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On-line methodology for the trace level determination of the chlorinated phenol family in water samples

Ana María Núñez-Gaytán,[1,2] Luz Elena Vera-Ávila*[1] and María del Rosario Covarrubias-Herrera[1]

[1] Facultad de Química, Departamento de Química Analítica. Universidad Nacional Autónoma de México, Ciudad Universitaria, México D.F. 04510, México. luzelena@servidor.unam.mx

[2] Facultad de Ingeniería Química, Departamento de Química, Universidad Michoacana de San Nicolás Hidalgo. Ciudad Universitaria, Morelia, Michoacán 58060, México.

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Abstract. On-line solid-phase extraction – liquid chromatography (SPE-HPLC) methodology was developed for the trace level determination of phenol and 19 chlorophenols in water samples. A small precolumn packed with polymeric adsorbent was placed in a switching valve and used for sample extraction and preconcentration; the trapped compounds were further on-line eluted and analyzed by reversed phase HPLC with UV and coulometric detection. Because of wide differences in hydrophobic character, SPE of the least (phenol and monochlophenols), the medium (di- and trichlorophenols) and the most chlorinated phenols (tetra- and pentachlorophenol) was performed using different sample volume (15-25 mL) and sample composition (0-10% methanol addition). Under these conditions, solute recoveries were ≥ 82% (except phenol, 72%) for concentrations in the range ~3-75 ng/mL. Applying recovery factors, excellent accuracy (100%) and precision (RSD < 6.5%) were achieved for the 20 compounds of interest in replicate analysis (n = 7) of spiked reagent water samples. Method detection limits were 0.5-1 ng/mL with the UV detector and 0.1-0.3 ng/mL with the coulometric detector.
Keywords: chlorophenols, water analysis, on-line methodology, solid-phase extraction, liquid chromatography.

Resumen. Se desarrolló una metodología basada en el acoplamiento en línea de la extracción en fase sólida y la cromatografía de líquidos (EFS-CLAR), para la determinación de fenol y 19 clorofenoles al nivel de trazas en muestras de agua. La extracción y preconcentración de las muestras se realizó en una pequeña precolumna empacada con adsorbente polimérico y colocada en una válvula de conmutación; posteriormente, los compuestos atrapados fueron eluidos en línea y analizados por CLAR en fase reversa con detección UV y coulombimétrica. Debido a las grandes diferencias en carácter hidrofóbico, la EFS de los fenoles menos clorados (fenol y monoclorofenoles), los medianamente clorados (di- y triclorofenoles) y los más clorados (tetra- y pentaclorofenol) se realizó usando diferente volumen (15-25 mL) y composición de muestra (0-10% de metanol adicionado). Con estas condiciones la recuperación de los solutos fue ≥ 82% (excepto fenol, 72%) para concentraciones en el intervalo de ~3-75 ng/mL. Aplicando factores de recuperación, se obtuvo una excelente exactitud (100%) y precisión (< 6.5%) para los 20 compuestos de interés en análisis replicados (n = 7) de muestras de agua pura fortificadas. Los límites de detección del método fueron de 0.5-1 ng/mL con el detector UV y de 0.1-0.3 ng/mL con el detector coulombimétrico.
Palabras Clave: clorofenoles, análisis de agua, metodología en línea, extracción en fase sólida, cromatografía de líquidos.

Introduction

Chlorination is one of the most widely used processes for water disinfection in water treatment plants. However, chemical disinfection processes lead to the formation of undesirable disinfection by-products that can be potentially toxic or carcinogenic. Due to the abundance of phenolic structures in the humic and fulvic material of natural waters, a variety of chlorophenols are susceptible to form upon chlorination of surface or ground waters. These species may be degraded to haloforms and haloacetic acids, or may persist for long time in the treated water, depending on the chlorination conditions and the properties of the feed water [1]. The presence of chlorophenols in the drinking water network is of concern because they increase the organoleptic properties of water producing disagreeable tastes and odors. From the early work of Burttschell [2], it appears that 2-chlorophenol, 2,4-dichlorophenol and 2,6-dichlorophenol are mainly responsible for malodorous water properties in water supplies, as their threshold odor concentration is extremely low (2-3 ng/mL). In addition to this problem, some chlorophenols are suspected carcinogenic (2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol) or toxic for wildlife and human; severe restrictions have been thus established for their maximal concentration in drinking and natural waters [3-5]. A regular monitoring of chlorophenols in water treatment plants and water supplies is of importance, not only to satisfy official regulations, but also to improve chlorination processes and for a better understanding of the reaction paths and transformation of natural organic matter in the presence of disinfection agents.

