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Determination of polar priority phenols at parts per trillion levels in water using on-line liquid–solid extraction followed by liquid chromatography with coulimetric detection

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Abstract

A high-sensitivity method for the determination of polar phenolic compounds in water samples was developed. Water samples were preconcentrated using on-line solid-phase extraction with LiChrolut EN sorbent and afterwards were analyzed by liquid chromatography and dual coulimetric detection. The first electrode was set at low potential (250 mV) for sample clean up whereas the second one was used for analytical purposes. The system could not be used in its reductive form due to the lack of reversibility on the electrochemical behaviour of nitrophenols. Detection limits at part per trillion level were obtained using only 5 ml of water. Additionally, the large cell constant of the coulimetric detector lead to low values of coefficients of variation (±6) when working with river water. © 1997 Elsevier Science B.V.

Keywords: Water analysis; Coulimetric detection; Detection, LC; Phenols; Chlorophenols; Nitrophenols

1. Introduction

Toxic and persistent phenolic compounds are listed in the European Community Directive 76/464/EEC concerning dangerous substances discharged into the aquatic environment [1] and in the US-Environmental Protection Agency (EPA) list of priority pollutants [2,3]. Legislation in drinking waters in Europe is very strict and the directive 80/778/EEC states that maximum admissible concentration (MAC) of phenols should not exceed 0.5 μg/l for total phenols and 0.1 μg/l for any individual phenol [4].

Several protocols using both off-line and on-line liquid–solid extraction (LSE) followed by UV or diode array detection were reported [5–7]. However, the problem of the analysis of the more polar phenols, i.e., phenol and catechol, still remains unsolved because of their low breakthrough volume (below 10 ml) in most available sorbent materials. Amperometric detection overcomes this problem because its higher sensitivity which allows to reduce the sample volume [8,9]. Electrochemical detection (ED) of phenolic compounds requires the use of high potentials (around 1 V), so many matrix components are oxidized, thus increasing the background current and chromatographic interferences. In this way, the electrochemical cell often needs to be cleaned up when processing complex matrices. The development of pulse amperometric detection (PAD) has improved the signal stability [10] although the working electrode should often be cleaned up to recover its initial response.

Another alternative is the use of coulimetric detectors [11]. Coulometric array detectors are designed in a way that the eluent flows through a

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porous graphite electrode and leading to a large cell constants which result in an enhancement of both sensitivity and signal stability. The most sophisticated approach consists in a serial array of electrodes at increasing potentials providing three-dimensional chromatograms (similarly to diode array detectors) where analyte voltammograms facilitates peak identification. Up to three orders of magnitude increase in sensitivity was obtained as compared to diode array detectors and limits of detection (LODs) ranging from 0.03 ng/l to 0.38 ng/l were achieved for phenolic compounds when combined with off-line LSE [12]. In this paper, a less sophisticated device having only two working electrodes was used. Even though this approach was successfully used in the food and pharmaceutical industries, few applications were reported up until now in the environmental field [13,14].

In view of previous data the aim of this work is to evaluate the coulimetric detector performance for the analytical determination of 12 priority phenols in water samples when it is combined with on-line LSE. The data obtained will be compared to previous data of our group using conventional amperometric detection.

2. Experimental

2.1. Materials

HPLC-grade water, methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). All the solvents were passed through a 0.45 μm filter from Scharlau (Barcelona, Spain). Catechol and phenol were obtained from Sigma (St. Louis, MO, USA), 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 4-methylphenol and 2,4-dimethylphenol were purchased from Merck and 3-chlorophenol, was obtained from Aldrich (Milwaukee, WI, USA).

2.2. Apparatus

Experiments were performed using an automatic sample processor from Gilson (Villers-le-Bel, France). This system includes: one automatic sample processor model Aspec XL, equipped with two Reodyne six-port valves, one high-pressure preconcentration pump model 305, one low-pressure pump model 401C, one eight-port valve model 817 valve actuator. HPLC system was purchased from Gilson and consists in two pumps model 305, one 811c dynamic mixing chamber and 805 manometric module. Detection was carried out by an ESA Coulochem 5100A (ESA, Bedford, MA, USA) detector with a dual electrode analytical cell (ESA model 5100) equipped with two glassy carbon electrodes and a Pt reference electrode.

