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Short communication

A direct Capillary Liquid Chromatography with electrochemical detection method for determination of phenols in water samples

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ABSTRACT

A fast and direct method based on the use of Capillary Liquid Chromatography (LC) with electrochemical (EC) detection has been described for phenols pollutants in water samples. Concretely, phenol, o-cresol, 2-chlorophenol and bisphenol A have been selected as target analytes. The combination of Capillary LC with EC detection avoided the necessity of preconcentration steps typically used in environmental analysis. The sample injected volume was 2 μL. The achieved detection limits were between 1 and 2 μg/L and the linear dynamic range was up to 50 μg/L for all studied phenols. The precision and uncertainty were satisfactory. The analysis time per sample was 10 min. The proposed procedure has been proved useful for treated waters.

1. Introduction

European Water Framework Directive (2000/60/CE) [1], partially modified by Directive 2008/105/CE [2], watches over the protection of surface waters in all the Member States. Directly related with this legislation is the development of new analytical methodologies that reaches the needed sensitivity but with environmental friendly processes, *i.e.* minimization of the amount of solvents [3].

Phenols are one the families of compounds covered by this legislation as they are considered as hazardous substances for the environment and human health. Also, the Environment Protection Agency [4] classifies most of these compounds as priority pollutants because of toxicity, bioaccumulation and persistency. Those compounds at concentration levels of low $\mu g\,L^{-1}$ can be found in the environment mainly due to industrial wastes.

Solid-phase extraction (SPE) and liquid–liquid extraction (LLE) combined with liquid chromatography and different detection modes have been traditionally used for the analysis of phenolic compounds. HPLC-UV [5–7], fluorescence [8–10], electrochemical [11–14] and mass spectrometry (MS) [15,16] detection have been described for the analysis of several phenols. Although these methodologies have reported good detection limits, the main drawbacks have been the high volumes of samples processed and solvents employed as well as long analysis time in some cases. In an attempt to miniaturize and simplify the preconcentration process several procedures have been recently published. Liquid–liquid microextraction (LLME) [7], solid-phase microextraction (SPME) [6,17], ultrasound-assisted headspace liquid-phase microextraction (UAHS) [18] and stir bar sorptive extraction (SBDE) [19] are some examples of these new preconcentration techniques for phenolic compounds.

Particularly, HPLC with EC detection has been proven to be an excellent alternative to determine phenols at low concentration level. Preconcentration with SPE [20–22] has been described. On line SPE [23–25] has also been proposed. More recently, SPME has been used for the determination of different phenolic compounds in water samples [14]. By another hand, the use of surface modified electrodes has been also proposed to enhance sensitivity in the determination of phenolic compounds [26–28]. Recently, a review has been published with the new developments of non-traditional types of electrodes [29].

Capillary Liquid Chromatography [30] can be an excellent candidate to substitute the conventional HPLC. In this sense, an automated in-column-μ-HPLC-UV method has been developed for phenols [31]. Good sensitivity can be reached and the micro dimensions of those systems allow the reduction of the solvent volumes.

Table 1 compares some experimental details of different works published in the last 10 years. As can be seen different strategies for preconcentration or derivatization as well as the use of micro separation techniques have been proposed.

The combination of Capillary LC with EC detection can be a good choice for the analysis of electroactive compounds with high sensitivity and selectivity. The commercialization of EC detectors equipped with electrochemical cell compatible with Capillary LC systems has facilitated the mentioned coupling. Capillary LC with

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Table 1
Comparative of some experimental details and detection limits obtained with HPLC and Capillary LC for phenols in the last 10 years.

Sample	Pretreatment				Separation/detection technique	LOD (μg/L)	Ref.
	Preconcentration		Derivatization				
	Technique	Time (min)	Reagent	Time (min)			
Lake water	LLME	25	–	–	HPLC-UV	0.5–0.6	[6]
River and waste water	SPME	33	–	–	HPLC-UV	0.2–3.7	[7]
Sea and tap water	SPME	120	–	–	HPLC-UV	0.9–3.8	[17]
Lake, tap and pond water	UAHS	20	–	–	HPLC-UV	6–23	[18]
Sea and lake water	SBSE	60	–	–	HPLC-UV	1.0–2.6	[19]
Drinking and industrial water	–	–	C6SCl[a]	20	HPLC-Fl	0.1–0.9	[10]
River and waste water	SPME	32	–	–	HPLC–EC	0.02–4.5	[14]
Tap and mineral water	In-column	62	–	–	Capillary LC-UV	0.004–0.013	[31]
Treated waste water	Not required	–	Not required	–	Capillary LC–EC	1–2	This work

[a]Coumarin-6-sulphonyl chloride.