The determination of the 19 chlorinated monohydroxy benzenes at the parts per billion (ppb = ng/mL) level or less in treated water (tap water, drinking water), is not a trivial problem, despite the relative cleanliness of the matrix. A previous preconcentration of the sample is necessary to achieve the required sensitivity of analysis. However, the wide range of hydrophobicity in the chlorophenol family renders very difficult to find suitable extraction-preconcentration conditions for all the compounds [6,7].

Actually, solid-phase extraction (SPE) has become the preferred method for the extraction and preconcentration of organic compounds from aqueous matrices. Indeed, the on-line coupling of SPE with a chromatographic technique has demonstrated enhanced accuracy, precision and sensitivity for trace

analysis [8]. In previous works, on-line SPE-HPLC methods were developed for the determination of the 5 most hydrophilic priority pollutant phenols in ground water [9], and for the determination of 8 nitro and chlorophenols in the same matrix [10]. Although many other proposals have been presented for the determination of chlorophenols in aqueous matrices, using SPE in several formats (cartridges, membranes, fibers, sorptive stir bar) or modes (off-line, on-line) for sample pretreatment, most of them are limited to the study of only some members of the family [6-7,11-16]. As previously mentioned, the 19 chlorophenols are susceptible to form in water treatment plants and persist in the drinking water network, with still unknown long-term health effects for most of them. In this respect, the works by Rodríguez [17-18] and Sarrion [19] are outstanding because they report extremely sensitive methods (limits of detection in the pg/mL range) for the determination of 16 or 19 chlorophenols, respectively, in water samples. The first group used carbon and polymeric adsorbent cartridges for off-line SPE of the analytes, which were derivatized pre- or post-concentration for their further determination by gas chromatography (GC) with atomic emission detector, or other highly powerful detection systems. Main drawbacks of this method are large sample volumes (250-2000 mL) and the high risk of sample losses or contamination during the derivatization/solvent evaporation step. The second group used solid-phase microextraction (SPME) to preconcentrate analytes on a fiber, which was further desorbed in a solvent mixture and injected in an HPLC system with amperometric detector. A problem with SPME processes is that they are generally not conducted at equilibrium conditions and a rigorous control of experimental variables is necessary for reproducible results. Besides, the two described methods are not amenable to automation and this limitation renders them less attractive for routine analysis.

In a recent work, an original sorbent was proposed for the SPE of chlorophenols with very different hydrophobicity [20]. Silica particles with a layer of adsorbed cationic surfactant micelles were successfully used for batch extraction or column extraction and preconcentration of 5 chlorophenols, with different degree of substitution (1 to 5 chloro substituents), from an aqueous solution buffered at pH 9. Although this proposal seems very attractive because all compounds were well recovered (>95%), the stability of the solid sorbent in the alkaline media is questionable, and was not studied or informed in the report.

From the previous discussion, the aim of this work was to develop a reliable and robust methodology, allowing the routine monitoring of all members of the chlorinated phenol family in water treatment plants and water supplies at concentration levels conforming to Mexican regulations (established in the low ppb range for the most toxic chlorophenols). On-line SPE-HPLC technology was chosen because phenol derivatization is not necessary (as in GC) and sample manipulation is minimized. To ensure peak identity and peak purity and to confirm quantitative results, two detection modes were used, the robust UV detector and the more sensitive coulometric detector. An additional advantage of on-line methods is that they offer the possibility of full automation of analyses.

Experimental

Reagents and solutions

Chlorophenol and phenol standards were purchased from Chem Service with certified purity of at least 98%. The list of compounds and two of their most relevant properties are presented in Table 1. HPLC-grade methanol and acetonitrile were from Prolabo (France). Type 1 reagent water was obtained from a Nanopure deionizer (Barnstead Thermolyne, model 04747). Other common chemicals were analytical reagent grade from various furnishers. Stock solutions of individual phenols (500 mg/l) were prepared in acetonitrile and stored at -20°C. Working standards were mixtures of phenols at different concentrations prepared in acetonitrile-water 50:50 (v/v); these standards were kept in refrigeration (4°C) when not in use, and were renewed periodically.

Equipment and chromatographic conditions

Figure 1 shows a diagram of the experimental setup. The sample preparation and the sample analysis sections in this setup are on-line coupled by means of a Rheodyne 7000 switching valve (**SV** in Fig. 1). The chromatographic section consisted of a Perkin-Elmer Series 200 binary pump (**P_1**), a Rheodyne BIO-7125 injector (**i**) with a home-calibrated loop (20 ± 0.3 µl), a reversed phase

Table 1. Relevant properties of the chlorophenol family [21,22].