2.3. On-line liquid–solid extraction

Stainless steel precolumns of 10×0.2 mm were handpacked with a slurry system purchased from the Free University (Amsterdam, Netherlands). Li-Chrolut sorbent was a gift from Merck. The on-line experimental set up was similar to previous studies carried out by our group [7]. Conditioning of the precolumn was done with 5 ml of methanol and 1 ml water (pH=3) at 1 ml/min. 5–10 ml of spiked water samples, acidified to pH 2.5, were passed through the precolumn at 4 ml/min. After the interferences were eliminated by washing the sorbent with 1.5 ml of water at 1 ml/min, analytes were directly eluted by the mobile phase to the analytical column in back-flush mode. After elution and to avoid memory effects, the precolumn was washed with 5 ml of acetonitrile at 1 ml/min.

2.4. Hydrodynamic voltammograms

2.4.1. Screen mode

The first electrode (E1) potential was set at 0 mv and the potential value of the second electrode (E2) was changed from 0 to 1300 mV with increments of 50 mV. Mobile phase used was 25 m*M* monohydrogenphosphate buffer (pH 5.2)–acetonitrile (75:25). For basic pH experiments pH value of 11 was accomplished by performing post column addition of 15 m*M* NaOH at 0.1 ml/min. Afterwards the current intensity for each chromatographic peak was plotted against the applied potential.

2.4.2. Redox mode

E1 was set at 900 mV and E2 was changed from 100 to −400 mV with increments of 25 mV. Mobile

phase used was 25 m*M* monohydrogenphosphate buffer–acetonitrile (75:25). The pH was set to a value of 11 by performing post column addition of 15 m*M* NaOH at 0.1 ml/min. The data were plotted as reported above.

2.5. Chromatographic conditions

A 150×4.6 mm Hypersil green ENV (C_{18}) analytical column equipped with guard column from Shandon Scientific (UK) was used. Elution was performed isocratically by using 25 m*M* monohydrogenphosphate buffer (pH 5.2)–acetonitrile (75:25). Post column addition of 15 m*M* NaOH was carried out at 0.1 ml/min resulting in a final pH=11. For the screen mode E1 and E2 potentials were 300 mV and 650 mV, respectively and when working in reductive mode were 800 mV and −150 mV, respectively.

2.6. Quantitation

External standard calibration was used for quantitation of the extracts after LSE. Calibration was performed by plotting peak area (y) versus amount injected following on-line precolumn enrichment (x, μg/l). Calibration graphs for screen out mode were plotted using 6 points ranging from 0.01 to 5 μg/l and 5 ml of water sample. Response was linear within this range. LODs were calculated by diluting the water samples until a signal-to-noise ratio of 3 (the ratio between the peak intensity and the noise) was obtained. Table 1 shows the LODs obtained using coulimetric and amperometric detection when processing 5 and 10 ml of water, respectively. For calculation of LODs spiked water samples (50, 10 and 5 ng/l) were processed.

3. Results and discussion

Coulimetric detectors are usually equipped with two electrodes connected in series. In general they can be operated in two main operational modes called screen out and redox mode. When using the screen out approach, the first electrode (E1) is set at low potentials to eliminate interferences and only the second one (E2) is used for analytical purposes. Alternatively the system can be operated in redox mode. In this case E1 is set a high positive potentials to ensure the oxidation of all compounds of interest. Afterwards the oxidation product is reduced in a second step. This approach is interesting in the case of analytes which require high oxidative potentials such as phenols. Potentials around −200 mV can be applied with reduction of background and chromatographic interferences. Hydrodynamic voltammetry (HDV) experiments were performed to discern the more suitable operational mode. HDV was preferred

Table 1
Detection limits (ng/l) of phenolic compounds obtained after on-line LSE followed by coulimetric (screen out mode) and amperometric detection (AD)

Compound	Screen out mode (E1: 350 mV)(E2: 900 mV)	Screen out mode (E1: 250 mV)(E2: 650 mV)	AD (1000 mV)[a]
Phenol	1.8	0.4	20
Catechol	2	1.2	n.d.
4-Methylphenol	2.5	1.6	10
2-Nitrophenol	8.5	2	2000
4-Nitrophenol	8.8	2.3	3000
2,4 Dinitrophenol	1.3	3000	
2,4-Dinitro-2-methylphenol	9	2.5	n.d.
2-Chlorophenol	2	1,3	25
3-Chlorophenol	7	5.4	50
4-Chlorophenol	1.8	0.6	30
2,4-Dichlorophenol	2	0.9	30
4-Chloro-4-methylphenol	4	2.4	10

[a] From Ref. [9].
Sample volume 5 ml (10 ml for amperometric detector).
n.d.: not detected.

over cyclic voltammetry because it closely reproduces the HPLC conditions.

