environmental problems. However, a large part is also due to the remarkable recent development and improvement of analytical equipment and technology [23,24]. In particular, the distribution of SPE-LC-MS/MS, which combines solid phase extraction (SPE) and liquid chromatography – tandem mass spectrometry (LC-MS/MS), and is capable of highly sensitive and highly selective simultaneous detection and quantification, helped to reveal the presence of pollutants in trace concentrations (in the range of ng/L to μg/L) that had been difficult to detect by conventional analytical methods [23,24]. Here, we summarized the research reports that have used SPE, LC-MS, and LC-MS/MS to analyze pharmaceutical pollutants and discuss the future development of environmental analysis techniques.

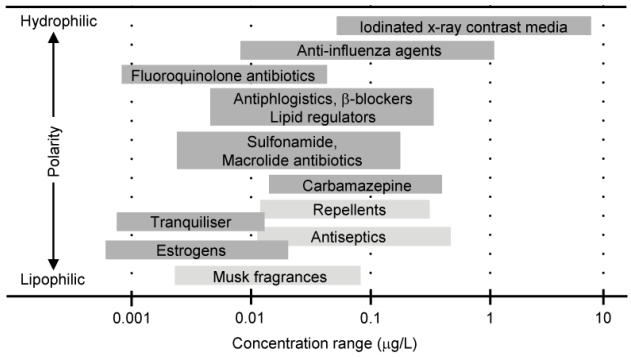

Figure 1: Ranges of measured concentration of pharmaceuticals (dark grey boxes) and personal care products (light grey boxes) in treated municipal wastewater [9,21,22].

Analytical Methods

A highly sensitive and highly selective analytical method is indispensable for accurate analysis of the pharmaceutical pollutants coexisting in trace concentrations in river and sewage water along with many other substances [23-25]. Combining the sample preparation process of SPE, which concentrates and purifies water samples, with chromatography and MS enables the applied analytical method highly sensitive. Therefore, selection of the type of SPE cartridge, the amount of adsorbent, the elution solvent and its operation condition is a prerequisite key to the method invaluable. After pretreatment with the appropriate SPE, the recovered pharmaceutical pollutants are separated by liquid chromatography (LC) and identified and quantified by tandem mass spectrometry (MS/MS). For analysis of pharmaceutical pollutants which are highly polar, nonvolatile, and thermally unstable, adequateness of use of the combined methods of LC-MS/MS [23,25,26] and LC/MS rather than gas chromatography (GC) and GC/MS is well known. Figure 2 summarizes the characteristics of the LC-MS(/MS) technique [27,28]. A resultant SPE-LC-MS/MS system has become an extremely versatile analytical method that is used not only analysis of pharmaceutical pollutants [7,23,24] including endocrine disrupting chemical residues [29] in the water environment, but also in the food [30] and pharmaceuticals [31] fields. Below, we summarized the combined SPE-LC-MS/MS system for analysis of the pharmaceutical pollutants in the environment.

Solid phase extraction

To analyze pharmaceutical pollutants coexisting in river and sewage water samples along with many other substances in concentrations in the range from ng/L to μg/L, samples need to be concentrated to the level of μg/L, which is the concentration detection limit of LC-MS and LC-MS/MS [26, 32]. In addition, even though LC-MS(/MS) has high sensitivity and superior selectivity, impurities in the samples can interfere with the ionization of the target substances and thus drastically lower the sensitivity and accuracy of the analytical method [29,33]. Therefore, it is highly important to clean up impurities from the samples during preparation.

SPE is a sample preparation process based on the separation mechanism of LC. In SPE, a sample solution is passed through a column containing selective stationary adsorbent. The target substance is retained in the adsorbent, the impurities are washed and removed, and the target substance is eluted from the adsorbent with a solvent and collected [25,34]. SPE is a superior method that is not only simple and fast but also uses a very small amount of solvent for extraction, has superior purification and reproducibility, and can process many samples simultaneously and more efficiently than with liquid phase extraction, which is a liquid-liquid separation method that uses organic solvents to extract target components [25,34].

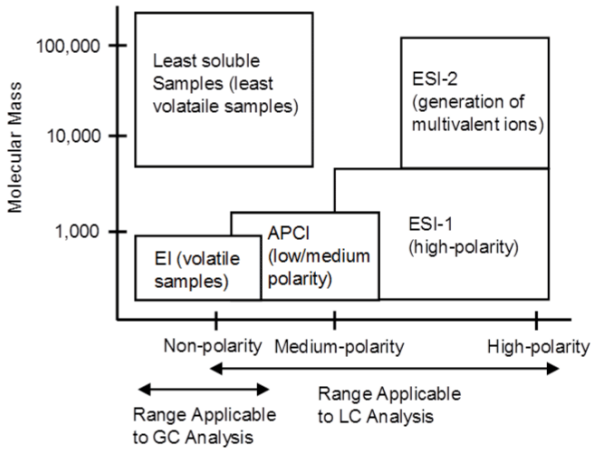

Figure 2: Ionization methods and applicable compounds [27,28]. (EI, electron ionization; APCI, atmospheric pressure chemical ionization; and ESI, electrospray ionization)

A wide variety of materials are used as the SPE stationary phase, beginning with the C_{18} stationary phase [35,36] and a stationary phase with ion exchange groups[25,35]. We presented a typical use of the SPE for simultaneous determination of four representative anti-influenza drugs, oseltamivir phosphate, oceltamivir carboxylate, zanamivir and amantadine by combination of strong-cationic SPE cartridge and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [9]. Special care must be taken for selection of the adsorbent to make the recovery rate higher because of the difference in adsorption-desorption profiles even if the same type ion-exchanger were used [41].