Compound	pKa	Log K_{ow}*
Phenol	9.89	1.57
2-chlorophenol	8.49	2.29
3-chlorophenol	8.85	2.64
4-chlorophenol	9.18	2.53
2,3-dichlorophenol	6.44	3.26
2,4-dichlorophenol	7.67	3.20
2,5-dichlorophenol	6.34	3.36
2,6-dichlorophenol	6.79	2.92
3,4-dichlorophenol	7.38	nf
3,5-dichlorophenol	6.92	3.60
2,3,4-trichlorophenol	7.65	nf
2,3,5-trichlorophenol	7.36	nf
2,3,6-trichlorophenol	7.13	nf
2,4,5-trichlorophenol	7.43	4.02
2,4,6-trichlorophenol	7.42	3.67
3,4,5-trichlorophenol	7.74	nf
2,3,4,5-tetrachlorophenol	6.95	nf
2,3,4,6-tetrachlorophenol	5.37	4.24
2,3,5,6-tetrachlorophenol	5.48	nf
pentachlorophenol	4.92	5.02

* K_{ow}: octanol-water partition coefficient; nf: not found.

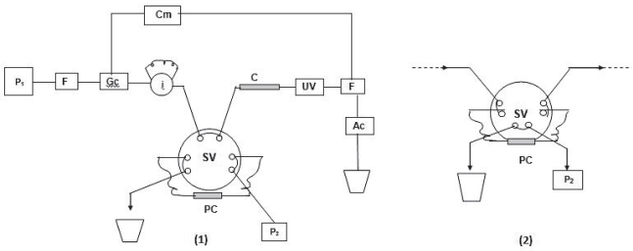

Fig. 1. Experimental setup: (1) sample loading, (2) on-line elution and analysis. $\mathbf{P_1}$: HPLC pump, $\mathbf{P_2}$: auxiliary isocratic pump, **i**: injector, **SV**: switching valve, **PC**: precolumn, **C**: analytical column, **UV**: UV detector, **Cm**: Coulometric detector, **Gc**: guard cell, **Ac**: analytical cell, **F**: filter.

analytical column (**C**), a Perkin-Elmer model 785-A variable wavelength UV detector (**UV**) set at 280 nm, and a Coulochem II model 5200-A coulometric detector (**Cm**) from ESA, equipped with a guard cell (**Gc**) set at 900 mV and an analytical cell (**Ac**) set at 850 mV. The guard cell was placed between the pump and injector to eliminate electroactive impurities from the mobile phase; the analytical cell was connected in series with the UV detector. Both cells were protected by on-line 0.5 μm filters (**F**) to prevent fooling of electrodes with micrometric particles that could be present in the liquid phase. A Turbochem PRO-SI system with an NCI-901 interface (Perkin-Elmer) was used for data acquisition and processing. The sample preparation section consisted of an isocratic Eldex-Duros model CC-100S pump ($\mathbf{P_2}$) and a reversed phase SPE precolumn (**PC**), which was placed in the position corresponding to the loop of the switching valve.

The analytical column (150 x 4.6 mm I.D.) was packed with 5 μm Hypersil ODS (Thermo-Hypersil-Keystone). The SPE precolumn (20 x 2 mm I.D.) was home-packed with the polymeric phase PLRP-S 100A, 10-15 μm (Polymer Laboratories). Three different separation conditions were used according to the hydrophobic character of the analytes. The phenols of lower hydrophobicity (Group 1: phenol + monochlorophenols) were separated with an isocratic mobile phase, consisting of acetonitrile-formiate buffer (0.07 M, pH 3.5) 27:73 (v/v). The compounds of medium hydrophobicity (Group 2: di- and trichlorophenols) were separated by gradient elution; mobile phase A (weak) was acetonitrile-methanol-formiate buffer (0.07 M, pH 3.5) 27:4:69 (v/v), mobile phase B (strong) was acetonitrile-formiate buffer (0.07 M, pH 3.5) 40:60 (v/v), the gradient program was: 0% B from 0-26 min, rapidly increased to 100% B in 3 min, and then constant for 21 min. The most hydrophobic phenols (Group 3: tetra- and pentachlorophenol) were separated under isocratic conditions with an acetonitrile-methanol-formiate buffer (0.1 M, pH 4.3) 29:28:43 (v/v) mobile phase. Separation conditions for Group 2 were designed in such a way that phenols of lower hydrophobicity (c.a. Group 1) were eluted at the beginning of the chromatogram without interfering with the compounds of the studied group. Indeed, the gradient elution program, designed for the separation of di- and trichlorophenols, was capable of separating phenol and monochlorophenols in the same run. A similar precaution was taken for Group 3, where separation conditions were designed to delay the elution of tetrachlorophenols and pentachlorophenol for >15 min, in order to leave enough time for the elution of less hydrophobic phenols (c.a. Groups 1 and 2), if eventually present in the same sample.

