22

Determination of Phenolic Compounds in Water

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22.1 Introduction

Phenolic compounds are ubiquitous in the environment, coming from different sources such as manufacturing processes used in the plastic, dye, drug, antioxidant, and pesticide industries. Chloro- and nitrophenols (NTPs) are the main degradation products of many chlorinated phenoxy acid and organophosphorus pesticides, respectively [1,2]. These compounds are of particular interest and concern to the environment because they are toxic to most aquatic organisms [3,4]. Moreover, they affect the taste and odor of both water and fish even at very low concentrations of phenolic compounds in water [5]. The U.S. Environmental Protection Agency (EPA) has listed 11 phenols as priority pollutants [6]. European Community (EC) legislation states that the maximum admissible concentration of phenols in water intended for human consumption is less than 0.5 μg/L for the total content and 0.1 μg/L for the individual compounds [7], while in bathing water, the maximum admissible value is 5 μg/L [8]. To evaluate the risks these compounds pose, a rapid and reliable process for their determination is therefore necessary.

Since the nature of these chemicals in water varies from polar compounds such as phenol to very non-polar compounds such as pentachlorophenol (PCP), it is a challenge to analytical chemists to determine
them collectively. The conventional analytical methods for these compounds are often extensive as they require numerous analytical steps to obtain significant results. The first and also one of the most important requirements is to find a suitable sample preparation technique that allows the separation of the substances of interest from the sample matrix. The analysis of phenols in water is normed by EPA Method 625 [9]. The main disadvantage of this time-consuming and cost-intensive method is the large sample volume required for the extraction and use of large volumes of toxic organic solvents. Therefore, current developments in the field of sample preparation aim for fast and low-cost treatment of environmental samples.

In line with this, this chapter seeks to give an overview of the major modern analytical techniques that can be applied to the analysis of phenolic compounds in environmental water samples. The chapter will preview the various chromatographic approaches for the determination of phenolic compounds in water. As sample preparation is crucial for trace-level determinations, the major sample preparation techniques for phenolic compounds will also be overviewed.

### 22.2 Classification and Chemical Characterization

Phenols are very heterogeneous group of compounds with varying chemical and physical properties. They are classified based on different kinds of substituents present on the aromatic ring of the phenolic moiety. Accordingly, there are alkyl-, chloro-, hydroxyl-, and nitrophenolic compounds belonging to this class of organic chemicals. Most of the substituted phenols are used or formed in different industrial processes. On the other hand, some phenols are used in pharmacopoeia, as for example, 4-acetamidophenol, widely used under the name of paracetamol, present in many drug formulations [10].

All phenols have weak acidic properties. Chlorophenols (CPs) are among the hardly biodegradable phenols and are difficult to remove from the environment—the half-life in water can reach 3.5 months in aerobic waters for PCP and for several years in organic sediments [11,12].

The determination of CPs in water has been studied extensively and they are most likely to be the group of phenols responsible for the largest impact on our aquatic environment. CPs vary from mono-substituted CPs to PCP. The PCP is considered to be the highest-priority pollutant within the phenolic group. The U.S. EPA listed 11 phenolic compounds as priority pollutants among different classes of these chemicals [12]. European Community directive 75/440/EEC states that the maximum levels of phenolic compounds in surface water for drinking purposes should lie within the range of $1^{-10}$ μg/L, depending on the required treatment [13].

### 22.3 EPA Methods and Other Official Methods

A large number of analytical methods found in the literature addressing the determination of phenolic compounds in water focus on the 11 priority pollutants of the phenolic compounds. Table 22.1 shows the 11 priority pollutant phenolic compounds.

The official methods for the determination of these compounds are based on liquid–liquid extraction (LLE) followed by separation with gas chromatography (GC). No official methods exist based on liquid chromatography (LC). The EPA Method 604 involves a serial extraction of an acidified sample with dichloromethane (DCM) [14]. An alternative description is found as Method 6420 in “standard methods” [15]. The extract is dried and the solvent is exchanged to 2-propanol. The phenols are then determined by GC using a packed column and flame ionization detection (GC/FID). The method also provides a derivatization procedure with pentafluorobenzyl bromide and column chromatographic cleanup followed by GC determination using electron capture detection (GC/ECD). This lowers the method detection limit (MDL) for some of the compounds. The MDL values are in the range of 0.14 (phenol) to 16 μg/L (4-methyl-4,6-dichlorophenol) for different compounds and the two GC procedures. An equivalent EPA method is 8040A [16].

Alternatively, EPA methods 625 [14], 6410 [15], and 8250A [16] for extractable bases/neutrals and acids can also be used for the determination of phenols in water samples. These methods are also based
on a serial extraction with DCM, first at pH > 11 and then at pH < 2. After drying the extract, the phenols are determined by GC using a packed column and mass spectrometry (GC/MS) detection. The MDL values for the above-mentioned methods are approximately 2 times larger than the values for Method 604. The alternative GC/MS methods using capillary columns are 1625C [14] and 8270B [16] and these methods are also applicable to soil and sludge. In these method descriptions, no MDL values are given. Method 1653 [17] provides the conditions for acetylation of the phenols with hexane before extraction and GC/MS determination. For the latter method, the detection limits are in the range of 0.15 μg/L (2,4-dichlorophenol [2,4-DCP]) to 0.71 μg/L (2,4,6-trichlorophenol [2,4,6-TCP]). Only a few of the compounds listed in Table 22.1 are covered here, but this technique provides MDL values using GC/MS in the same order of magnitude as with GC–ECD. The ISO methods 8165–1:1992 and 8165–2 [18] are generally equivalent to the aforementioned EPA methods.

These methods for the determination of phenols in water samples are regarded by many analysts as very time consuming and labor intensive, with many extraction and solvent-exchange steps. Also, the use of hazardous chlorinated solvents is regarded as a limitation of these methods, as DCM will be or is already forbidden for use in many countries. To overcome these limitations, EPA Method 528 recommends the use of solid-phase extraction (SPE) and capillary column GC/MS for the determination of phenols in drinking water.

According to this method, analytes and surrogates are extracted by passing a 1 L water sample through an SPE cartridge containing 0.5 g of a modified poly(styrene–divinylbenzene) copolymer. The organic compounds are eluted from the solid phase with a small quantity of methylene chloride. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high-resolution fused silica capillary column of a GC/MS system. Compounds eluting from the GC column are identified by comparing their measured mass spectra and retention times to the reference spectra and retention times in a database. The reference spectra and retention times for analytes are obtained by the measurement of calibration standards under the same conditions used for samples. The concentration of each identified component is measured by relating the MS response of the quantification ions produced by that compound to the MS response of the quantification ions produced by a compound that is used as an internal standard. Surrogate analytes, whose concentrations are known in every sample, are measured with the same internal standard calibration procedure [19].

Another method based on liquid–solid extraction is the extraction of PCP, which together with other organic compounds, can be measured by GC/ECD (Method 515.2) or GC/MS (Method 525.2) [20]. For these methods, the MDL values for PCP are given as 0.16 μg/L (GC/ECD) and 0.72–1.0 μg/L (GC/MS). A method involving the direct injection of water samples into a GC column is described in ASTM standard D2580 [21]. This method obviously bypasses the use of solvents for extraction, but the lowest concentration for which this method can be used is 1 mg/L, which is considerably larger when compared with the extraction methods already mentioned.

<table>
<thead>
<tr>
<th>Analyte Priority Pollutant Phenols</th>
<th>CAS Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>108-95-2</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>95-57-8</td>
</tr>
<tr>
<td>2-Methylphenol</td>
<td>95-48-7</td>
</tr>
<tr>
<td>2-Nitrophenol</td>
<td>88-75-5</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>105-67-9</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>120-83-2</td>
</tr>
<tr>
<td>4-Chloro-3-methylphenol</td>
<td>59-50-7</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>88-06-2</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>51-28-5</td>
</tr>
<tr>
<td>4-Nitrophenol</td>
<td>93951-79-2</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>87-86-5</td>
</tr>
</tbody>
</table>
The total content of phenols in natural waters and wastewaters (WWs) can be determined by using the 4-aminoantipyrine (4-AAP) colorimetric procedure. There are essentially many descriptions of the same method: EPA Methods 420 [22] and 9065 [16], standard methods 5530 [15], ASTM D1783-91 [21], and ISO 6439:1990 [18]. The different procedures involving, for example, chloroform extraction and distillation, in some cases using automated flow injection analysis (FIA), are described in these standards. Another reagent, 3-methyl-2-benzothiazolinone hydrazone (MBTH), is also used for the same purpose in EPA Method 9067 [16]. The 4-AAP reacts with phenol and ortho- and metasubstituted phenols and, under proper pH conditions, also with phenols with an alkyl, aryl, nitro, benzyl, nitroso, or aldehyde group. The methods can be used in the low micrograms per liter range. However, they cannot differentiate between the different substituted phenols and thus, they give the total phenol content (or phenol index) provided that the phenols present react with the reagent. Owing to this low specificity, detailed descriptions of these methods are beyond the scope of this chapter.