EC detection is surely a success for the development of analytical procedures that accomplished the requirements of the environmental analysis. This technique has been used for the analysis of L-dopamine in food samples [32] and serotonin [33] in clinical samples. Since our knowledge, this is the first time that Capillary LC with EC detection has been used in the environmental field.

This paper has been focussed on the development of a direct analytical procedure based on the use of Capillary LC with EC detection for the analysis of phenolic compounds, such as phenol, o-cresol, 2-chlorophenol and bisphenol A in treated water samples. Phenol and o-cresol were selected by their abundant use in the world, bisphenol A since it is a component of polycarbonate plastics and epoxy resins and 2-chlorophenol because it can be a by-product of phenol in chloride water treatment. Phenol and 2-chlorophenol are listed as EPA priority pollutants and bisphenol A is under evaluation by EPA for action plan development owing to its harmful effects as endocrine disruptor.

The combination of Capillary LC with EC detection avoided the necessity of preconcentration steps typically used in environmental analysis. Improved analysis time and minimized consumption of organic solvents were the main characteristics achieved in reference to previously published methods for estimating phenols in waters.

2. Experimental

2.1. Reagents

Phenol, o-cresol, 2-chlorophenol, bisphenol A and chloride potassium were purchased from MERCK (Schurchardt, Germany). Acetonitrile was obtained from J.T. Baker (Deventer, Holland).

Working standard solutions of the analytes between 5 and 50 $\mu g\,L^{-1}$ were prepared by dilution of the stock solutions with ultrapure water obtained from a Nanopure II System (Sybron, Barnstead).

2.2. Apparatus and chromatographic conditions

The capillary chromatographic system consisted of a capillary pump (Agilent 1100 Series, Waldbronn, Germany) equipped with a high-pressure six port injection valve with an internal loop of 2 μL (Rheodyne model 7725). The detection was carried out with an INTRO electrochemical detector (Antec Leyden, Netherlands) with a wall-jet configuration μVTO3 flow cell which was operated in DC mode. A 0.7 mm glassy carbon electrode and an Ag/AgCl (ISAAC) reference electrode and a 25 μm spacer were used for the electrochemical measurements. We used 2 mM KCl in the mobile phase because of the use of an ISAAC reference electrode, thus the analytes were eluted at oxidation potential in the range of 0.70–1.0 V. Filter and range values were also optimized in the range of 0.1–5 and 1–10 nA/V, respectively.

The surface of the working electrode was cleaned by wiping the electrode surface with a tissue wetted in ethanol once a day. The electrodes were polished with the conventional method one a week. The ISAAC reference electrode was also polished once a week.

A Zorbax SB C18 (150 mm × 0.5 mm i.d., 5 μm particle diameter) column (Agilent) was used for the analytes separation. The studied mobile phases were acetonitrile–potassium chloride 2 mM (35:65) and (30:70) in isocratic elution mode at a flow rate 10 μL/min. All solvents were filtered through 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain) and degassed with a vacuum degasser on-line (Agilent 1100 Series).

In the process 2 μL solution volumes were injected in the chromatograph, and chromatograms were obtained for the electrochemical condition above mentioned.

2.3. Water samples

Waters samples were sampled at different effluents just before discharging to the sea along the coast of the Comunidad Valenciana. Samples were directly processed.

3. Results and discussion

3.1. Optimization of the chromatographic method

The preliminary studies were carried out with phenol 10 $\mu g\,L^{-1}$, 2 μL was used as injection volume and mobile phase acetonitrile:2 mM KCl (35:65) for optimizing EC detection. The range was studied between 1 and 10 nA/V (filter and potential were fixed at 1 and 0.80 V, respectively). Fig. 1 shows the influence of the range value on the shape of the baseline for blank solutions. The range affected the recorder output voltage and it helped to control the zero compensation, the chromatograms in Fig. 1 shows that 1 nA/V resulted in the best baseline. Fig. 2A shows the normalized signal vs range for the testing solution, the best signal was obtained for a range value of 1 nA/V. Filter was studied between 0.2 and 5 (range and potential were fixed at 1 nA/V and 0.80 V, respectively), although the results did not reveal any significative difference between these values, the value 1 for filter resulted in the best signal as can be seen in Fig. 2B.

