Journal of Chromatography A, 1229 (2012) 288–292

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Determination of phenols with ion chromatography–online electrochemical derivatization based on porous electrode–fluorescence detection

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ARTICLE INFO

Article history:
Received 15 November 2011
Received in revised form 7 January 2012
Accepted 11 January 2012
Available online 18 January 2012

Keywords:
Phenols
Ion chromatography
Porous electrode
Fluorescence detection

ABSTRACT

The current study describes the determination of phenols using ion chromatography–online electrochemical derivatization–fluorescence detection (IC/ED/FD). Six model phenols including 4-methylphenol (pMP), 2, 4-dimethylphenol (DMP), 4-tert-butylphenol (TBP), 4-hydroxylphenolacetic acid (pHPA), 4-acetamidophenol (pAAP), and phenol (P) were well separated on an anion-exchange column under ion exchange mode using NaOH with small amount of acetonitrile added as eluent. Online electrochemical derivatization performed via a laboratory-made electrolytic cell (EC), consisting of porous titanium electrode and cation-exchange membrane (CEM), allows the oxidation products that are strongly fluorescent to be detected by the fluorescence detector. NaOH eluent used in the present method matches well with the maximal fluorescence intensity obtained at alkaline condition for oxidized phenols, thus the addition of specific buffer solution after oxidation encountered in previous report could be eliminated. This process leads to a simplified procedure. The proposed method was sensitive to the limits of detection in the range of 0.4 μg/L and 3.8 μg/L and the limits of quantification between 1.2 μg/L and 13 μg/L due to the strong electro-oxidation capacity of porous titanium electrode, as well as the implementation of time-programmed potential over EC. The linear ranges were 2.0–1.0×10^4 μg/L for pAAP and DMP, and 10–1.0×10^4 μg/L for P, pMP, pHPA, and TBP, respectively. The relative standard deviations range from 0.9% to 4.8%. The utilization of the method was demonstrated by the analysis of real samples. The average spiked recoveries of target analytes in pool water were 81.0–118%.

1. Introduction

Phenolic compounds have attracted great concern in recent years due to their high toxicity and bio-recalcitrant effect in the ecosystem water cycling process [1]. Hence, numerous techniques have been studied and developed to determine phenols. However, most of these detection techniques focuses on high performance liquid chromatography (HPLC) equipped with various kinds of detectors, such as UV, electrochemical, fluorescence, and mass spectroscopy (MS) [2–5]. Among these detection techniques, fluorescence detector is a better choice in terms of selectivity and sensitivity. Therefore, HPLC combined with fluorescence detector (HPLC/FD) have been used in numerous applications in trace analysis. However, some phenols have weak fluorescent property, and post-column derivatization is often required to convert these compounds into strong fluorescent substances that can then be efficiently detected by the fluorescence detector [6,7]. Using online electrochemical derivatization, Karst et al. presented a method to determine mono-substituted phenols via HPLC equipped with fluorescence detector (HPLC/ED/FD) [7]. This method addressed the problems on phenols that could not be detected via fluorescence detector. However, the separation was performed by common silica-based C_{18} separation column. Unfortunately, the silica column works well only in the pH range of 2–8 (pH < 3 in the report), whereas the optimum pH for producing the fluorescence of oxidized phenols is basic (pH ~ 10). Obviously, the separation condition could not match well with that of downstream detection. Therefore, buffer solution of NH_3/NH_4Cl at pH 9.5 had to be added to the effluent from the column to perform the electrochemical conversion to enhance the fluorescence signal. Additional pump and mixing tee were also required, which made the procedure more complicated.