### 22.4 Liquid Chromatographic Determination of Phenolic Compounds in Water

High-performance liquid chromatography (HPLC) is the most suitable technique to determine phenolic compounds in water using ultraviolet (UV) or diode-array detection (DAD) [23–29] or using electrochemical detection (ECD) [30–32] but, although amperometric detection is more sensitive than UV detection, a preconcentration step is necessary in both cases to achieve the low levels allowed in real samples. Nowadays, LC is often the choice over GC, as it is more suitable for aqueous samples and as no derivatization step is needed for phenolic compounds. The online connection between SPE and the HPLC column is fairly straightforward; this approach appears very suitable for the analysis of these compounds. However, the conventional UV detector is much less sensitive than most of the GC detectors. This has promoted the search for more sensitive HPLC detection devices as well as improvements and alternative methods in sample preparation. Several detectors, more or less sensitive toward phenolic compounds are used today in the liquid chromatographic determination of phenolic compounds in water: UV, DAD, electrochemical, fluorescence, and MS detections. Their advantages and disadvantages together with some applications are summarized later.

The separation of phenols with LC is normally carried out with reversed-phase liquid chromatography (RPLC). The mobile phase consists of a mixture of a polar organic solvent (methanol or acetonitrile) and an aqueous buffer, and in most cases, different types of hydrophobically modified silica, C$_{18}$, or C$_{8}$ columns are used as analytical columns.

The separation and retention of 29 phenolic and related compounds on different RPLC columns has been investigated by Marko-Varga and Barceló [33]. The columns studied were LiChrospher 100, PLRP-S, Vydac, and Hypercarb. Also, the effects of various acetonitrile/buffer mixtures and the pH of the mobile phase on the retention and also separation of the phenolic compounds on the different columns were evaluated. For this application, it was found that the silica C$_{18}$ column (LiChrospher 100) gave the best separation, probably due to a mixed retention mechanism.

Nitro- and chlorophenols were monitored at eight sampling sites along the coast of Pieria province (northern Greece) [34], from October 2003 to September 2004. The target compounds were 2-nitropheno, 4-nitropheno (4-NP), 2,4-dinitropheno, 2-chloropheno (2-CP), 3-chloropheno, 2,4-DCP, and PCP. SPE followed by HPLC was used for the determination of the compounds. Among the selected phenolic compounds, the most frequently detected compound was PCP. The maximum concentration for PCP was 8.04 μg/L, and for 2,4-DCP, the maximum concentration was 6.11 μg/L. The geometric mean concentrations for PCP during the 12-month survey ranged from nondetectable to 1.06 mg/L.

#### 22.4.1 Liquid Chromatography with Ultraviolet and Diode-Array Detections

Most phenols are relatively polar and their separation may be carried out using LC with UV detection at 280 nm [35–38]. However, UV detection, besides being nonspecific for phenols, has detection limits that do not permit a direct analysis in water matrices [39]. Despite the fact that it is inferior to other detectors,
it is frequently used for phenolic determination [40–43], often together with an SPE sample preparation step (for more discussion, see Section 22.8).

DAD, where a large span of wavelengths are monitored at the same time, has also been frequently used for the determination of phenols in water in combination with an SPE sample work-up step [44–49]. Moving from UV detection at a single wavelength to DAD, a small sacrifice in sensitivity is made up for a much better peak identification with the available spectral libraries to confirm the presence of analytes.

LC with DAD was used together with online SPE for the determination of phenolic compounds in the river Meuse [49]. Sample volumes of 10 mL gave detection limits of below 0.1 μg/L for phenol and m-cresol in surface water.

Sharma et al. [50] developed a method consisting of SPE of phenol and CPs, their derivatization to methyl ethers, headspace single-drop microextraction (HS-SDME) of methyl ethers using 1-butanol as the extraction solvent, and direct transfer of the drop into the injector for HPLC–DAD.

Dynamic liquid–liquid–liquid microextraction (LLLME) coupled with ion-pair liquid chromatography (IP-LC) and photodiode array detection was developed and used for the extraction and analysis of chlorinated phenoxyacetic acids (CPAs) and CPs from water samples [51]. The applicability of this newly developed method was illustrated by the determinations of CPAs and CPs in environmental water samples.

LLLME was utilized to extract CPs from water [52]. The extracted CPs, present in anionic form, were then separated, identified, and quantified by ion-pair high-performance liquid chromatography with photodiode array detection (HPLC/DAD).

### 22.4.2 Liquid Chromatography with Electrochemical Detection

As phenols are electrochemically reactive on carbon electrodes, liquid chromatography coupled with electrochemical detection (LC–ECD) can provide a more selective and sensitive analysis [24,53–55]. A further increase in sensitivity can be obtained by using a preconcentration technique such as SPE [56–58]. Several different modes of ECD have been used, with amperometric detection [5,53,55,59–62] being the one most frequently employed. Coulometric detection [63,64] has also been used (for a summary of different ECD of phenols in water, see Table 22.2).

The coulometric detector converts 100% of the analyte since the oxidation of phenols occurs in the high-porosity electrode, whereas an amperometric detector normally converts only about 10% at the electrode surface [68]. Amperometric detection is used in conjunction with HPLC separation with a glassy carbon working electrode at an oxidizing potential around +1000 mV versus Ag/AgCl reference electrode. However, this type of ECD exhibits the problem of phenols fouling the electrode. The problem can be partly solved by cleaning the electrode using two additional pulses: one oxidizing pulse and one reducing pulse between each measurement pulse (pulsed amperometric detection).

LC with different modes of amperometric detection has been frequently used for the determination of phenols in water, with or without a preconcentration step. Without preconcentration, amperometric detection at +1150 mV versus Ag/AgCl was tested with six different RPLC columns [62]. Of the studied columns, the Spherisorb C₈ gave the best chromatographic behavior of the 11 tested phenols.

Pulsed amperometric detection using a glassy carbon electrode (+1200 mV vs. Ag/AgCl) in combination with online SPE with C₁₈ material has been used for the determination of the 11 priority pollutant phenols at submicrogram per liter levels [5]. Another application where pulsed amperometric detection has proved to be successful is for phenols in seawater [59]. After passing 1000 mL of seawater through a polymeric SPE material and detecting at +1.25 V versus Ag/AgCl, the phenols could be quantified at nanogram per liter levels.

The use of multielectrode ECD in combination with SPE using C₁₈ material and HPLC separation was described for the identification of 27 phenolic compounds in water samples [64]. The multielectrode consisted of four coulometric array cells, each containing four electrochemical detector cells. These employed porous graphite-working sensors with palladium as the reference and counter electrodes were arranged in series after the analytical column. Tap water and mineral water were analyzed; the authors reported very low detection limits for the phenols.
Dual coulometric detection was used with online SPE with LiChrolut EN [63] for the determination of polar priority phenols at nanogram per liter levels. The first electrode was intended for sample cleanup (normally set at a low potential), and the detection of the phenols was made at the second electrode.

For the determination of phenols in seawater after enrichment using SPE cartridges and disks, the HPLC detection was performed using a large-surface-area colorimetric electrode at +750 mV versus the Pd reference electrode [30].

Furthermore, biosensors have been used for the ECD of phenols in combination with FIA and HPLC separation [69,70]. The biosensors are normally working at a much lower potential, and they are also very analyte specific, since several enzymatic steps may be involved in the detection. Normally, the enzyme is immobilized onto solid graphite electrodes or in carbon paste electrodes.