3.1. Screen out mode

Hydrodynamic voltammograms of selected phenols and background current at acid pH are shown in Fig. 1. E1 potential was set at 0 mV and E2 was changed from 0 to 1200 mV. The limiting currents were around 900 mV except for nitrophenols with values higher than 1200 mV. Background currents higher than 1500 mA were found in this latter case thus leading to an unacceptable signal-to-noise ratio. As regards to the oxidation pathway of phenolic compounds [15,16], lower limiting currents could be expected at basic pH. This usually involves a radical mechanism with two successive electron transfers in which a deprotonation occurs either between the two steps or after the second electron transfer. Hence, the limiting current will be easier achieved when working at basic pH because reaction intermediates will be more stable and proton transfer will not occur. Fig. 2 shows the HDV of selected phenolic compounds in basic media. Limiting currents were in general lower than in acid media thus allowing reduction of the E2 potential to 650 mV. In this case values of background currents of around 130 nA were found which lead to a better signal to noise ratio. Hence oxidation in basic media will be selected in most cases. On the other hand from HDV it could also be concluded that maximal value of E1 should be around 250–300 mV.

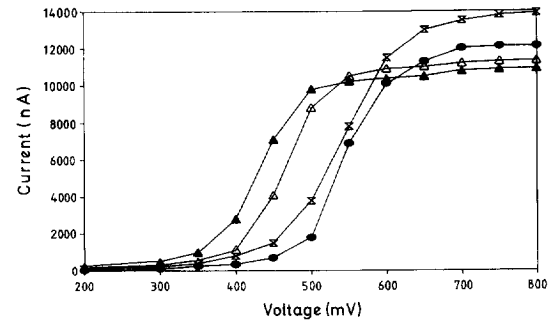

Fig. 2. Hydrodynamic voltammetries of selected phenols in basic conditions using the screen out approach. E1 potential was set at 0 mV and the potential value of E2 was changed from 0 to 1300 mV with increments of 50 mV. Mobile phase: 25 m*M* monohydrogenphosphate buffer–acetonitrile (75:25). pH was set at 11 by performing post column addition of 15 m*M* NaOH at 0.1 ml/min.

3.2. Redox mode

HDV of the reduction step was carried out in a similar way than for the oxidative mode. In order to ensure complete oxidation of phenols, E1 voltage was set over the limiting current reported above (900) mV and E2 value was changed from 100 to −400 mV. Fig. 3 shows the resulting voltammograms for selected phenols. A value around −150 mV was found as a optimal potential for catechol, phenol and chlorophenols although profiles were not as clear as

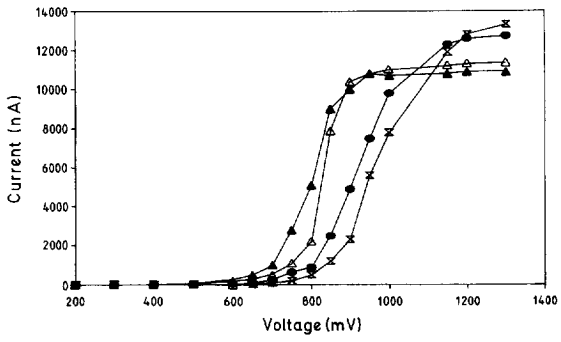

Fig. 1. Hydrodynamic voltammetries of selected phenols in acidic conditions using the screen out approach. E1 potential was set at 0 mV and the potential value of E2 was changed from 0 to 1300 mV with increments of 50 mV. Mobile phase: 25 m*M* monohydrogenphosphate buffer (pH 5.2)–acetonitrile (75:25).