Ion chromatography (IC) has now been a well-established technique for the analysis of ionic compounds because of the rapid developments, such as stationary phase and hyphenated technique [8]. The polymer-based stationary phases (e.g., divinylbenzene/ethylvinylbenzene, DVB/EVB) in IC dominate most of the applications due to their wide pH tolerance (0–14). Moreover,

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doi:10.1016/j.chroma.2012.01.025

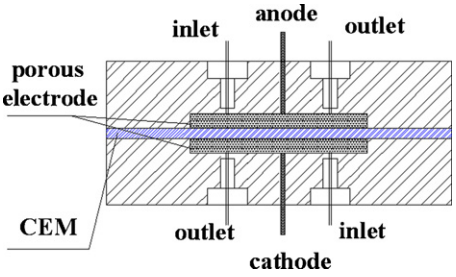

Fig. 1. Schematic diagram of self constructed electrolytic cell. CEM: cation exchange membrane.

the polymer-based column can work well in alkaline solution (e.g., pH ~ 10). The choice of alkaline eluent matching with the downstream fluorescence detection will not be a barrier if the phenols could be well separated by IC. Based on these considerations, a method to determine phenols, where their separation is performed using IC combined with online post-column electrochemical derivatization and fluorescence detection (IC/ED/FD), has been developed in the current study. In other words, the separation of phenols is carried out in the anion exchange column with basic eluent, and the electro-oxidation of phenols is performed using a laboratory-made electrolytic cell (EC) consisting of porous titanium electrode and cation exchange membrane (CEM). The present method eliminates the use of additional setup and greatly simplifies the operating procedures.

2. Experimental

Phenols, including 4-methylphenol (pMP), 2, 4-dimethylphenol (DMP), 4-tert-butylphenol (TBP), 4-hydroxylphenolacetic acid (pHPA), 4-acetamidophenol (pAAP), 4-nonylphenol (pNP), and phenol (P) were purchased from Aladdin Co. (Shanghai, China). The 2(3)-t-butyl-4-hydroxyanisol (BHA), 2, 4, 5-trihydroxybutrophenone (THBP), 2,6-di-tert-buyl-4-hydroxymethylphenol (Q), 3,5-di-tert-4-butylhydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), 2-chlorophenol, 2,6-dichlorophenol, 2,4,5-trichlorophenol, 4-nitrophenol, and 2,6-nitrophenol were purchased from Huipu Co. (Hangzhou, China). All the above reagents were of analytical grade. Acetonitrile (HPLC grade, TEDIA Company, Inc., Fairfield, OH, USA), sodium hydroxide (analytical grade, Huipu Co., Hangzhou, China), and Milli-Q water (Millipore, Molsheim, France) were used as chromatographic eluents. All other reagents or solvents used in the current work were of analytical grade.

Stock standard solutions of phenols were prepared at 200.0 mg/L with acetonitrile, and were further diluted with water–acetonitrile (90:10) to prepare a series of mixed working standard solutions. The stock standard solutions were then stored in the refrigerator at −4 °C. The working standard solutions were freshly prepared every day.

Isocratic elution was performed on a Dionex ion chromatographic system (Dionex, Sunnyvale, CA, USA) consisting of a P680 pump, an ASI-100 automated sample injector, a UVD340U diode array detector, and an IonPac AS11 column (4 mm i.d. × 250 mm long) preceded by an IonPac AG11 guard column (4 mm i.d. × 50 mm long). The fluorescence signal was recorded from a RF-535 fluorescence detector (Shimadzu Co. Japan) via Dionex UCI 100 universal chromatography interface. Data collection and processing were performed with a personal computer equipped with Dionex Chromeleon 6.8 software. The mobile phase was 10 mmol/L NaOH with 10% (v/v) acetonitrile. The flow rate was 1.0 mL/min and the injection volume was 20 μL.