### 22.4.3 Liquid Chromatography with Fluorescence Detection

LC in conjunction with fluorescence detection has been used to improve the sensitivity and selectivity for the determination of phenolic compounds in water. All the different techniques for the determination of phenolic compounds in water that use HPLC and fluorescence detection are summarized in Table 22.3.
Determination of Phenolic Compounds in Water

Precolumn dansylation with dansyl chloride in combination with postcolumn photolysis has been described in a couple of papers [73,74]. In one of the papers, peroxyoxalate chemiluminescence detection was also used, yielding detection limits as low as 0.01–0.1 μg/L for several phenols in surface water [73]. Either way, the phenolic anions are extracted as an ion pair with tetrabutylammonium into an organic phase containing dansyl chloride. Precolumn derivatization with 2-(9-anthrylethyl) chloroformate has been described for the determination of phenols in industrial WW [72].

A postcolumn reaction with 4-AAP and potassium ferricyanide has been used in combination with SPE sample preparation for phenols in WW [76]. The reagent 4-AAP was employed in a similar manner for the fluorescent derivatization of 22 monohydric phenols [75]. However, this setup merely showed a minor improvement (16-fold) compared to the conventional UV detection. The postcolumn reaction of N-methylbenzothiazole-2-hydrazone and Ce(NH₄)₂(SO₄)₃ and detection at 500 nm are other approaches that were used to determine 30 hydroxyaromatic compounds in WW [71].

A reversed-phase high-performance liquid chromatography (RP-HPLC) method is proposed by Suliman et al. [77] for the analysis of some environmentally important phenols in water. The use of coumarin-6-sulfonyl chloride (C6SCI) as a fluorescence-labeling reagent has been investigated. The compound reacts with phenols within 20 min under mild conditions (ambient temperature, pH 9.0) to give sulfonates that can be separated by RP–HPLC employing fluorescence detection at lambda(ex) = 360 nm and lambda(em) = 460 nm.

Liquid Chromatography with Mass Spectrometric Detection

The combination of HPLC separation and MS is described in several papers and the number of applications where HPLC–MS is used is rapidly increasing with the availability of less-expensive benchtop instruments. The superiority of the mass spectrometer compared to other HPLC detectors is undisputed for it offers unsurpassed selectivity and also, to some degree, structure identification, thus being a powerful tool for the characterization of complex water samples. Several ionization techniques, such as atmospheric pressure chemical ionization (APCI), electrospray/ion spray (ESP/ISP), and thermospray (TSP) [78], have been employed in the MS determination of phenols at low concentration.

A comparison between positive- and negative-ion modes in TSP HPLC–MS with a quadrupole instrument showed that the negative-ion mode gave better sensitivity for the CPs than did the positive mode [79]. The APCI and ISP techniques in the negative-ion mode were used for the identification of 19 priority phenols [80]. Some of these phenols (phenol, 4-methylphenol, and 2,4-dimethylphenol [2,4-DMP]) could be detected only with ISP–MS. Following the preconcentration of 50–100 mL river water with SPE, detection limits for the different phenols from 0.1–5 μg/L to 0.1–25 ng/L were found using full-scan and time-scheduled single-ion monitoring modes, respectively (for chromatogram, see Figure 22.1).

### TABLE 22.3

<table>
<thead>
<tr>
<th>Derivatization Reagent</th>
<th>Mode</th>
<th>Type of Water Sample</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Methylbenzothiazole-2-hydrazone and Ce(NH₄)₂(SO₄)₃</td>
<td>Postcolumn</td>
<td>Wastewater</td>
<td>[71]</td>
</tr>
<tr>
<td>2-(9-Anthrylethyl) chloroformate</td>
<td>Precolumn</td>
<td>Wastewater</td>
<td>[72]</td>
</tr>
<tr>
<td>Dansyl chloride</td>
<td>Precolumn</td>
<td>River water</td>
<td>[73,74]</td>
</tr>
<tr>
<td>4-Aminoantipyrine</td>
<td>Precolumn</td>
<td>Wastewater</td>
<td>[75]</td>
</tr>
<tr>
<td>4-Aminoantipyrine and potassium ferricyanide</td>
<td>Postcolumn</td>
<td>Wastewater</td>
<td>[76]</td>
</tr>
<tr>
<td>Diazotized sulfanilic acid</td>
<td>Postcolumn</td>
<td>River water</td>
<td>[38]</td>
</tr>
<tr>
<td>Coumarin-6-sulfonyl chloride</td>
<td>Precolumn</td>
<td>Different types of water</td>
<td>[77]</td>
</tr>
</tbody>
</table>

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Both APCI–MS and ESP–MS were used in the negative-ion mode for the determination of chloro- and NTPs in tap water and seawater [81]. After the extraction of 250 mL seawater with polymeric SPE disks, detection limits in the low micrograms per liter range were found for most of the phenols using HPLC–APCI–MS. PCP and 2,4-dinitrophenol were determined together with acidic pesticides in river water and drinking water using ESP–MS in combination with SPE using graphitized carbon-packing material [82].

Jin et al. [83] developed a method for the simultaneous determination of three monochlorophenols (MCPs) in drinking water samples by ion chromatography (IC) coupled with an atmospheric pressure

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chemical ionization mass spectrometry (APCI–MS) in the negative mode. Drinking water was pre-concentrated by SPE. The IC separation was carried out with an IonPac AS18 analytical column (250 × 4.0 mm) and an IonPac AG18 guard column (250 × 4.0 mm) using 15 mmol/L KOH containing 10% acetonitrile as the mobile phase in isocratic mode at a constant flow rate of 1.0 mL/min.

The same authors also proposed a determination method of nine mono-MCPs and dichlorophenols (DCPs) in water samples using the eluent generator IC (IQ coupled with an APCI–MS in the negative mode) [84]. The IC separation was carried out on an IonPac AS11 analytical column (250 × 4.0 mm) using gradient KOH containing 15% acetonitrile as the organic modifier.

These authors also developed a method for the simultaneous determination of 19 CPs in water by liquid chromatography, coupled with atmospheric pressure chemical ionization mass spectrometry (LC–APCI–MS), and an SPE utilizing Oasis HLB cartridges [85]. A carbamate analysis C8 column (250 × 4.0 mm; 5 mm) was used.

### 22.5 Gas Chromatographic Determination of Phenolic Compounds in Water

As described earlier, all the official methods for the determination of phenolic compounds in water are based on GC. The GC methods are normally more sensitive than the HPLC methods, but because of the high polarity and low vapor pressure of the phenols, a derivatization step is normally necessary before the final GC analysis. GC separation of underivatized phenols using capillary columns with conventional phases is difficult, for phenols (in particular, NTPs) exhibit severe tailing. Highly deactivated capillary columns have been used for the direct separation of phenols [86,87], but in most cases, the phenols are derivatized to improve their chromatographic performance. Several different derivatization agents have been used, for example, pentafluorobenzyl bromide [88], pentafluorobenzyl chloride [89], acetic anhydride [90,91], and heptafluorobutyric anhydride [92].

Several different detectors have been used in combination with GC for the determination of phenols, for example, the FID [90,93], the electron capture detector (ECD) [89,91,92,94,95], and the MS detector [88,96–98].

In a series of papers, Lee and coworkers described the use of pentafluorobenzyl bromide as a derivatization agent for the determination of 22 phenols in water samples [88]. Before derivatization, the phenols were extracted from the water sample into DCM. In the first paper, six different columns were tested, and the OV-101 fused silica capillary column with carbowax-deactivated surface was found to give the most efficient separation. The detection was carried out using both the ECD and MS. A similar approach using derivatization with pentafluorobenzyl chloride and ECD, was described for the analysis of mono-chlorinated and brominated phenols in aqueous samples [89].

The use of acetic anhydride for the acetylation of phenols has been described in some papers [90,91]. The determination of CPs in freshwater, WW, and seawater using acetylation and ECD was reported by Abrahamsson and Xie [91]. They compared two derivatization procedures, pentafluorobenzoylation versus acetylation, and concluded that the acetylated derivatives gave better separation on the capillary column. Derivatization using heptafluorobutyryl in combination with GC–ECD [92] involves an extraction of the acidified sample into benzene before derivatization. Another method describes the conversion of eight phenols (phenols, cresols, and xylenols) into the corresponding bromophenols after reaction with bromine followed by an analysis using GC–ECD [95].