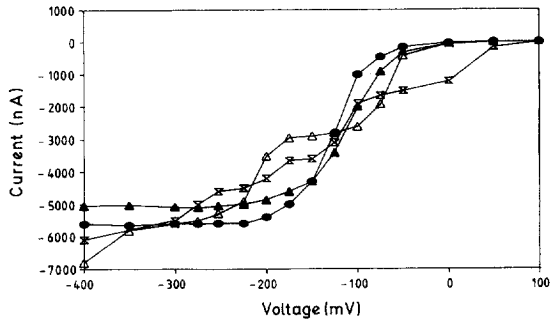

Fig. 3. Hydrodynamic voltammetries of selected phenols in using the redox approach. E1 was set at 900 mV and E2 was changed from 100 to −400 mV with increments of 25 mV. Mobile phase: 25 m*M* monohydrogenphosphate buffer–acetonitrile (75:25). pH was set to a value of 11 by performing post column addition of 15 m*M* NaOH at 0.1 ml/min. The data was plotted as reported above.

in the oxidative mode. Background currents at these range of potentials were around 40–50 nA thus allowing to improve signal to noise ratio. However, the presence of several oxidation products was found for nitrophenols (Fig. 3). The mechanism of the oxidation of phenols reported above is quite complex and involves the formation of cation radicals. In addition, the presence of electron withdrawing and electron resonant substituents will reduce the stability of these intermediates favoring collateral reactions such as hydroxylation or dimerization. This lack of reversibility makes the redox mode unsuitable for nitrophenols although it could be a good approach for the rest of target phenols since better chromatographic profiles could be expected.

3.3. On-line coupling with LSE

A styrene–divinylbenzene sorbent (LiChrolut EN) was selected because it was found to be the most suitable material to effectively trap phenolic compounds from water matrices in a earlier work of our group [17]. However a problem arises from the need to perform the oxidation of the phenolic compounds in basic media since acid pH is required for LC. This was accomplished by using monobasic ammonium phosphate. LC was performed in acidic conditions (pH 5.2) and the eluent pH was changed by carrying out post-column addition of 15 m*M* NaOH at flow-rate 0.1 ml/min thus resulting in pH 11. Pump pulsation was avoided since it could increase background current thus overcoming the positive effect of the basic media oxidation.

When using coulimetric detection, only 5–10 ml water sample were required to achieve the LODs of current EC legislation. Fig. 4 shows a typical chromatographic profile of a ground water sample spiked at 0.1 μg/l level obtained using the screen out mode. In general, at least one order of magnitude improvement was found when using coulimetric detection as compared with amperometric detection. Table 1 compares LODs after on-line LSE in acid and basic media and those found using typical amperometric detection at 1 V potential. This improvement in LODs should be attributed to the complete oxidation of the analytes in the coulimetric detector. On the