Extraction, preconcentration and on-line analysis

All samples were acidified to pH 2 with some μL of concentrated perchloric acid (12 M) prior to analysis. Besides, 1% or 10% (v/v) methanol was added to the samples assigned for the determination of phenols from Groups 2 and 3, respectively. Using the experimental setup shown in Fig. 1, a 6-step procedure was finally adopted for extraction, preconcentration and on-line analysis of samples. Table 2 shows the position of the switching valve and the injector, as well as the status of pumps at each step of the procedure. The flow lines corresponding to the "load" (L) and "inject" (I) positions of the switching valve (**SV**) are shown in diagrams (1) and (2) of Fig. 1, respectively.

Experimental procedure:

1) With the switching valve in load position (**SV**=L), condition the precolumn with 15 ml of: aqueous $HClO_4$ (pH 2) for Group 1, methanol-$HClO_4$ (pH 2) 1:99 (v/v) for Group 2, or methanol-$HClO_4$ (pH 2) 10:90 (v/v) for Group 3 (using pump $\mathbf{P_2}$).
2) Load a sample volume of: 15 ml for Group 1, or 25 ml for Groups 2 or 3 (pump $\mathbf{P_2}$). Simultaneously equilibrate the HPLC column with the mobile phase (pump $\mathbf{P_1}$).
3) Rinse the precolumn with 0.5 ml of reagent water (Pump $\mathbf{P_2}$). Continue the equilibration of the HPLC column (pump $\mathbf{P_1}$).
4) Switch the valve to inject position (**SV** = I) for on-line elution and HPLC analysis of the preconcentrated sample (pump $\mathbf{P_1}$).
5) Inject a working standard as reference for quantitation (pump $\mathbf{P_1}$).
6) Return the valve to load position (**SV** = L) and regenerate column and precolumn with water and acetonitrile (15 mL each; pumps $\mathbf{P_1}$ and $\mathbf{P_2}$).

During steps 1-4, the injector (**i** in Fig. 1) is maintained in the load position and the loop is filled with a working standard at the end of sample analysis. The injector is then turned to inject position (step 5) and the standard mixture is sent through the precolumn and column for separation and analysis. In this way, the standard is subjected to the same interactions and spreading than the sample. To shorten the analysis time, the HPLC column is equilibrated with the appropriate mobile phase (according to the phenol group under study) in one circuit of the setup, while simultaneously the precolumn is loaded with the sample and rinsed in the other circuit (steps 2 and 3). After step 4, and only for Group 2, the HPLC column must be

re-equilibrated with the initial mobile phase of the gradient program prior to injection of the reference standard. At the end of the procedure, the column and precolumn are rinsed and regenerated, independently of each other, using the two pumps and flow circuits. A flowrate of 1 mL/min is used throughout. Although the system was manually controlled in this work, the whole 6-step procedure can be fully automated using a sample-prep module and an autosampler.

Results and discussion

Method development

The adsorbent type, the sample composition and the sample volume are critical parameters for the optimization of SPE processes, especially in the on-line mode because a very small precolumn is used for preconcentration of the compounds of interest.

In the case of aqueous samples, probably the best compromise for the extraction of analytes with very different polarity or hydrophobicity is a polymeric reversed phase adsorbent, such as PLRP-S. The styrene-divinylbenzene matrix of this adsorbent is more retentive than conventional C-18 phases because of a larger hydrocarbonaceous area. Thus, PLRP-S was used instead of C-18 to increase breakthrough volumes and preconcentration factors of the least hydrophobic phenols in the precolumn. Although graphitized carbon has the strongest retention properties of all reversed phases, it cannot be on-line coupled to analytical C-18 HPLC columns because excessive band broadening occurs during the transfer of analytes from a highly retentive precolumn to a less retentive column [11].

The problem with polychlorophenols was not a weak retention but a too high hydrophobicity that resulted in significant losses of the most chlorinated compounds prior to extraction and preconcentration. It is known that hydrophobic compounds have a strong tendency to adsorb on the walls of vessels, tubing or any other material in contact with the aqueous sample. This "extra-column adsorption" can be eliminated or drastically reduced by addition of organic solvent to the water sample, but at the expense of also reducing the retention of all analytes in the SPE precolumn [10]. Recovery problems for phenol, 2-chlorophenol and/or pentachlorophenol have been quite common and are reported in several works [6-13]. Therefore, to avoid excessive losses of the less chlorinated or the most chlorinated phenols due to opposite effects (breakthrough from the precolumn or extra-column adsorption, respectively), it became necessary to group the compounds according to their hydrophobic character and to develop appropriate conditions for each group.