We evaluated the analytical signal in the range between 0.7 and 0.9 V (range and filter were fixed at 1 nA/V and 1, respectively). We selected 0.85 V as the optimum potential (see Fig. 2C). The best values for range, filter and potential (1 nA/V, 1 and 0.85 V, respectively) were assayed for mixtures of phenol, o-cresol, 2-chlorophenol and bisphenol A. Because all analytes presented good signals and the

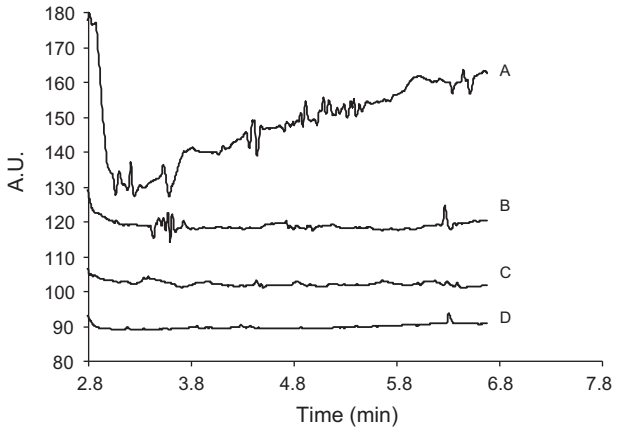

Fig. 1. Influence on the baseline of the EC detector range value for a blank injection (A) 10, (B) 5, (C) 2 and (D) 1 nA/V. (*A.U. arbitrary nits*). Injection volume: 2 μL, mobile phase: acetonitrile:2 mM KCl (35:65). Detector parameters: filter 1 and potential 0.80 V.

scope was testing all analytes at once no further optimization was carried out.

The injection volume was studied by processing different volumes between 2 and 20 μL (mobile phase of acetonitrile:2 mM KCl (35:65)). This study was carried out by injecting a mixture of phenol, o-cresol, 2-chlorophenol and bisphenol A (10 μg L^{-1}). The resolution values, *Rs*, obtained with 2 μL as injection volume were 1.9, 0.3 and 3.5 for $Rs_{\text{phenol–o.cresol}}$, $Rs_{\text{o-cresol–2.chlorophenol}}$ and $Rs_{\text{2-chlorophenol–bisphenol A}}$, respectively. The other injection volumes provided worse resolution than 2 μL as they were too high for Capillary LC. These results were in agreement with the results obtained by Parrot et al. [33]. An injection volume of 2 μL was selected as optimum value.

The composition of the mobile phase was also studied. This composition was chosen taking into account the optimal use of the ISAAC reference electrode using KCl in the mobile phase. Mixtures of acetonitrile and 2 mM KCl 35:65 and 30:70 were tested. By another hand, we also avoided the use of buffer solution in the mobile phase in order to eliminate clogging of the Capillary LC system. The resolution values were increased from 1.9 to 4.8, from 0.3 to 2 and from 3.5 to 8.5 for $Rs_{\text{phenol–o.cresol}}$, $Rs_{\text{o-cresol–2.chlorophenol}}$ and $Rs_{\text{2-chlorophenol–bisphenol A}}$ when the mobile phase was acetonitrile: 2 mM KCl 30:70. Thus, we selected this mobile phase as the mixture of the analytes was completely resolved.

Flow rate was also studied as it is an important parameter in Capillary LC with EC detection. We selected 10 μL/min for further studies. Lower flow rates resulted in a loss on the efficiency as dispersion of signal was higher. An increase on the flow resulted in the loss of *Rs* between o-cresol and 2-chlorophenol.