GC with Fourier transform infrared spectroscopy (FTIR) has been used for the determination of CPs in drinking water [99]. Before the GC–FTIR analysis, the phenols were acetylated with acetic anhydride followed by off-line SPE using a graphitized carbon cartridge. GC with microwave-induced plasma atomic emission spectroscopy was used in combination with two different off-line SPE procedures [100]. Derivatization with 3,5-bis(trifluoromethyl)benzylimethylphenylammonium fluoride in combination with MS detection in the negative chemical ion mode has been used for the determination of CPs in industrial WW [98]. As seen earlier, SPE sample preparation is a commonly integrated part of the overall system setup in GC analysis. The technique is treated in more detail in the following section.

Many of the papers already cited describing GC determination of phenols are fairly old (from the end of the 1970s to the beginning of the 1980s). Puig and Barceló remarked that there has been a general
trend to change the overall procedure, that is, the use of LLE and separation by GC is being replaced by SPE and HPLC procedures [68]. This seems to be a general trend and not just a change in the analysis of phenols. One of the reasons for this is that the derivatization step is regarded as very tedious and time consuming. On the other hand, the sensitivity and separation power of GC is still unsurpassed by even the latest developments in HPLC.

Phenolic compounds from waters were purge-and-trap preconcentrated after in situ acetylation [101]. They were quantified by GC/MS in selected ion monitoring (SIM) mode. The final optimized preconcentration conditions were as follows: 10.0 g of NaCl, 0.40 g of Na₂HPO₄, and 400 μL of acetic anhydride were added to a 35 mL sample, which was then heated at 55°C for 20 min and was purged for 20 min while the trap remained at room temperature. Once the purge step was over, the trap was desorbed at 260°C for 2 min and was then baked at 270°C for 6 min.

Elci et al. [102] evaluated SPE followed by derivatization and gas chromatography–atomic emission detection (GC–AED) for the determination of five CPs in water samples. The derivatization was based on the esterification of phenolic compounds with ferrocenecarboxylic acid. The determination of the derivatized phenols was carried out by GC–AED in the ion-selective detection mode.

Hossain et al. [103] developed a GC–MS method for the analysis of some environmentally important highly toxic phenols in water. The concentration level of phenol was determined in water at the sampling stations of Savar, Dhaka Export Processing Zone (DEPZ), and Bank Colony of the Bangsai River, Bangladesh. Water samples were collected from different depths of the sampling stations. The phenolic compounds were extracted with DCM, which was further preconcentrated by evaporation. The concentration of highly toxic phenol was found in the range of 0.01–0.998 μg/L.

A rapid and simple method is described by Kovacs et al. [104] for the simultaneous determination of six phenols (phenol, o-, m-, p-cresol, catechol, and resorcinol) and 19 CPs (all mono-, di-, tri-, and tetrachlorophenol isomers and PCP) present in aqueous samples. The method is based on derivatization with trimethylsilyl-Ν,N,N-dimethylcarbamate (TMSDMC). In contrast to other derivatization agents, TMSDMC instantaneously reacts with the phenolic compounds at room temperature and no further sample processing is necessary prior to instrumental analysis. The determination of the derivatives was performed by capillary GC–MS.

An oil-in-water emulsion (OWE) method was developed for the extraction of four phenolic pollutants in environmental water samples followed by GC and FID [105]. The column used was a BP21-FFAP capillary column (25 m length and 0.52 mm i.d.).

### 22.6 Alternative Separation Techniques

Other separation techniques, such as capillary electrophoresis (CE) and supercritical fluid chromatography (SFC), have been shown to perform well for the separation of phenols. Several papers describing the use of CE for the separation of phenolic compounds in water samples have been published lately [66,106,107]. The majority of these compounds employ UV detection, but ESP–MS in the negative-ion mode [108] and indirect fluorescence detection [109] have also been used. In one study, a comparison between HPLC and CE was carried out to assess their suitability for the determination of the 11 priority pollutants in water [16]. The authors claim that CE gave a shorter analysis time and smaller matrix effects. However, it was not possible to achieve the desired detection limits without a preconcentration on the solid-phase material.

Micellar electrokinetic chromatography (MEKC) has been used by several authors for the separation of phenolic compounds [110] and in some cases for the determination in water [111]. Off-line SPE using polymeric sorbents and MEKC with ECD were used for the determination of chlorinated phenols in a river at a low microgram per liter level [111]. Generally, one problem associated with miniaturized techniques such as CE when combined with UV detection is its limitation to small injection volumes. Therefore, efficient enrichment steps in the sample preparation are necessary. The plates were developed vertically up to a distance of 80 mm.

Stege et al. [112] describe a method for the simultaneous determination of p-nitrophenol (PNP), p-aminophenol (PAP), and hydroquinone (HQ) by micellar electrokinetic capillary chromatography after
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preconcentration by cloud point extraction. The optimized procedure was applied to the determination of phenolic pollutants in natural waters from San Luis, Argentina.

SFC has also been used for the separation of phenols [113]. The supercritical fluid normally used is carbon dioxide with some modifier, for example, methanol or chlorodifluoromethane (Freon 22). Berger and Deye tested binary and tertiary supercritical mixtures; among them, methanol/carbon dioxide mixtures contained very polar additives [113]. Ong et al. used chlorodifluoromethane as the supercritical fluid. In most studies, UV detection was used and did not measure up to the required sensitivity. To bypass this problem, an online system with SPE connected to the SFC instrumentation was designed. Some of these systems are presented in the following section.

A liquid-phase microextraction (LPME) method coupled with high-performance thin-layer chromatography (HPTLC) for preconcentration, separation, and determination of six phenolic compounds in water samples has been developed [114]. The target analytes were extracted using a microliter volume of 1-undecanol, which floated on the surface of the aqueous sample and were separated on an RP-18 F254 HPTLC plate using a mixture of methanol–water 2:1 (% v/v).

An electrokinetic flow analysis (EFA) system was developed for the determination of phenolic compounds in environmental water samples [115]. The analyte preconcentration and matrix cleanup was carried out by online LLLME.

22.7 Nonchromatographic Techniques

For direct measurements of o-nitrophenol, a selective optical chemical sensor has been developed by Wang et al. [116]. The determination of o-nitrophenol in tap water was presented, but the sensitivity of the sensor is poor compared to the earlier-presented chromatographic systems.

For detection of phenolic compounds in environmental water samples, Kochana et al. [117] propose an amperometric biosensor based on tyrosinase immobilized in titania sol gel. The analytical characteristics toward catechol, p-cresol, phenol, p-chlorophenol, and p-methylcatechol were determined.

An immunoassay kit for the measurement of PCP has been developed with a limit of detection around 60 μg/L [118]. The sample matrix had little influence on the immunoassay, but 2,4,5,6-tetrachlorophenol and 2,3,4,6-tetrachlorophenol show some cross-reactivity. The methodology can be used as an initial screening of phenols, and it normally does not require any sample preparation. The immunoassay methodology has also been applied for the determination of 4-NP and for substituted 4-NPs [119].

Phenol-specific immunoassay has also been used as a detection system in liquid chromatographic separation system [120]. The connection between HPLC and immunoassay detection was regarded by the authors to be more labor demanding than the conventional HPLC–UV, but the payoff in selectivity and sensitivity is claimed to be immense.

Spectrophotometry utilizing the reagents 4-aminoantipyrine and MBTH is the classical technique for the nonspecific determination of phenolic compounds. It is the basis for several official methods and was discussed earlier.

A method based on the reaction of phenolic compounds with 4-AAP in the presence of peroxodisulfate at pH 10 to form antipyrine dye and the SPE of a dye with a Varian Bond Elut Plexa cartridge was proposed by Morita et al. [121]. The dye collected on the cartridge is eluted with acetonitrile and the absorbance is measured at 475 nm. The recovery ratios of >90% were obtained for phenol, o-aminophenol, m-aminophenol, o-methoxyphenol, m-methoxyphenol, p-methoxyphenol, o-cresol, m-cresol, o-chlorophenol, m-chlorophenol, p-chlorophenol, 2,5-dimethylphenol, and 2,4-DCP.

Morita et al. [122] reported a methyl benzoate extraction procedure for determining small amounts of phenol compounds in WW with 4-AAP spectrometry. The detection limit was 0.004 μg as phenol/mL. Good results were obtained in recovery tests using both wastewater and river water.