The schematic diagram of EC is shown in Fig. 1. The fabrication of EC was as follows. Briefly, two quadrate PVC blocks (93 mm × 42 mm × 12 mm) were held together by screws (not shown in Fig. 1). One chamber in each block was machined with the size matching the porous titanium electrodes (56 mm × 14 mm × 1 mm), which permitted perfect incrustation of electrodes to the block. The porous electrode with an average pore diameter of 50 μm and thickness of 1 mm was cut from the commercially available porous titanium plate (Taixin Metal Co., LTD., Baoji, China). Cation-exchange membrane (CEM, Shanghai Chem. Co., Shanghai, China) was soaked in purified water for 24 h before use. It was then cut to the desired shape (93 mm × 42 mm × 0.5 mm) and was sandwiched between the two electrodes. Moreover, the electrical connection with the porous working electrode was created using a very thin Pt wire (0.1 mm) piercing through the PVC block. Power supply was provided with Hull cells (Xianke Scientific Instrument Co., Taishan, China). The time-programmed working potential implemented throughout the experiment was at 5.0 V for the first 4.5 min, and was then adjusted to 1.0 V for the rest of the procedure.

The water samples used for real sample analysis were obtained from a pool in Hangzhou and from a refinery company in Hangzhou (Hangzhou, China), respectively. The samples were first filtered through a 13 mm membrane syringe filter (Xiboshi, pore size 0.45 μm, Tianjin Fuji Tech Co., Tianjin, China), and then directly injected into the IC system.

3. Result and discussion

Six phenols were chosen to be the target analytes. Each analyte was determined whether its oxidation product had fluorescence property using flow injection analysis/electrochemical derivatization/fluorescence detection (FIA/ED/FD). pMP, BMP, TBP, as well as phenol were tested to be satisfactory at some fixed potential. Moreover, pHPA and pAAP were also investigated and have been proven to be credible based on literature [6,7]. However, 4-nonylphenol (pNP), which was asserted by [7] to be fluorescent after oxidation, yielded no response in the current initial experiments. Thus, it could not be selected for analysis.

Methodologies dealing with the separation of phenols via IC have been reported previously [9,10]. In the current study, attempts have been made to obtain optimal separation conditions by testing different anion exchange columns as well as eluent types. Initially, Dionex IonPac AS12A column, which were packed with DVB/EVB particle functionalized by alkyl quaternary amine group, was chosen for the separation of pMP, DMP, and phenol, with carbonate/bicarbonate buffer plus acetonitrile used as eluent. Bad resolutions (<0.8) and poor peak shapes (peak width at half height of every analyte was > 2.0 min) were observed. The plate numbers (N) of these three analytes were less than 300, which indicate poor separation efficiency. Moreover, some analyte (TBP) could not be eluted from the column at a suitable time even with the addition of 40% acetonitrile as organic modifier.

AS11 column, a hydroxide-selective column commercially available from Dionex Corp, has been widely used for the separation of anions using NaOH or KOH as eluent. Subsequently, small amount of organic solvent can be added to the eluent to modify the selectivity when organic anions are separated due to unspecific interaction. On the other hand, phenols are known to be in the form of weakly acidic anions in the alkaline solution. The dissociation constant (pK_a) of pAAP, pMP, and phenol is 9.51, 10.27, and 10.0, respectively. The current experiment revealed that these phenolic anions with high pK_a value could be eluted from the AS11 column under ion exchange mode using 10 mmol/L NaOH + 10% ACN as eluent. Chromatographic parameters, such as retention time, resolution,

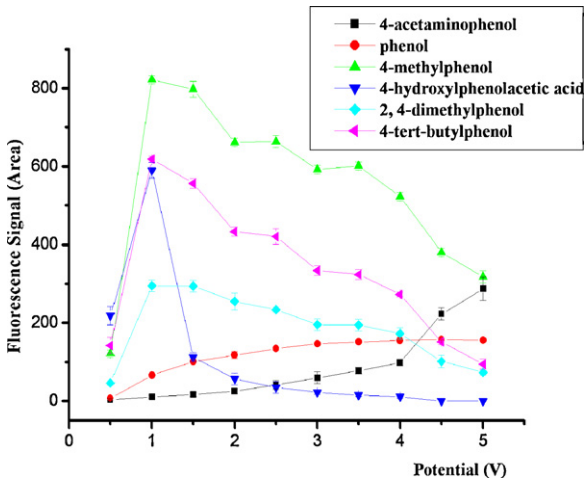

Fig. 2. The effect between the fluorescence signal of phenols (~10 mg/L) and potential. Experimental conditions: $\lambda_{ex}/\lambda_{em}$ = 320/410 nm.