First, several samples fortified with phenols of one group were acidified (pH 2) to maintain the compounds unionized, and different methanol aliquots were added to each sample (0-10%, v/v, of organic solvent). Then, different volumes (5 to 25 mL) of the fortified samples were loaded in the precolumn, on-line eluted and analyzed. From the obtained recoveries, the appropriate sample composition and sample volume were determined for that group. The same series of experiments was then repeated for the other groups. For the group of lowest hydrophobicity (Group 1), only 15 ml of the plain aqueous sample could be preconcentrated, mainly because of the weak retention of phenol and 2-chlorophenol on PLRP-S and their rapid breakthrough from the precolumn. Di- and trichlorophenols (Group 2) were well retained in the precolumn, but a 1% (v/v) content of methanol in the sample was necessary to avoid some losses of the trichlorinated derivatives that were observed in plain water. Under these conditions, the maximum assayed sample volume (25 mL) could be preconcentrated with good recoveries for all compounds of the group. Although larger sample volumes could probably be preconcentrated without recovery problems for Group 2, no attempt was made to increase this volume because 25 mL was sufficient to achieve the determination of these compounds at concentrations in the low ppb range. Besides, the preconcentration of larger sample volumes also represents longer analysis times. The highly hydrophobic tetra- and pentachlorophenol (Group 3) have the strongest retention on reversed phases but, paradoxically, the measured recoveries from plain water samples were too low (~ 45-60%). The addition of up to 10% (v/v) methanol to the samples was not sufficient to completely eliminate the losses due to extra-column adsorption; indeed, the obtained recoveries with this organic solvent content remained in the range 85-89%, independently of the preconcentrated sample volume. The latter indicates that breakthrough from the precolumn did not occur when 25 mL of sample containing 10% (v/v) methanol was preconcentrated, so this condition was kept for the determination of Group 3. Higher methanol contents were not assayed because the increase of recoveries could not compensate the loss of sensitivity associated to sample dilution.

The efficient on-line transfer of preconcentrated phenols from the small precolumn to the analytical C-18 column was performed with isocratic mobile phases of appropriate strength (Groups 1 and 3) or by gradient elution (Group 2). Figures 2, 3 and 4 show typical chromatograms obtained from the analysis of spiked reagent water samples, according to the procedure and conditions described in the experimental section for each

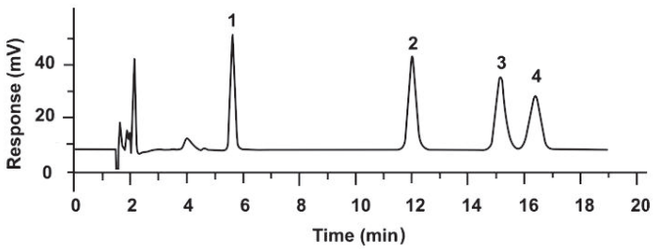

Fig. 2. Coulometric detector chromatogram of Group 1, obtained from preconcentration and on-line analysis of a reagent water sample spiked at 5 ng/mL of each compound. Solutes: 1) phenol, 2) 2-chlorophenol, 3) 4-chlorophenol, 4) 3-chlorophenol.

group. As previously mentioned, the gradient elution program was capable of separating the 16 phenols corresponding to Groups 1 and 2 (Fig. 3). However, the two groups were independently determined because the SPE conditions for di- and trichlorophenols were not adequate for a good recovery of Group 1, in particular for phenol.

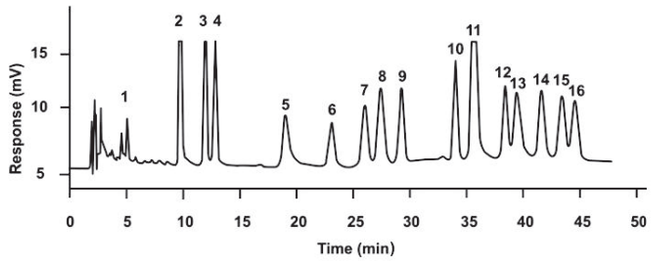

Fig. 3. UV chromatogram of Groups 1 and 2, obtained from preconcentration and on-line analysis of a reagent water sample spiked at 20 ng/mL of each compound. Solutes: 1) phenol, 2) 2-chlorophenol, 3) 4-chlorophenol, 4) 3-chlorophenol, 5) 2,6-dichlorophenol, 6) 2,3-dichlorophenol, 7) 2,5-dichlorophenol, 8) 2,4-dichlorophenol, 9) 3,4-dichlorophenol, 10) 3,5-dichlorophenol, 11) 2,3,6-trichlorophenol, 12) 2,3,4-trichlorophenol, 13) 2,4,6-trichlorophenol, 14) 2,4,5-trichlorophenol 15) 3,4,5-trichlorophenol, 16) 2,3,5-trichlorophenol.