Di Nezio et al. [123] propose an analytical method to determine directly and simultaneously five phenolic compounds (4-NP, 2-nitrophenol, phenol, 2,4,6-TCP, and 4-chlorophenol [4-CP]) in seawater (Ria de Bahia Blanca, Argentina).
Shan et al. [124] developed a biocomposite based on polycrystalline bismuth oxide (Bi$_2$O$_3$) film and polyphenol oxidase (PPO) for the construction of a mediator-free amperometric biosensor for phenolic compounds in environmental water samples.

Trap-and-release membrane introduction mass spectrometry (T&R-MIMS) with a removable direct insertion membrane probe (DIMP) is used to quantify a variety of trace phenolic compounds in water after acetylation [125].

### 22.8 Sample Preparation Procedures

The fact that different substituted phenols even at very low concentration affect our aquatic environment demands selective and sensitive determination systems. The first step in such a system is an efficient sample preparation. Over the last two decades, much focus has been given to sample preparation in chromatographic analysis since this step is regarded as critical, error prone, and normally time consuming.

There are two main objectives with the sample preparation step:

1. Cleanup of the sample to avoid deterioration of the chromatographic system (column, detectors, etc.) and degradation of the analytes
2. Concentration enrichment of the analytes, which is normally necessary before introduction to the final chromatographic instrument

Therefore, the need for fast, selective, and sensitive sample preparation technique has never stopped. In line with this, the most common sample preparation methods are described as follows.

An excellent review was published in 2012 by de Morais et al. [126] on extraction and preconcentration techniques for the chromatographic determination of CPs in environmental and food samples.

#### 22.8.1 Liquid–Liquid Extraction

LLE is the classical sample preparation technique. The reviews of LLE are found in general papers on sample preparation [127,128], and it is still frequently used by the environmental analyst. This is mainly due to the fact that LLE is used in many of the official methods. The technique is based on partitioning of the analyte between an aqueous and an organic phase, contained inside a bottle or a separatory funnel. The analyte is extracted from the aqueous phase to the water-immiscible organic phase, and after extraction, the two phases are allowed to separate. If necessary, the organic phase is dried with a suitable drying agent. Before introduction into the analytical instrument, the organic extract can be concentrated by a volume reduction. Also, solvent change is often made after evaporation to dryness. The selectivity in LLE can be controlled by changing the organic solvent, by using ion pairing or derivatization reagents, and by adjusting the pH in the aqueous phase.

For polar analytes (e.g., phenol and monosubstituted phenols), polar solvents such as ethyl acetate and methyl chloride are favored, whereas for nonpolar analytes (e.g., higher substituted phenols), more nonpolar solvents such as hexane and toluene are used. However, conventional LLE is often regarded as having some severe drawbacks:

- It is laborious and time consuming
- It uses large quantities of organic, often hazardous, solvents
- It is difficult to automate

#### 22.8.1.1 LLE in Combination with HPLC

LLE with DCM has been used before HPLC with UV and fluorescence detection [129]. Dinitrophenols are detected with UV absorption followed by oxidation with cerium(IV) in an open tubular reactor, allowing fluorescence measurement of cerium(III).
2,4,5-Trichlorophenols and 4-NP, both degradation products of pesticides, together with some pesticides have been extracted with an online continuous-flow extraction in combination with HPLC with UV and MS detection [71]. The enrichment factors were lower than those obtained with online precolumn systems using solid adsorbents, but the LLE suffered less from memory effects.

The combination of LLE and normal-phase HPLC with UV fluorescence has been used for phenol and cresols in rain [130]. The rain samples were adjusted to pH 2 with sulfuric acid and were continuously extracted with DCM giving detection limits at the microgram per liter level.

### 22.8.1.2 LLE in Combination with GC

The combination of LLE and GC separation has frequently been applied to phenolic compounds in water samples. As already discussed, many of the official methods for phenol determination in water are based on this combination.

In combination with derivatization with pentafluorobenzyl bromide and GC determination, LLE has been used for the determination of phenolic compounds in water [88]. A similar approach for phenol and monochlorinated/monobrominated phenols in complex aqueous samples has been described by Booth and Lester [89].

Continuous LLE has been connected online to GC for the determination of phenols in aqueous samples [131]. The technique has been used in two different modes. The first mode involves simultaneous extraction and derivatization. Acetate esters of phenol, cresols, and CPs were formed by continuous extraction into n-hexane containing acetic anhydride. Another approach was to first derivatize the phenols with acetic anhydride. The esters were extracted with a pentane/diethyl ether mixture before capillary GC determination with both FID and ECD [132].

Continuous LLE with ethyl acetate in combination with direct GC separation using highly deactivated capillary columns has been used for the determination of NTPs in groundwater [87]. When GC–MS was used for the identification of NTPs and with nitrogen–phosphorus detection, limits of detection (LOD) in the microgram per liter range were found.

Preconcentration and determination of eight phenolic compounds in water samples has been achieved by in situ derivatization and using a new liquid–liquid microextraction-coupled GC–MS system [133].

### 22.8.2 Solid-Phase Extraction

SPE is widely used instead of a liquid–liquid process to concentrate phenolic compounds from environmental water samples. Chemically bonded silica, C18, or C8 modified are the most commonly used sorbents for SPE [134,135]. Porous polystyrene resins [54] and graphitized carbon black [136,137] have been used as well, but many polar compounds are not well extracted or a nonspecific adsorption takes place. The low recovery of low-molecular-weight phenolic compounds leads to the use of poly(styrene–divinylbenzene) (PSDVB) copolymers, modified with the hydrophilic groups (acetyl- or hydroxymethyl group) [138]. A mixture (20:80, w/w) of keto-derivatized/underivatized PSDVB was used for preconcentration of the phenols from mineral and tap waters [139]. In general, low breakthrough volumes were obtained with alkylsilica-bonded phases, mainly for the most polar compounds. On the other hand, PSDVB involves higher breakthrough volumes, which are even higher with the highly cross-linked sorbents. Di Corcia et al. [140] obtained better results with a reversible graphitized carbon black cartridge because of the higher retention capacity of the carbon. Membrane extraction disks, either with C18 or with PSDVB adsorbent, have also been used for the extraction of phenolic compounds [141–143] and have the advantage of a faster elution rate and hence a shorter extraction time.

The SPE technique has been developed in the off-line and online mode. Both have their own advantages and limitations [144]. When comparing with the off-line mode, online SPE coupled to HPLC system allows easy automation with high sample throughput and good reproducibility [145], and the whole analysis can be completed within a shorter time.

Therefore, the online approach is generally preferred to the off-line mode. Online SPE has been applied to the determination of phenols in water samples using small precolumns with different adsorbents such as octadecyl-bonded silica [30,31,53,146], styrene–divinylbenzene (DVB) copolymers PLRP-S or PRP-1...
Molecularly imprinted polymers (MIPs) are other types of sorbents that have been investigated by different researchers for SPE. MIPs are polymers produced in a process where functional and cross-linking monomers are copolymerized in the presence of a target analyte molecule, which acts as a molecular template. The functional monomers initially form a complex with the imprint molecule, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric structure. By removing the imprint molecule, it is possible to have binding sites that are complementary in size and shape to the analyte. Then, a molecular memory is introduced into the polymer that is now capable of selectively rebinding the analyte from different matrices. MIPs can be prepared by three different protocols [156,157].

Caro et al. [158] synthesized three polymers using 4-CP as the template, following different protocols (noncovalent and semicovalent), and used different functional comonomers: 4-vinylpyridine (4-VP) and methacrylic acid (MAA). They have evaluated the selectivity of the polymers as MIP sorbents in SPE coupled online to LC. They found out that the 4-VP noncovalent polymer was the only polymer that showed a clear imprint effect. This MIP also showed cross-reactivity for the 4-chloro-substituted phenols and for 4-NP from a mixture containing the 11 priority EPA phenolic compounds and 4-CP. The MIP was applied to selectively extract the 4-chloro-substituted compounds and 4-NP from river water samples. Figure 22.4 shows the chromatograms obtained by online molecularly imprinted solid-phase extraction (MISPE) with the 4-VP noncovalent 4-CP imprinted polymer of 10 mL standard solution (pH 2.5) spiked at 10 mg/L with each phenolic compound.