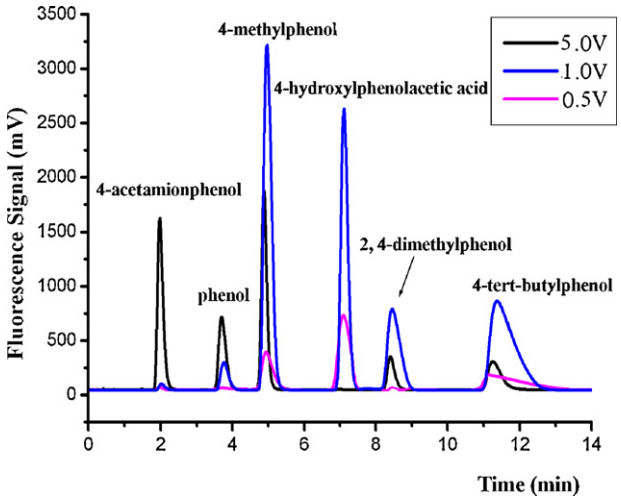

Fig. 3. Chromatograms of phenols (~10 mg/L) at different potential. Column: IonPac AG11 (4 mm i.d. × 50 mm long) and AS11 (4 mm i.d. × 250 mm long). Eluent: 10 mmol/L NaOH with 10% (v/v) acetonitrile at the flow rate of 1.0 mL/min, fluorescence detection: $\lambda_{ex}/\lambda_{em}$ = 320/410 nm.

and peak efficiency, are listed in the Supplementary data (SI-Table 1). All selected phenols could be well separated on AS11 column for 15 min with good resolution and peak shapes. On the other hand, maximum fluorescence signals could be obtained in this alkaline eluent condition, and the addition of post-column buffer solution for electrochemical oxidation used in previous report could be avoided. From this point, the use of IC mode for the separation of phenols is advantageous relative to the HPLC mode, in which the common silica-based column could not bear alkaline solution and post-column addition of buffer solution is required.

The fluorescence signal of phenols after oxidation was optimized by manually presetting the emission and excitation wavelength ranging from 260 nm to 440 nm and 350 nm to 490 nm, respectively. The potential was preliminarily fixed at 1.0 V. The maximum excitation and emission wavelength ($\lambda_{ex}/\lambda_{em}$) of pMP, DMP, TBP, pHPA, pAAP, and phenol was 320/420, 300/410, 320/410, 320/430, 320/410, and 320/410 nm, respectively. Moreover, no marked difference of the emission and excitation wavelengths between analytes was observed (e.g., <7% loss of the fluorescence intensity at 320/410 nm was found for pMP, DMP, and pHPA) compared with the fluorescence intensity at 320/410 nm. Thus, 320/410 nm was finally chosen to be the optimal emission and excitation wavelengths for all analytes. This condition was consistent with previous studies [7].

An electrolytic cell was fabricated using porous Ti electrode to make electrochemical derivatization efficient. Porous Ti electrode has been widely employed in the fabrication of electrochemical sensors and detectors in flowing analytical systems (e.g., flow injection analysis and ion chromatography) since 1980s [11–16]. The porous Ti plate is cheap, commercially available, and electrochemically stable, thus it is a good electrode for flowing electrochemical reaction. The porous electrode has been reported to exhibit effective surface area, which is four times larger than that of the planar Ti electrode [17,18]. Hence, it was selected as the working electrode for the current work. Electro-oxidation of phenols can occur in both the inner and the surface of the porous electrode [19]. As a result, strong oxidation capacity could be obtained, which greatly improves the detecting sensitivity.