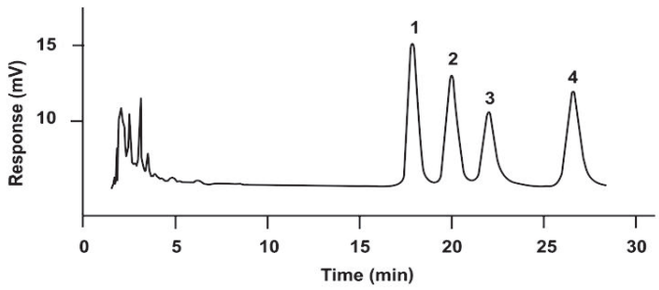

Fig. 4. UV chromatogram of Group 3, obtained from preconcentration and on-line analysis of a reagent water sample spiked at 50 ng/mL of each compound. Solutes: 1) 2,3,4,5-tetrachlorophenol, 2) 2,3,4,6-tetrachlorophenol, 3) pentachlorophenol, 4) 2,3,5,6-tetrachlorophenol.

Method evaluation

The general procedure described in the experimental section and summarized in Table 2, was used for the analysis of water samples spiked with standard mixtures of the phenols of interest at known concentration. Quantitation was performed by comparing the peak areas of sample chromatograms with those of directly injected working standards. However, other experimental variables should also be considered for determination of solute concentrations or solute recoveries when preconcentrated samples are on-line analyzed. Equations E-1, E-2, and E-3 were used to calculate the different parameters required for method evaluation.

- Mass (ng) of phenol "*i*" in the studied sample (Qi_S):
  $Qi_S = Ci_S * V_S * F$ (E-1)
- Mass (ng) of phenol "i" recovered or determined (Qi_R):
  $Qi_R = (Ai_S * Ci_E * V_E) / Ai_E$ (E-2)
- Percent recovery of phenol "*i*" (%R*i*):
  $\%Ri = (Qi_R / Qi_S) * 100$ (E-3)

Where, Ai_S and Ai_E are the peak areas of phenol "*i*" in the sample chromatogram and in the external standard chromatogram, respectively; Ci_S and Ci_E are the concentrations (ng/ml) of phenol "*i*" in the sample and in the external standard, respectively; V_S is the volume of sample preconcentrated in the precolumn (15 mL for Group 1, or 25 mL for Groups 2 and 3); V_E is the volume of injected external standard (0.020 ml for the calibrated loop used in this work); F is a correction factor that accounts for sample dilution due to the addition of methanol (F=1 for Group 1, F=0.99 for Group 2, and F=0.90 for Group 3).

The method linearity was evaluated from the recovery curves (Qi_R versus Qi_S) obtained for each phenol. The parameters derived from application of linear regression analysis to the experimental data of chromatograms obtained from UV and Coulochem detectors are reported in Table 3. Determination coefficients (r^2) were ≥ 0.997, demonstrating linear behavior of the 20 phenols when determined under appropriate conditions (Groups 1, 2 and 3) and using either UV or electrochemical detection. Indeed, the variance of the adjusted curves obtained for each phenol with the two detectors showed no significant difference (F-test) in the range of studied concentrations

Table 2. On-line preconcentration – analysis procedure

Step	Operation	Switchingvalve[a](**SV**)	Injector[a](**i**)	Auxiliarypump[b](**P_2**)	HPLCpump[b](**P_1**)
1	precolumn conditioning	L	L	+	-
2	sample loading and HPLC column equilibration	L	L	+	+
3	precolumn rinsing and HPLC column equilibration	L	L	+	+
4	on-line elution and analysis	I	L	-	+
5	injection of standard	I	I	-	+
6	column/precolumn regeneration	L	I	+	+

[(a)] position of valves: L = load, I = inject [(b)] status of pumps: + = run, - = stop

Table 3. Adjusted parameters of Recovery Curves[a]. Concentration range (ng/mL) 3-75, except Group 1: 1.5-75 (UV detector) and 0.5-75 (Coulochem detector).

Compound[b]	UV		Coulochem		Group
	Ordinate (ng)	Slope	Ordinate (ng)	Slope	
Phenol	0.39	0.72	-0.01	0.72	1
2-chlorophenol	-0.9	0.83	-0.72	0.82	1
4-chlorophenol	-3.5	0.92	0.21	0.88	1
3-chlorophenol	0.04	0.92	0.01	0.92	1
2,6-dichlorophenol	25	0.97	-2.7	0.98	2
2,3-dichlorophenol	6.1	0.99	11	0.95	2
2,5-dichlorophenol	8.2	0.99	8.3	0.94	2
2,4-dichlorophenol	29	0.93	-1.8	0.96	2
3,4-dichlorophenol	-25	1.00	-25	0.96	2
3,5-dichlorophenol	4.4	0.95	5.3	0.93	2
2,3,6-trichlorophenol	6.8	0.94	6.3	0.93	2
2,3,4-trichlorophenol	-1.4	0.99	0.72	0.96	2
2,4,6-trichlorophenol	12	0.97	3.1	0.94	2
2,4,5-trichlorophenol	23	0.92	8.4	0.93	2
3,4,5-trichlorophenol	-7.6	0.98	2.3	0.96	2
2,3,5-trichlorophenol	11	0.95	0.12	0.96	2
2,3,4,5-tetrachlorophenol	1.2	0.88	-1.8	0.88	3
2,3,4,6-tetrachlorophenol	0.8	0.89	-1.7	0.88	3
Pentachlorophenol	-11	0.89	-6.8	0.88	3
2,3,5,6-tetrachlorophenol	-2.9	0.86	-6.3	0.89	3