In research into general pharmaceutical pollutants in the water environment, Oasis HLB (Waters, Milford, MA, USA) is the stationary phase most frequently used in sample preparation [3,37,38]. Oasis HLB is a polymer sorbent; the stationary phase is a divinylbenzene-N-vinylpyrrolidone copolymer in which hydrophilic and lipophilic polymers are polymerized in a fine balance [38]. This property of the Oasis HLB makes it possible, in a single operation, to simultaneously analyze a wide variety of pharmaceutical pollutants that have not only combined lipophilic and hydrophilic properties but also a wide range of physicochemical qualities that are difficult to analyze simultaneously with C_{18} or other stationary phases based on only one type of interaction [38,39]. To perform an efficient SPE with a satisfactory rate of collection of the target substance it is indispensable to optimize the extraction conditions. Therefore, it is important to optimize the pH of the water sample loaded onto the stationary phase [34,38,40] and the volume of the sample loaded onto the stationary phase [35,40,41]. It is also essential to clean up impurities [25,38,41] and choose an eluent well suited to elution of the target component

[34,40,41]. After the completion of SPE sample preparation, the processed sample is measured by either LC-MS or LC-MS/MS.

An application of online SPE-LC-MS was recently introduced in the field of food science and technology by Willenberg et al. [42]. In this system importance of the selection of the SPE column and the optimization of online-SPE procedure regarding extraction efficacy, reduction of ion suppressing matrix and carry over was also noted by Willenberg et al. [42] in addition to the case for analysis of human exposure to triclocarban [43].

Liquid chromatography – mass spectrometry and liquid chromatography – tandem mass spectrometry

In LC-MS, the target component is separated out during the LC part by using the difference in the target component's affinities for the stationary and mobile phases. The target component is then loaded onto the MS part along with the mobile phase and fragmented under atmospheric pressure, after which the fragment ions are measured by the detector for qualification and quantification of the target component [23,26].

In contrast, in LC-MS/MS, in which the MS parts are connected in tandem mode, the first MS selects precursor ions, which are then further selected according to their mass-to-charge ratios (m/z). The further selected ions are fragmented by collision energy, and the fragment ions of the resulting product are measured by the second MS [23,26]. Thus the ions are selected twice by the MS/MS, allowing it to scan and analyze specific pairs of parent and daughter ions for each compound. This enables multiple reaction monitoring (MRM) with much higher analytical sensitivity and selectivity than with LC-MS [42, 43]. Electrospray ionization, which is a comparatively soft ionization technique, is used in the MS part of the detector [43,44]; a tandem quadrupole type or ion-trap type is usually used to separate the ions [45, 46]. In addition, in recent years, to shorten measurement times and increase efficiency, ultra-performance liquid chromatography (UPLC) [42,45,46] has become the major LC method used. It uses a column with a much smaller particle diameter (1.7 μm) than that used in conventional LC (3 to 5 μm) and has superior pressure resistance.

In measurement using LC-MS/MS, optimization of the measurement conditions of the MS part is crucial. For this purpose, the first MS part performs an MS scan and determines the m/z of the parent ions and the cone voltage at which the peak intensity of the parent ions is highest. Next, a daughter scan is performed to determine the m/z of the daughter ions, and the collision energy is optimized so that it results in the highest peak intensity of the daughter ions. Finally, the combination at which the peak intensity for both parent ions and daughter ions is highest is determined for each component [44,47]. As a result of this procedure, a single MRM analysis can simultaneously analyze close to 100 components [48,49]. To optimize the LC part, the column that best suits the separation of the target component is chosen and the gradient conditions are optimized [42,43]. After optimization of the measurement conditions is completed, the samples are detected and quantified under these conditions.

Further instrumental development is now progress such as an online-SPE-LC-MS system [42,47]. Joining of the method presented above with such an innovative development would be useful in future for the evaluation of wide-spread PPCPs and pharmaceuticals including pollutants, suggesting the potential use for environmental evaluation needs and possible risk management.

Conclusions

Here, I have presented a literature review of the use of the highly sensitive and highly selective analytical method SPE-LC/MS/MS to detect pharmaceuticals, which are causing new problems with environmental pollution. SPE-LC/MS/MS is the main method used to assess the status of this pollution. I have also organized and clarified the knowledge accumulated in published analytical cases and discussed the future prospects of this environmental analysis technique.

Research into pharmaceutical pollutants in the water environment by using SPE-LC-MS/MS is progressing day by day. Recently, analyses using this method have begun to reveal that among these pharmaceutical pollutantss are antibacterial agents that could intensify the development of drug-resistant bacteria in the water environment [8]. Anti-influenza drugs in the water environment could foster the development of drug-resistant viruses carried by wild waterfowl and their spread to humans [22]. There is also the issue of the detection of narcotics and stimulants [48-50] in the water environment.

Research that aims to automate and mechanize the series of operations needed to perform SPE and LC-MS/MS is also being attempted [45,51,52]. Further future development of SPE-LC-MS/MS analytical techniques joining with online SPE-LC-MS and such a kind of innovative technology is expected to promote more detailed assessment of the environmental risks posed by wide range pollutants derived from PPCPs and pharmaceuticals.

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