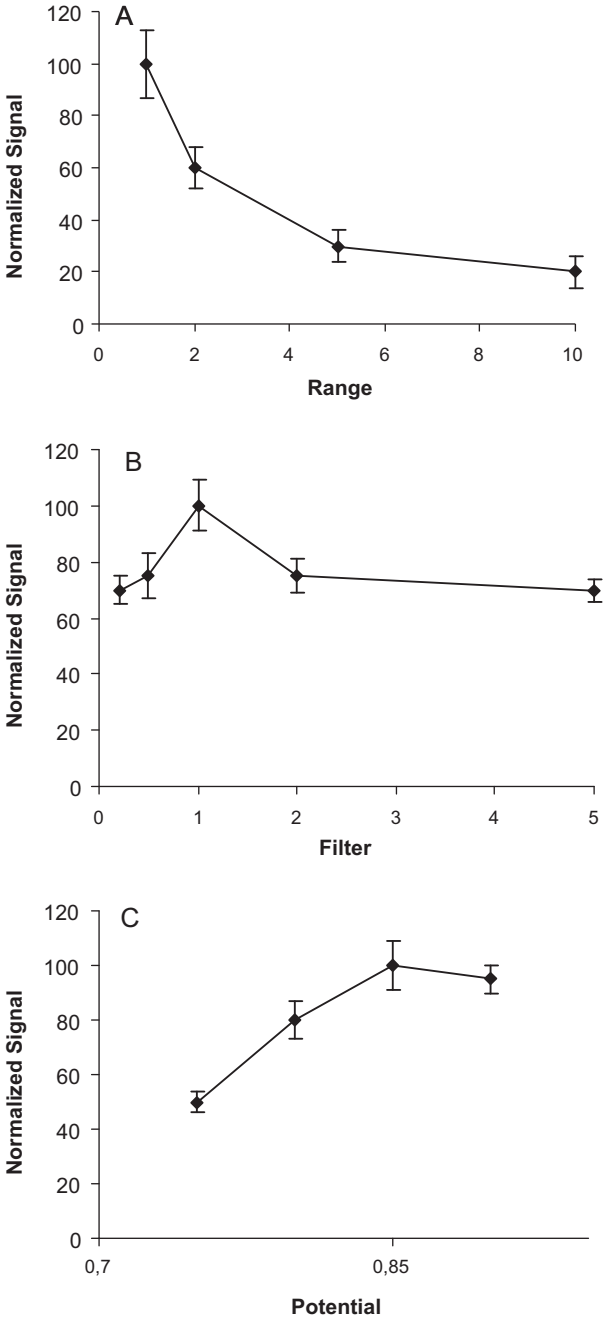

Fig. 2. Normalized signal as function of (A) output range, (B) filter values and (C) potential (for conditions, see text).

3.2. Analytical parameters

Table 2 summarizes the figures of merit of the proposed method, namely, linear range, regression equation, detection limit (LoD) and quantification limit (LoQ). The linear interval was from low μg/L to 50 μg/L for all the analytes tested. The procedure detection limits were calculated experimentally by injecting successive diluted solutions (from 5 to 1 μg L^{-1}) of the analytes. Intraday relative standard deviations (%RSD) for a mixture of phenol, o-cresol, 2-chlorophenol and bisphenol A (10 μg/L) was also calculated and the values were between 4 and 2% ($n = 3$). Fig. 3A and B shows the chromatogram obtained for a blank and a mixture of phenol, o-cresol, 2-chlorophenol and bisphenol A (10 μg L^{-1}), respectively.

Table 2
Figures of merit for the Capillary LC–EC detection method.

	Linear range (μg/L)	Calibration curve $y = (b_1 \pm s_{b_1})x + (b_o \pm s_{b_o})$ (μg/L)	R^2	LoD (μg/L)	LoQ (μg/L)
Phenol	3–50	$y = (520 \pm 10)x + (1600 \pm 80)$	0.9998	1	3
o-Cresol	3–50	$y = (240 \pm 30)x + (300 \pm 200)$	0.9860	1	3
2-Chorophenol	3–50	$y = (680 \pm 70)x + (4000 \pm 900)$	0.9891	1	3
Bisphenol A	6–50	$y = (138 \pm 5)x + (1600 \pm 80)$	0.9988	2	6