The cation exchange membrane isolates the anode and cathode and prohibits the diffusion of phenolic anions and eluent. As a result, oxidized phenols were confined in the anode chamber, avoiding their decomposition at the cathode.

NaOH solution with acetonitrile served as both the eluent for separation and the supporting electrolyte for electro-oxidation. Typically, the resistor between the anode and cathode of EC increased with the increase of organic solvent content in the supporting electrolyte, thus, the potential over the EC needs to be increased as well. In the current experiment, fluorescence signals were not observed until the potential was set at 0.5 V. When the potential was increased to 1.0 V, except pAAP and phenol, the signal of other phenols had a significant increase and reached a maximal value and then decreased when further increasing the potential. In addition, the fluorescence signal of pHPA disappeared at 4.5 V. In comparison, the fluorescence signal of pAAP and phenol continuously increased with the increase of the potential in the range of 0.5–5 V, and the signal at 5.0 V was ~ 290 times higher than that of 0.5 V. Moreover, electrodialytic gases were found to interfere with the stable signal when the potential exceeded 5.5 V. The relationship between potential and fluorescence signal of phenols is shown in Fig. 2. The fluorescence signal at every potential was the mean of three determinations, and the standard deviations were from 0% to 4.8%. The background signal for baseline was from 47.47 mV at 0.5 V to 49.08 mV at 5.0 V, in which, only 3.3% increase was observed. This phenomenon indicated that the potential had a negligible effect upon the background noise level. Fig. 3 shows the chromatograms of selected phenols at typical potentials, such as 0.5, 1.0, and 5.0 V. Flat baselines were observed at different potential. However, peak tailing of TBP was observed at 0.5 V. This observation might have resulted from the incomplete decomposition of TBP at such a low potential and the strong interaction with the stationary phase. However, better peak shape was observed with the increase of potential (see Fig. 3). Figs. 2 and 3 show that this potential-dependent relationship of the fluorescence signal gave a clue that time-programmed potential is necessary during the analysis procedure: the potential was set at 5.0 V at the beginning of 4.5 min for pAAP and phenol, and then 1.0 V was fixed for the remaining time to obtain maximum signals for every phenolic compound.

The oxidation products of phenols were biphenols or oligomers. The fluorescence signal changed dramatically compared with monomeric phenols [7,20]. Moreover, the mechanism of the electro-oxidation of some phenols was also discussed in literature [7]. However, the claim that all phenols reached their highest peak value under the potential of 900 mV was different from the potential effect of the current work. The effect of potential on the fluorescence property might have resulted from the different structure of phenols. pAAP and phenol might have the same reaction mechanism and the oxidized products might have similar

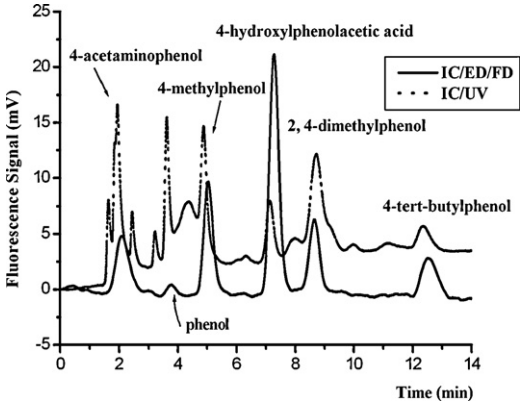

Fig. 4. Interference effect of phenols with IC/ED/FD and IC/UV. *Conditions*: UV-DAD detection: λ = 254 nm; potential: 5.0 V in the first 4.5 min and 1.0 V in the rest of the procedure. Other conditions are same as Fig. 3.

structure. Similar conclusion could be obtained from the rest of the phenols in the current work. However, detailed reasons for this electrochemical oxidation mechanism need to be further explored.