Sirvent et al. [159] evaluated the efficiency of a new SPE cartridge, Spe-ed Advanta, in the extraction and preconcentration of four phenolic compounds (phenol, 2-CP, 2-nitrophenol, and 2,4-DCP) from ultrapure spiked water at different concentration levels and natural waters (river waters and groundwaters). They have compared the recoveries achieved with those obtained using a polystyrene–DVB cartridge. They have used GC with an FID and LC with a DAD to analyze organic and aqueous extracts. They have obtained very high preconcentration factors and they found that Spe-ed Advanta cartridges gave better results than those obtained with Isolute ENV+, showing that the presence of polar groups in the polymer improves the extraction of the phenols.

A novel approach for the sample pretreatment and determination of a number phenolic compounds in environmental water samples has been developed by hyphenating SPE and LPME techniques based on solid organic drop combined with GC–MS [160]. After preconcentration and purification of the sample through the column containing 60 mg of β-CDS particles as the stationary phase, under optimum conditions, LPME technique has been performed on the eluent solution.
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FIGURE 22.2 Chromatograms obtained from online SPE–HPLC system. The mobile phase for chromatographic separation consisted of 38% CH$_3$CN, 61% water, and 1% (v/v) acetic acid, with the apparent pH adjusted to 6.0. The flow rate is 1 mL/min. (a) The sample solution consisted of 10 mL double-distilled water spiked with phenols at 10 μg/L, without washing with acetonitrile; (b) the sample solution consisted of 100 mL double-distilled water spiked with phenols at 1 μg/L, without washing with acetonitrile; (c) the sample solution consisted of 100 mL double-distilled water spiked with phenols at 1 μg/L, washing with 0.6 mL acetonitrile; (d) the sample solution consisted of 100 mL double-distilled water spiked with phenols at 1 μg/L, washing with 0.6 mL methanol. (1) Phenol, (2) 4-nitrophenol, (3) 3-nitrophenol, (4) 2-nitrophenol, (5) 2,4-dimethylphenol, (6) 4-chloro-3-methylphenol, (7) 2,4-dichlorophenol. (Reprinted from Y. Fan, Y. Qi Feng, and S. Lu Da. Anal. Chim. Acta, 484: 145, 2003. With permission.)
A method has been developed for the simultaneous extraction of 13 phenolic compounds, including CPs, NTPs, cresols, and alkylphenols (APs) in different types of WW effluents [161]. The SPE method has been optimized prior to the determination by gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC–QqQ–MS/MS). The Oasis HLB cartridges were conditioned with 5 mL of acetone followed by 5 mL of MeOH and 3 × 5 mL of ultrapure water without allowing the cartridges to dry out. Then, the filtered WW sample (250 mL) was passed through the cartridges under vacuum at a flow rate of 10 mL/min. The cartridges were dried for 2 h and the phenolic compounds were eluted sequentially with 3 mL of acetone and 2 mL of DCM. The extracts were collected into 5 mL volumetric flasks, adjusting the total volume with DCM, without any evaporation step. Owing to the complexity of

**FIGURE 22.3** Chromatograms obtained with Donghu Lake water samples. (a) CDS sorbent, sample solution consisted of 20 mL Donghu Lake water spiked with phenols at 5 μg/L, without washing with acetonitrile; (b) CDS sorbent, sample solution consisted of 20 mL Donghu Lake water spiked with phenols at 5 μg/L, washing with 0.6 mL acetonitrile; (c) superclean LC18 sorbent, sample solution consisted of 20 mL Donghu Lake water spiked with phenols at 5 g/L, without washing with acetonitrile; (d) superclean LC18 sorbent, sample solution consisted of 20 mL Donghu Lake water spiked with phenols at 5 μg/L, washing with 0.6 mL acetonitrile; (e) CDS sorbent, sample solution consisted of 20 mL Donghu Lake water without spiking, without washing with acetonitrile. The peak designations are given as in Figure 22.2. (Reprinted from Y. Fan, Y. Qi Feng, and S. Lu Da. *Anal. Chim. Acta*, 484: 145, 2003. With permission.)
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the matrix, a comparison study of matrix-matched calibration (MMC) and standard addition calibration (SAC) was carried out for quantification purposes.

MIP may not selectively recognize a small template of a limited number of functional groups, such as 2-CP. El-Sheikh et al. [162] proposed a novel method to improve the recognition ability of the MISPE of 2-CP from environmental waters. This was achieved by derivatization of 2-CP with 4-amino-antipyrine (4-AAP) to enlarge its molecular size and add more binding sites. For that purpose, two MISPE methods of 2-CP were developed. In method 1, a polymer imprinted with 2-CP was used as the extracting sorbent but it suffered from low selectivity and high detection limit of 2-CP (7.10 ng/L). In method 2, a polymer imprinted with 4-AAP-derivatized 2-CP (2-CP–4-AAP) was used as the extracting sorbent. Method 2 showed high recognition ability/selectivity toward 2-CP–4-AAP with a lower detection limit of 0.05 ng/L for 2-CP–4-AAP. Method 2 was able to detect the presence of 2-CP–4-AAP in unspiked real water samples and almost full spike recovery was achieved.

FIGURE 22.4 Chromatograms obtained by online MISPE with the 4-VP noncovalent 4-CP imprinted polymer (P1) of 10 mL standard solution (pH 2.5) spiked at 10 μg/L with each phenolic compound. (a) Without washing step, and (b,c) with washing step using 0.1 and 0.3 mL of dichloromethane, respectively: (1) Ph, (2) 4-NP, (3) 2,4-DNP, (4) 2-CP, (5) 4-CP, (6) 2-NP, (7) 2,4-DMP, (8) 4-C-3-MP, (9) 2-M-4,6-DNP, (10) 2,4-DCP, (11) 2,4,6-TCP, and (12) PCP. For details, see Ref. [158]. (Reprinted from E. Caro, et al. J. Chromatogr. A, 995: 233, 2003. With permission.)
In-house synthesized polymeric SPE sorbents in the form of monodisperse, hypercross-linked polymer microspheres with diameters in the low micrometer range (similar to 4 μm) were evaluated by Fontanals et al. [163]. More specifically, their performance in the online SPE of a group of polar pollutants has been investigated thoroughly. The novel hypercross-linked materials were compared with satisfactory results to commercial SPE sorbents with similar chemical and morphological properties, albeit that the commercial materials had higher particle sizes and broader particle size distributions. The online SPE method developed using these novel particles as the packing material was successfully applied to ultrapure, mineral, tap, and Ebre river water samples.

Mixed hemimicelle SPE based on cetyltrimethylammonium bromide (CTAB)-coated nanomagnets Fe₃O₄ was investigated for the preconcentration of four CPs in environmental water samples prior to HPLC–spectrophotometry determination [164]. Under optimized conditions, four analytes of 2-CP, 2,4-DCP, 2,4,6-TCP, and PCP were quantitatively extracted. The method was then used to determine four CPs in five real environmental water samples.

An MIP was prepared using 2,4-DMP as the template [165]. The synthesis is optimized by using three different porogens, chloroform, acetonitrile, and toluene. The MIP was used as a class-selective sorbent in molecularly imprinted solid-phase extraction (MIP-SPE) for preconcentration and determination of phenolic compounds from environmental water. The difference in the selectivity of recognition of the polymer columns was observed by HPLC.

The MIP with 2,4,6-TCP as the template molecule and MAA with DVB as the functional monomer and the cross-linker, respectively, has been prepared and used as molecularly imprinted microsolid-phase extraction (MIMSPE) procedure for the selective preconcentration of phenolic compounds from environmental water samples [166].

The same authors [167] prepared molecularly imprinted bulk polymer with 2,4,6-TCP as the template molecule and MAA, ethylene glycol dimethacrylate (EGDMA) as the functional monomer and the cross-linker, respectively, and applied to the MIP-SPE procedure for selective preconcentration of phenolic compounds from environmental water samples.

### 22.8.3 Solid-Phase Microextraction

Solid-phase microextraction (SPME), first described by Pawliszyn and coworkers [168–172], is a recent upcoming sample preparation method for phenolic and other organic compounds from water and air samples. It is a novel solvent-free sample preparation technique. SPME has the advantage of simplicity, low cost, and rapid extraction. It has been successfully coupled with various techniques such as GC, HPLC, CE, and MS.