[a] Determination coefficient, $r^2 \geq 0.997$

[b] Compounds listed according to elution order

(specified in Table 3). However, accurate and precise determination of chlorophenols at concentrations < 1 ng/mL, can only be achieved with the more selective and sensitive coulometric detector, especially when complex samples (surface water or waste waters) are analyzed [7,11,12,19]. Concentrations over 75 ng/mL were not examined because they are not likely to be found in natural waters or treated water. The ordinates and slopes of the adjusted linear curves also are important indicators of method performance. From statistical analysis (t-test), it was demonstrated that the ordinate of all curves was equivalent to zero; therefore, no systematic errors were detected in the proposed method. The slope of each curve represents the mean recovery of the corresponding compound in the range of studied concentrations. As observed in Table 3, all recoveries are over 85%, with the exception of 2-chlorophenol (82-83%) and phenol (72%). It is obvious that breakthrough volumes of these phenols in the PLRP-S precolumn were less than 15 mL (analyzed sample volume for Group 1). However, due to the relatively extended profile of frontal curves in polymeric adsorbent precolumns, the fraction of non retained compound commonly is largely surpassed by the fraction of retained compound when the percolated sample volume is a little larger than the breakthrough volume. Therefore, the moderate losses of phenol and 2-chlorophenol with the processed 15-mL sample volume were largely compensated by increased preconcentrated amounts and higher sensitivity of analysis for all phenols of Group 1.

The recoveries for Group 3 (86-90%) were a little lower than those of Group 2 (> 90%). This result was expected as extra-column adsorption of tetrachlorophenols and pentachlorophenol could not be completely eliminated by addition of 10% methanol to samples.

The previous results clearly show that it was not possible to determine the 20 members of the chlorophenol family with only one analysis of the water sample at fixed conditions. Chromatographic separation was not a problem because the developed gradient elution program was already capable of separating 16 phenols and could be easily modified to include the 4 remaining compounds. On the contrary, extraction and preconcentration of the sample, which is an unavoidable step for ppb concentration levels, could not be performed using the same sample volume and sample composition for the 20 phenols. Very low recoveries and poor quantitative results would be obtained for several phenols in that case. Use of off-line SPE, instead of the on-line mode, may ameliorate the recovery of the least chlorinated phenols because of the much larger sorbent amount in cartridges compared to precolumns; however, recovery problems would persist for the most hydrophobic compounds [6,13,17]. Liquid-liquid extraction was another alternative, but the drawbacks associated to this technique are discouraging and it is uncertain whether phenol and monochlorophenols would be acceptably recovered. Interestingly, we observed that monochlorophenols were not too badly recovered using the conditions developed for di- and trichlorophenols, as their measured recoveries were: 80% for 2-chlorophenol, 82% for 3-chlorophenol, and 84% for 4-chlorophenol. On the contrary, the recovery of phenol was lower than 40% under these conditions,

and presented a great variability in replicate analysis of the same sample. Therefore, if the determination of phenol is not necessary for a given sample, mono- di- and trichlorophenols could be determined together, simplifying and speeding the analysis.

In general, it has been considered that recoveries of the order of 70% are sufficiently good in environmental trace analysis, if the required sensitivity is attained [23]. In the present work, preconcentration factors with the proposed methodology were about 1000 (by comparison with a 20 µL injection), and all compounds could be adequately determined at low ppb levels. Method detection limits (MDL) using the UV detector at 280 nm were in the range 0.5-1.0 ng/ml (s/n = 3) in spiked reagent water samples. Electrochemical detection is more sensitive and the MDL with our coulometric detector conditions were 0.1-0.3 ng/mL (s/n = 3) for the same samples. These detection limits are similar to those reported in recent works, where SPME on fibers or sorptive bar was used for the preconcentration of some chlorophenols from water samples [14-16]. Although SPME is a rapid and simple sample preparation technique, analytical methods based on this technique are in general less robust than those based on on-line SPE.

Method accuracy and precision were evaluated from replicate analysis (n = 7 or 8) of samples spiked with standard phenol mixtures at various concentrations. Equation E-4, derived from rearrangement of previous mathematical relations (E-1 to E-3), was used to determine phenol concentrations in the analyzed samples ($Ci_{S(exp)}$).

$$Ci_{S(exp)} = (Ai_S * Ci_E * V_E) / (Ai_E * V_S * F * \%Ri/100) \qquad \text{(E-4)}$$

Where, $\%Ri/100$ is the mean recovery of phenol "i", as determined from recovery curves (slope of adjusted linear curve, reported in Table 3), and all other variables have been previously defined. Tables 4, 5 and 6 summarize the results of this study for the phenols of Groups 1, 2 and 3, respectively. A good agreement can be observed between the determined mean concentration ($\Sigma Ci_{S(exp)} / n$), and the spiked or "true" concentration, $Ci_{S(true)}$, for all phenols in the analyzed

Table 4. Evaluation of method accuracy and precision for Group 1 (n = 7).