The data for IC/ED/FD and IC/UV, such as limits of detection (LODs), limits of quantification (LOQs), linear ranges, and regression coefficients for the plot vs. concentration, are summarized in Table 1. The LODs were determined as a signal-to-noise ratio (SNR) of the three and LOQs were set at SNR = 10. In the method of IC/ED/FD, the LODs were between 0.4 μg/L and 3.8 μg/L, whereas LOQs were from 1.2 μg/L to 13 μg/L. LODs and LOQs of every phenol determined by the proposed method were about one or two order of magnitude lower than that of IC/UV, which indicated that fluorescence detection after electrochemical derivatization was more sensitive than UV.

The described IC/ED/FD was compared with other techniques reported in literature with respect to the determination of phenols, as shown in Table 2. Normally, the proposed method was less sensitive than the amperometric detection [21]. However, the LOD of pAAP of the proposed method was one order of magnitude lower than postcolumn oxidation with H_2O_2 [6]. In addition, the LODs and LOQs for all phenols have been found to be comparable with those of previous reports [7]. Moreover, for some analytes, e.g., pHPA, the LOD in the current work was ~30 times lower than that of HPLC/ED/FD [7]. In summary, the proposed method was comparably sensitive, or even more sensitive, than the postcolumn derivatization techniques reported previously. This condition was partly because of the stronger oxidation capacity of the porous electrode in the self constructed EC. Meanwhile, the use of time-programmed potential over EC attributed partly to the improvement of sensitivity. The proposed method was much simple compared to that of previous reports [7].

In the current work, the interference effects of some related phenols were investigated. Some chlorophenols and nitrophenols without native fluorescence, such as 2-chlorophenol, 2,6-dichlorophenol, 2,4,5-trichlorophenol, 4-nitrophenol, and 2,6-nitrophenol [22], could not emit fluorescence signals after electrochemical oxidation. In addition, BHA, THBP, Q, BHT, and TBHQ were added to the standard solution to test the interference effects, which were simultaneously carried out by IC/ED/FD and IC/UV, as shown in Fig. 4. The concentration of interference phenols, such as BHA, THBP, Q, BHT, and TBHQ was about 1.0 mg/L, whereas the concentration for the target analyte was 10 μg/L for pAAP, 30 μg/L DMP, 100 μg/L for phenol, 200 μg/L for pMP and TBP, and 600 μg/L for pHPA, respectively. Severe interference was obtained using IC/UV, whereas no interference was observed in IC/ED/FD. This observation is another demonstration that the much stronger selectivity, compared with UV detection, can be

Table 1
Analytical figures for the determination of phenols by using IC/ED/FD and IC/UV.

	IC/ED/FD					IC/UV				
	Linear range (μg/L)	R^2	[a] RSD (n = 7)	LOD (μg/L)/(M)	LOQ (μg/L)/(M)	Linear range (μg/L)	R^2	[a] RSD (n = 7)	LOD (μg/L)	LOQ (μg/L)
pAAP	2.0–1.0×10^4	0.9972	3.7	$0.4/2.4 \times 10^{-9}$	$1.2/8.2 \times 10^{-9}$	10–1.0×10^4	0.9992	3.2	2.5	8.3
P	20–1.0×10^4	0.9986	4.6	$3.8/3.9 \times 10^{-8}$	$13/1.3 \times 10^{-7}$	1.0×10^2–1.0×10^4	0.9995	3.1	19	63
pMP	10–1.0×10^4	0.9992	2.0	$1.2/1.0 \times 10^{-8}$	$4.0/3.7 \times 10^{-8}$	1.0×10^2–1.0×10^4	0.9995	3.1	29	96
pHPA	10–1.0×10^4	0.9994	0.9	$1.4/8.5 \times 10^{-9}$	$4.6/3.0 \times 10^{-8}$	1.0×10^2–1.0×10^4	0.9999	2.4	29	96
DMP	2.0–1.0×10^4	0.9992	3.2	$0.5/4.3 \times 10^{-9}$	$1.8/1.5 \times 10^{-8}$	1.0×10^2–1.0×10^4	0.9994	2.7	26	90
TBP	10–1.0×10^4	0.9988	4.8	$1.9/1.3 \times 10^{-8}$	$6.3/4.2 \times 10^{-8}$	1.0×10^2–1.0×10^4	0.9990	4.6	21	70

[a] The relative standard deviations (RSD) were studied by seven replicates run of analytes with the concentration of 50 μg/L.