The crucial part of the SPME is the fiber coating, which provides the enrichment of the solute of interest selectively from the matrix components. The typical SPME fibers are [173,174] poly(dimethylsiloxane) (PDMS), polyacrylate (PA), PDMS–divinylbenzene (PDMS/DVB), carboxen–PDMS, carbowax–divinylbenzene (CW/DVB), and carbowax-templated resin (CW/TPR). According to the principle of alike dissolves like, the more polar fibers (PA, PDMS–DVB, CW–DVB, and CW-TPR) have been found suitable for the extraction of phenols, whereas the PDMS fiber was not satisfactory because of its relative nonpolar nature. According to many publications, the most favorable extraction of phenols is performed using a PA fiber.

SPME involves both absorption and desorption steps. In the absorption step, a coated fused silica fiber extracts the analytes from the sample matrix. In the desorption step, the analytes are desorbed from the fiber and are introduced into the analytical column for separation.

A fused silica fiber coated with an immobilized phase of the above coatings is fixed inside a syringe. For the analysis of aqueous samples, the fiber is exposed to the liquid and the analytes are accumulated in the stationary phase until equilibrium is reached. The fiber is then removed from the solution and the extracted organic substances are thermally desorbed in a split–splitless injector or on a column injector of a gas chromatograph or are desorbed by organic solvents and injected into HPLC system. As indicated earlier at the beginning of this section, SPME has been successfully coupled with various techniques such as GC, HPLC, CE, and MS [175–179].
SPME and HPLC were first coupled in 1995 [180], and the system has been commercially available since 1996. An organic solvent (static desorption) or the mobile phase (dynamic desorption) is used to desorb the analytes from the SPME fiber. SPME has been successfully used to determine phenols and NTPs in water [181–183].

As indicated above, the fiber coatings can range from a rather nonpolar PDMS to the more polar PA film. The PDMS phase is used for the determination of nonpolar volatile compounds in water samples [184,185]. In comparison, the PA fiber is preferred for the extraction of more polar compounds. The first application of SPME to the analysis of polar compounds was the determination of phenols carried out by Buchholz and Pawliszyn [186]. These authors reported a detection limit at the nanogram per liter level for the GC/FID and GC/MS procedures using a saturated sodium chloride solution at pH 4 to increase the SPME sensitivity. In connection with the analysis of a sewage sample, the authors found significant differences in the recoveries of phenols [182]. The 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol could only be recovered in the sewage sample if the acid conditions and salt saturation were adjusted. The recovery of the CPs was also unsatisfactory. To quantify the content of phenols despite the remarkable influence of the organic matrix, the standard addition technique was applied.

Peñalver et al. [187] have applied SPME coupled to HPLC with ECD and UV detection to determine the 11 phenolic compounds considered as priority pollutants by the U.S. EPA. In this work, 85 mm PA fibers were used to extract the analytes from the aqueous samples. They have compared static and dynamic desorption modes. They found out that the static desorption showed better recoveries for the phenolic compounds. The authors evaluated the performance of the SPME–HPLC–UV–ECD with river water and WW samples. The method enabled the determination of phenolic compounds at low levels in these water samples. Figure 22.5 shows [187] the chromatograms for the Ebro river water sample spiked with 1 mg/L for ECD, and 0.1 mg/L for UV detection.

Bagheri et al. [188] prepared an aniline-based polymer and applied it as a new fiber coating for SPME of some priority phenols from water samples. They investigated the efficiency of this new coating using a laboratory-made SPME device and GC with FID for the extraction of some phenols from the headspace of aqueous samples. The results obtained proved the ability of this polymer as a suitable SPME fiber coating for trapping the selected phenols. The authors have optimized the influential parameters affecting the extraction process and an extraction time of 50 min at 50°C gave maximum efficiency, when the aqueous sample was saturated with NaCl and was adjusted at a pH of 2. The optimized method was successfully applied to some real-life water samples.

Zhou et al. [189] used a laboratory-made fiber with 25,27-dihydroxy-26,28-oxy (2′,7′-dioxo-3′,6′-diazaoctyl)oxy-p-tert-butylcalix[4]arene/hydroxy-terminated silicone oil (amide bridged-C[4]/OH-TSO) coating in headspace SPME coupled to GC–FID for the determination of phenolic compounds in WW matrices. They have compared the extraction ability of this new fiber with the commercially available PA (85 μm) fiber. They showed that the new calixarene fiber had high affinity for the phenolic compounds due to the introduction of the polar amide bridge in calix[4]arene. The authors have applied the method to determine the phenolic analytes in real WW samples and found recoveries ranging from 89.7% to 103.2%.

SPME of CPs [2-CP, 2,4-CP, 4-chloro-3-methylphenol (4,3-CP), 2,4,6-trichlorophenol (2,4,6-CP), and PCP] followed by direct mass spectrometric analysis has been performed by fiber introduction mass spectrometry (FIMS) [190]. Two SPME fibers (65 μm PDMS/DVB and 85 μm PA fibers) were tested, and FIMS was performed via selective ion monitoring (SIM). The extractions were evaluated at 10% ionic strength and pH 1. The best extraction times were determined for both fibers. LOD and limits of quantification (LOQ) for both fibers were in the low microgram per liter range.

A dodecylsulfate-doped polypyrrole (PPy-DS) film was prepared by electrochemical fiber coating (EFC) technique and applied as a new fiber for headspace solid-phase microextraction (HS-SPME) of phenolic compounds from water samples [191]. The efficiency of this fiber for microextraction of phenols was evaluated using an HS-SPME device coupled with gas chromatography–flame ionization detection (GC–FID).

A novel analytical method is presented by Santana et al. [192] for the determination of CPs in water. This method involves preconcentration by SPME and an external desorption using a micellar medium,
the nonionic surfactant polyoxyethylene 10 lauryl ether (POLE), as the desorbing agent. The final analysis of the selected CP compounds was carried out by HPLC with DAD.

### 22.8.4 Liquid-Phase Microextraction

LPME as a sample preparation technique for chromatography and electrophoresis is one of the recently developing techniques. In LPME, the principles of LLE and the miniaturized nature of SPME are combined to realize the advantages of both techniques.

LPME has been accomplished by extraction into a small droplet of organic solvent hanging at the end of a microsyringe needle [193–202] (microdrop) or into small volumes of the acceptor solution present inside the lumen of porous hollow fibers [203–205]. In both the microdrop concept and in the hollow
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fiber format, the analytes of interest are extracted and preconcentrated into a few microliters of appropriate solvents. Because of this, LPME may be very effective for analyte enrichment and may result in a major reduction in the use of organic solvents. A detailed review based on hanging droplets in two- and three-phase LPMEs has been presented by Psillakis and Kalogerakis [206].

The application of LPME with backextraction (LPME/BE) combined with HPLC for the determination of phenols in the aqueous sample was shown by Zhao and Lee. They investigated the parameters affecting the extraction efficiency (solvent selection, solvent volume, phase ratio between donor solution and acceptor phase, extraction time, and composition of the donor and acceptor solutions) [207]. They extracted the target phenolic compounds from 2 mL aqueous sample adjusted to pH 1 (donor solution) through a microliter-size organic solvent phase (400 μL n-hexane), confined inside a small polytetrafluoroethylene (PTFE) ring, and finally into a 1 mL basic aqueous acceptor microdrop suspended in the organic solvent phase from the tip of a microsyringe needle. After extracting for a prescribed time, the microdrop was taken back into the syringe and was directly injected into an HPLC for detection. At the optimized conditions of extraction, they have found a large enrichment factor (more than 100-fold) for most of the phenols within 35 min.

Saraji and Bakhshi [208] carried out a trace analysis of phenolic compounds in water by coupling single-drop microextraction (SDME) with in-syringe derivatization of the analytes and GC–MS analysis. The analytes were extracted from a 3 mL sample solution using 2.5 μL of hexyl acetate. After extraction, derivatization was carried out in the syringe barrel using 0.5 μL of N,O-bis(trimethylsilyl)acetamide. To investigate the applicability of the proposed SDME method in real-sample analysis, they have carried out the determination of phenols in water samples of Kashkan and Zayandeh-Rud rivers by the standard addition technique. Figure 22.6 shows the chromatograms obtained by in-syringe derivatization procedure of these river water samples spiked with phenolic compounds.