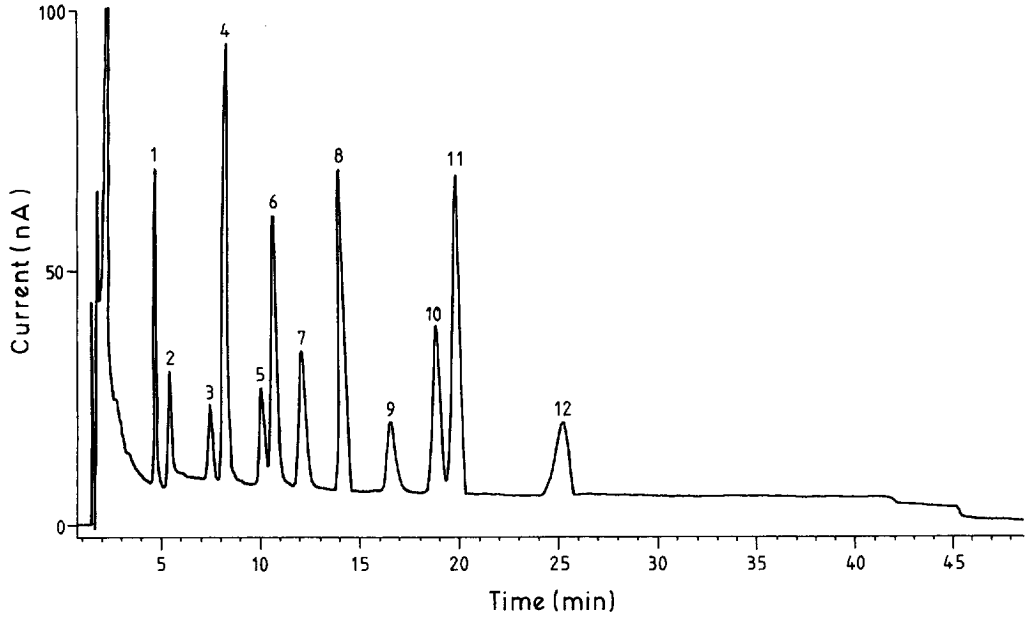

Fig. 4. Chromatographic profile obtained after on-line LSE when processing 5 ml of spiked ground water sample at 0.1 μg/l level using coulimetric detection. 1=catechol; 2=phenol; 3=4-methylphenol; 4=4-nitrophenol; 5=2-nitrophenol; 6=2,4-dinitrophenol; 7=2-chlorophenol; 8=3-chlorophenol; 9=4-chlorophenol; 10=4-choro-3-methylphenol; 11=4,6-dinitro-2-methylphenol; 12=2,4-dimethylphenol. E1, 300 mV; E2, 650 mV. Other experimental conditions, see Section 2.

other hand a cleaner chromatogram profiles were found when using the redox mode due the lower applied working potential. However, less sensitivity was found for some analytes such as 2,4-dichlorophenol and mononitrophenols that were not detected at those levels. This was attributed to the uncompleted reversibility of the electrochemical processes due the existence of parallel reactions. Hence, additional work using this mode was discarded.

Table 2 reports the recoveries and relative standard deviations (R.S.D.s) obtained when processing only 5 ml of river water using coulimetric and amperometric detection. Recovery values higher of 85% were found for all phenols since no breakthrough occurs at these sample volumes. R.S.D. values were improved as compared with those of amperometric detection and it can be attributed to the progressive fouling of the amperometric electrode when processing dirty water samples. To recover initial response a careful cleaning protocol is often applied, and a new calibration of the system should be carried out. Contrary to that, coulimetric detection shows excellent reproducibility caused mainly by the large surface area of the electrode. Reaction rate or cell constant values up to 500 s^{-1} which are higher than those of the amperometric detectors (1–10 s^{-1}) could be obtained.

The main drawback of coulimetric detection arises from the difficulties of performing gradient elution. The high porosity of the electrode leads in general to a rather large equilibration times and high signal instability when mobile phase composition is changed. So gradient elution is unsuitable and it is a problem when processing dirty samples because flushing the analytical column with 100% of organic solvent will not be feasible. The other drawback, in the analysis of phenols, is that the analysis of high chlorinated phenols (tri, tetra and pentachlorophenol) will need to be performed in a different chromatographic run than the analysis of the rest of priority phenols.

4. Conclusions

An automated method using on-line LSE followed by LC and coulimetric detection for the determination of phenolic compounds in water samples was optimized. Oxidation was carried out in basic media because working potential could be reduced, thus improving signal to noise ratio. LODs ranging from 0.1 to 5.4 ng/l were found when processing only 5–10 ml of water. Even though gradient elution was not feasible, coulimetric detection showed several

Table 2
Mean recoveries (%) (REC) and R.S.D.s (n=6) of phenolic compounds in ground water when processing 5 ml of ground water at 0.5 μg/l level

Compound	REC (±R.S.D.)	
	Screen out mode (E1:250 mV)(E2:650 mV)	Amperometric (1000 mV)[a]
Phenol	85±4	74±8
Catechol	80±5	n.d.
4-Methylphenol	83±3	94±7
2-Nitrophenol	102±5	n.d.
4-Nitrophenol	99±6	n.d.
2,4 Dinitrophenol	103±6	n.d.
2,4-Dinitro-2-methylphenol	100±5	n.d.
2-Chlorophenol	97±3	91±6
3-Chlorophenol	98±4	95±7
4-Chlorophenol	98±4	93±7
2,4-Dichlorophenol	101±3	104±6
4-Chloro-3-methylphenol	97±5	90±8

[a] From Ref. [9].
Sample volume: 5 ml (10 ml for amperometric detection).
n.d.: not detected.

advantages over classical amperometric devices. In general up to one order of magnitude enhancement on sensitivity and better R.S.D.s were obtained because of their higher cell constant. The present system is a good approach for the determination of polar phenols with low breakthrough volume which are difficult to analyze using conventional LC conditions.

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