Compound	$Ci_{S(true)}$ [(a)] (ng/mL)	$Ci_{S(exp)}$ [(b)] (ng/mL)	S [(c)] (ng/mL)	%RSD [(d)]
Phenol	1.5	1.39	0.077	5.6
	75	73	1.14	1.6
2-chlorophenol	1.5	1.42	0.088	6.2
	75	73.1	1.11	1.5
4-chlorophenol	1.5	1.56	0.069	4.4
	75	72.1	0.84	1.7
3-chlorophenol	1.5	1.64	0.062	3.8
	75	73.7	0.92	1.2

[(a)] $Ci_{S(true)}$= concentration in spiked sample;
[(b)] $Ci_{S(exp)}$ = determined concentration (mean);
[(c)] S = standard deviation; [(d)] RSD = relative standard deviation

Table 5. Evaluation of method accuracy and precision for Group 2 (n = 7).

Compound	$Ci_{S(true)}$ [(a)] (ng/ml)	$Ci_{S(exp)}$ [(b)] (ng/ml)	S [(c)] (ng/ml)	%RSD [(d)]
2,6-dichlorophenol	20	19.5	0.83	4.2
2,3-dichlorophenol	20	20.6	0.95	4.6
2,5-dichlorophenol	20	18.8	0.63	3.4
2,4-dichlorophenol	20	19.1	0.86	4.5
3,4-dichlorophenol	20	18.2	0.84	4.6
3,5-dichlorophenol	20	19.1	0.84	4.4
2,3,6-trichlorophenol	20	18.9	1.04	5.5
2,3,4-trichlorophenol	20	18.8	0.86	4.6
2,4,6-trichlorophenol	20	18.3	0.98	5.4
2,4,5-trichlorophenol	20	20.6	0.60	2.9
3,4,5-trichlorophenol	20	19.1	0.68	3.6
2,3,5-trichlorophenol	20	20.5	0.64	3.1

[(a)] $Ci_{S(true)}$= concentration in spiked sample;
[(b)] $Ci_{S(exp)}$ = determined concentration (mean);
[(c)] S = standard deviation; [(d)] RSD = relative standard deviation

Table 6. Evaluation of method accuracy and precision for Group 3 (n = 8).

Compound	$Ci_{S(true)}$ [(a)] (ng/mL)	$Ci_{S(exp)}$ [(b)] (ng/mL)	S [(c)] (ng/mL)	%RSD [(d)]
2,3,4,5-tetrachlorophenol	20	19.1	0.76	4.0
	50	49.3	0.76	1.5
2,3,4,6-tetrachlorophenol	20	19.3	0.90	4.7
	50	49.6	1.17	2.4
pentachlorophenol	20	19.4	0.73	3.7
	50	49.1	1.04	2.1
2,3,5,6-tetrachlorophenol	20	19.7	1.10	5.6
	50	49.7	1.03	2.1

[(a)] $Ci_{S(true)}$= concentration in spiked sample;
[(b)] $Ci_{S(exp)}$ = determined concentration (mean);
[(c)] S = standard deviation; [(d)] RSD = relative standard deviation

samples. Statistical comparison (t-test) demonstrated that the determined and true concentrations were equal, at the 5% significance level. Besides, the relative standard deviation of the determined concentration was less than 6.5% in all cases, which is an excellent precision for trace analysis.

Application of the proposed methodology to the speciation and determination of chlorophenols formed from the chlorination of phenol, at conditions commonly found in water treatment plants, was very successful.

Conclusions

A methodology was developed for the determination of the chlorophenol family (phenol and 19 chlorinated monohydroxy benzenes) at low ppb concentration levels in water samples. This methodology is based on the on-line coupling of solid-phase extraction and liquid chromatography with UV detection and electrochemical detection. The experimental procedure is relatively simple and can be fully automatized; however, three fractions of the water sample (different volume and organic modifier content) must be analyzed to achieve good recoveries and the required sensitivity for the 20 phenols. Under the proposed conditions for the three phenol groups (low, medium and high hydrophobicity), solute recoveries obtained in the analysis of fortified reagent water samples were ≥ 82%, except for phenol (72%), the method accuracy (100%) and precision (%RSD < 6.5%) were excellent, and the method detection limits were 0.5-1 ng/ml with the UV detector, and 0.1-0.3 ng/mL with the coulometric detector.

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