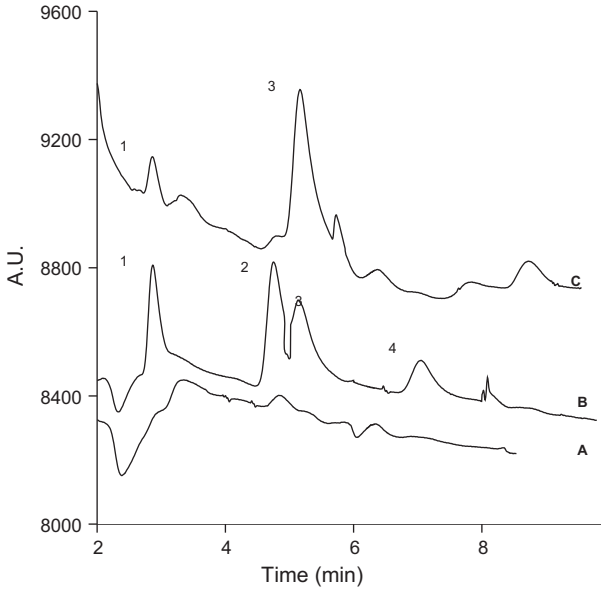

Fig. 3. Chromatograms obtained in the optimum conditions for (A) blank, (B) mixture of phenol, o-cresol, 2-chlorophenol and bisphenol A (10 μg/L) and (C) water sample. 1: phenol, 2: o-cresol, 3: 2-chlorophenol and 4: bisphenol A. (*A.U. arbitrary units*). Injection volume: 2 μL, mobile phase: acetonitrile:2 mM KCl (30:70). Detector parameters: range 1 nA/V, filter 1 and potential 0.85 V.

The results showed that the combination of the Capillary LC with the EC detection seemed to be a good alternative for the direct analysis of phenols with a low time-consuming procedure and with sensitivity comparable with previously reported methods that uses preconcentration techniques. Some experimental parameters as well as the LoD obtained are compared with previously published methods in Table 1. The main advantage of the proposed method was the elimination of the preconcentration step simplifying the analytical procedure and reducing the analysis time to a minimum. Besides, the proposed procedure reached to detection limits comparable with many of the detection limits obtained with the previously published procedures [6,7,14,17–19].

3.3. Analysis of water samples

Five water samples were analysed using the proposed method. These samples were collected in five different effluents just before discharging to the sea. Under these conditions the total analysis time per sample was 10 min. Firstly, we evaluated the recovery of the analytes with spiked water samples with a mixture of the analytes (10 μg/L). The mean recoveries obtained for each analyte in the five samples was 90 ± 4%, 86 ± 2%, 93 ± 4%, 89 ± 5% and 87 ± 3% for phenol, o-cresol, 2-chlorophenol and bisphenol A ($n = 5$), respectively. These results indicated satisfactory recoveries independently of the sampling point. Thus we concluded that matrix effect could be depreciated.

Only phenol and 2-chlorophenol were found in one of the processed samples at concentrations of 3.7 ± 0.1 and 14.9 ± 0.6 μg L^{-1}, respectively. The corresponding chromatogram appears in Fig. 3C.

3.4. Contribution and future trends

The development of analytical methods suitable for large scale environmental monitoring of pollutants is one of the most important tasks of Analytical Chemistry. In this sense, the development of direct analytical methodologies in which the pretreatment steps are eliminated, minimizing the analysis time, simplifying the global analytical procedure and using easy-handling equipment are of special interest for the environmental analysis. This paper is focussed in this direction, as we have used Capillary Liquid Chromatography combined with EC detection for the analysis of phenols in environmental water samples. This combination has provided good sensitivity without the necessity of any preconcentration step. From our point of view this paper contributes to the development of simplified methodologies by using Capillary Liquid Chromatography with EC detection and we think it could be extensive their use in environmental analysis.

4. Conclusions

We have demonstrated that the combination of Capillary LC with EC detection is an attractive alternative for the analysis of phenols in environmental analysis, concretely for the analysis of water samples. We have developed an analytical method that does not need any additional preconcentration step to reach the sensitivity required for those analytes in water samples. This sensitivity was comparable to that reported in previously published work that uses different preconcentration strategies. In addition, the proposed methodology yielded to numerous advantages such as low time consuming and low solvent consumption owing to the use of Capillary LC system.

The combination of Capillary LC with EC detection avoided the necessity of preconcentration steps typically used in environmental analysis, such as liquid–liquid microextraction (LLME) or solid-phase microextraction (SPME), providing an excellent sensitivity (<2 μg L^{-1}). Thus, we have simplified the analysis procedure to a minimal, reducing not only the analysis time but also the amount of solvents.

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