Rasmussen and Pedersen-Bjergaard reviewed the developments in hollow fiber-based LPME [209]. In hollow fiber-based LPME, the analytes of interest are extracted from aqueous samples, through a thin layer of an organic solvent immobilized within the pores of a porous hollow fiber, and into an acceptor solution inside the lumen of the hollow fiber. Subsequently, the acceptor solution is directly subjected to a final analysis by capillary gas chromatography (CGC), HPLC, CE, or MS without further efforts. In this review, it has been indicated that hollow fiber-based LPME may provide high analyte preconcentration and excellent sample cleanup, and has a broad application potential within areas such as drug analysis and environmental monitoring. In the review are discussed the basic extraction principles, technical setup, recovery, enrichment, extraction speed, selectivity, applications, and future trends in hollow fiber-based LPME.

Hollow fiber-based extraction can be used for the determination of freely dissolved phenols or total concentration of phenols in environmental water samples. Liu et al. [210] applied hollow fiber-based supported liquid membrane (SLM) coupled with HPLC for the determination of freely dissolved CPs in water samples. In this equilibrium sampling through membranes, freely dissolved CPs were successfully determined in model solutions of humic acids and at low parts per billion levels in river and leachate waters.

Berhanu et al. [211] developed a hollow fiber-SLM extraction method for the liquid chromatographic determination of dinitrophenolic compounds at parts per trillion levels in environmental water samples.

A novel method for the determination of phenolic compounds (phenol, o-cresol, m-cresol, 2,4-DMP, 2,3-dimethylphenol, and 3,4-dimethylphenol) in water samples was developed by combined continuous-flow liquid-phase microextraction (CFME) with GC–FID by Chen et al. [212].

22.8.5 SLM Extraction

The SLM extraction technique can serve as an alternative sample preparation whenever dealing with difficult matrices and dirty samples, this being the case with many water samples [213,214]. The SLM extraction utilizes a porous hydrophobic membrane impregnated with a water-immiscible organic solvent. The membrane is placed between two blocks in which sample channels are formed on both sides of the membrane: the donor and the acceptor. The analytes are extracted from the aqueous donor phase into the membrane and are then backextracted to the second aqueous phase, the acceptor. The process is normally driven by differences in pH between the two aqueous phases. By pumping the water sample in the donor and by keeping the acceptor stagnant, an enrichment of the analytes in the acceptor is achieved.
The technique has been used in combination with HPLC using ECD for the determination of phenolic compounds with a large variety in polarity, namely, phenol, 4-CP, 2,5-dichlorophenol, 2,4,5-trichlorophenol, 2,3,5,6-tetrachlorophenol, and PCP, in natural water samples [65]. The membrane provides very efficient cleanup, and detection limits below 0.1 μg/L for the phenols were obtained (for chromatogram, see Figure 22.7). The technique has also been used for the extraction of five NTPs (2-nitrophenol, 3-nitrophenol, 4-NP, 2,3-dinitrophenol, and 2,4-dinitrophenol) [215].

A static extraction mode of hollow fiber-SLM was developed for the field of sample passive pretreatment of environmental water samples [216]. The extraction device was prepared by immobilizing dihexyl ether on the wall of a polypropylene hollow fiber membrane (60 cm length, 50 mm wall thickness, and 280 mm id) as the liquid membrane and filling the lumen of the fiber with 0.1 M NaOH as the acceptor, and closing the two ends of the fiber with an aluminum foil. Passive extraction was conducted by immersing the device into 15 mL water samples modified with 0.01 M HCl and 20% m/v NaCl. The

![FIGURE 22.6 GC–MS–SIM chromatograms obtained by SDME followed by in-syringe derivatization procedure of (a) Zayandeh-Rud river water and (b) Zayandeh-Rud river water spiked with 0.45–0.8 μg/L of phenols, (c) Kashkan river water and (d) Kashkan river water spiked with 0.45–0.8 μg/L of phenols. IS, internal standard: (1) 2-CP, (2) 2,4-DMP, (3) 4-CP, (4) 4-C,2-MP, (5) 2,4-DCP, (6) 2-NP, (7) 2,4,6-TCP, (8) 4-NP, and (9) PCP. For details, see Ref. [187]. (Reprinted from A. Peñalver et al. J. Chromatogr. A, 953: 79, 2002. With permission.)](image-url)
model analytes, including 4-CP, 2,4-DCP, and 2,4,6-TCP, were transferred into the acceptor with extraction efficiencies over 79% for 10 h at room temperature, and were determined by HPLC.

1-Octyl-3-methylimidazolium hexafluorophosphate ([CsMIM][PF6]) ionic liquid was immobilized in the pores of a polypropylene hollow fiber for hollow fiber-protected LPME [217]. The analytes, including 4-CP, 3-chlorophenol (3-CP), 2,4-DCP, and 2,4,6-TCP, were extracted into this ionic liquid membrane, and were backextracted into 10 μL sodium hydroxide acceptor solution in the lumen of the hollow fiber. Then, the acceptor solution was withdrawn into the HPLC microsyringe connected to the hollow fiber, and was directly injected into the HPLC system for analysis.

### 22.8.6 Others

Supercritical fluid extraction (SFE) has been mostly used in environmental analysis [143,218] for the extraction of nonpolar organic pollutants from solid samples, for example, sediments. The technique has also been used together with SPE disks (silica C<sub>18</sub>, polymeric, and ion exchanger disks) for the extraction of phenols from water samples followed by GC–MS [219]. After adsorbing the phenols onto the SPE disk, they are eluted with a supercritical fluid (carbon dioxide).

Another way of desorbing adsorbed phenolic compounds from silica C<sub>18</sub> SPE disks was given by Chee et al. [220]. They used closed-vessel microwave extraction before the final HPLC–UV analysis.

A poly(vinyl pyridine–ethylene dimethacrylate) monolithic material was synthesized and selected as stir bar sorptive extraction (SBSE) medium [221]. The influences of polymerization conditions on the extraction efficiency were investigated using phenol and PNP as the target analytes. On the basis of this, six strong polar phenols in water were directly concentrated by the new SBSE and were determined with HPLC equipped with DAD. The proposed method was successfully applied to the determination of phenolic compounds in lake and seawaters.

The directly suspended droplet liquid–liquid–liquid-phase microextraction was used in this research for the determination of three CPs in environmental water samples [222]. The analytes (2-CP, 3-chlorophenol, and 4-CP) were extracted from 4.5 mL acidic donor phase (pH 2, P1) into an organic phase, 350 μL of benzene/1-octanol (90:10 v/v, P2), and were then backextracted into a 7 μL droplet of a basic (pH 13) aqueous solution (acceptor phase, P3). In this method, contrary to the ordinary single-drop LPME technique, an aqueous large droplet is freely suspended on the surface of the organic solvent,
without using a microsyringe as the supporting device. This aqueous microdroplet is delivered at the top-center position of an immiscible organic solvent that is laid over the aqueous donor sample solution while the solution is being agitated. Then, the acceptor phase containing CPs was withdrawn back into an HPLC microsyringe and neutralized by adding 7 μL of 0.1 M HCl. The total amount was eventually injected into the HPLC system with UV detection at 225 nm for further analysis.

A multiwalled carbon nanotube–polyaniline composite (MWCNT–PANI) film-coated platinum wire was fabricated through electrochemical deposition [223]. The as-made fiber was used for the HS-SPME of some phenolic compounds (i.e., 2-CP, 2,4-DCP, 2-methylphenol, 3-methylphenol, 2,6-dimethylphenol, and 2-nitrophenol), followed by gas chromatographic analysis.

Huang et al. [224] developed a method for polar phenols in water matrix by using SBSE based on a hydrophilic poly(vinylpyrrolidione–divinylbenzene) (VPDB) monolithic material and HPLC analysis.

Fan et al. [225] determined the phenols in water by ionic liquid. 1-Butyl-3-methylimidazolium hexafluorophosphate ([C-4 mim][PF6]) based on liquid–liquid extraction was coupled with HPLC.

An LLLME technique has been published based on the principle of single-drop LLLME [226]. In the technique, a vial insert was first utilized as the acceptor phase container. Since the diameter of the bottom of the vial insert was small, the contact area between the acceptor phase and the vial insert was bigger than that between the microsyringe and the microdrop of the acceptor phase in single-drop LLLME. This method was successfully applied to determine four phenolic compounds in real aqueous samples.

22.9 Conclusion

An overview is given of the many possibilities for determining phenolic compounds in various water matrices. The area of phenolic analysis in water has been thoroughly investigated in the past, as is readily seen in this chapter, and the field will surely be a focus of future research as well, especially with the environmental concerns of today.

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