Table 2
Comparison of methods for the determination of phenols with direct amperometric detection and with postcolumn oxidation and fluorescence detection.

Separation	Detection	Postcolumn oxidation	LOD (mol/L)	Ref
RP-HPLC	Amperometric	–	5.4×10^{-11} for TBP	[21]
RP-HPLC	Fluorescence, 329/435 nm	H_2O_2	2×10^{-8} for pAAP	[6]
RP-HPLC	Fluorescence, 320/410 nm	Electrochemical	2.7×10^{-7} for pHPA	[7]
IC	Fluorescence, 320/410 nm	Electrochemical	1.3×10^{-8} for TBP, 2.4×10^{-9} for pAAP, 8.5×10^{-9} for pHPA	This work

Table 3
Analytical results of sample from wastewater.

Sample no.	Analytes	[a] Found	Added	Reclaimed	Recovery (%)	RSD (%) ($n = 3$)
1 (IC/ED/FD)	pAAP	[b] N.D.	5	5.4 ± 0.002	108	0.6
	P	N.D.	20	16.2 ± 0.005	81.0	5.7
	pMP	N.D.	10	11.8 ± 0.004	118	0.5
	pHPA	N.D.	20	18.4 ± 0.003	92.0	2.1
	DMP	N.D.	5	4.8 ± 0.012	96.0	0.6
	TBP	N.D.	20	21.4 ± 0.003	107	4.6
2 (IC/ED/FD)	pMP	0.95 ± 0.001	2.0	2.73 ± 0.034	93.0	1.3
2 (IC/UV)	pMP	0.99 ± 0.004	2.0	3.40 ± 0.010	114	0.3

[a] Each represents the value (mean ± SD) of three determinations.
[b] N.D., not detectable.
The concentration unit is μg/L for sample no. 1 and mg/L for sample no. 2.

generated by the combination of the electrochemical derivatization and fluorescence detection.

To demonstrate the utilization of the proposed method, real sample of wastewater from a pool and a refinery factory was collected to determine whether some pollutants, such as phenol, pMP, and DMP exist. The results are shown in Table 3, where sample no. 1 was from a pool and sample no. 2 was from a refinery factory in Hangzhou, China. Results show that no phenols were detected in sample no. 1 using the proposed method or IC/UV. However, pMP was found in sample no. 2, and the results determined by IC/ED/FD were with relative errors of 4.0% compared with the results detected by IC/UV. The chromatograms of the spiked phenols in sample no. 1 and pMP in sample no. 2 were listed in the Supplementary data of SI-Figs. 1 and 2.

4. Conclusion

Analysis of phenols was successfully carried out using anion exchange chromatography combined with post-column electrochemical derivatization and fluorescence detection. The alkaline eluent under anion exchange mode eliminated the use of additional buffer electrolyte, thus simplifying the analysis procedure without sacrificing the sensitivity compared with the previous method using HPLC/ED/FD. The applied time-programmed potential on a laboratory-made electrochemical cell greatly enhanced the oxidation capacity, leading to high sensitivity.

Acknowledgements

This research was financially supported by National Natural Science Foundation of China (Nos. 20775070, 20911140271, J0830413), Zhejiang Provincial Natural Science Foundation of China (Nos. R4080124, Y4090104, Y4090078), National Science Foundation of Anhui Provincial Education Department (Nos. KJ2011B211, 2010SQRL189) and the opening foundation of the State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital of Medical College, Zhejiang University (grant No. 2010KF07). We thank Hu Liu (West Anhui University, China) for his help of English.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2012.01.025.

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