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Determination of phenols in environmental waters using liquid chromatography with electrochemical detection

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Abstract

The development of a liquid chromatographic method for the determination of priority pollutant phenols at low μg/l levels in environmental waters is discussed. The phenols were separated using a Nova-Pak Phenyl column with concave gradient elution from 20 m*M* ammonium acetate (19% acetonitrile) to 20 m*M* ammonium acetate (54% acetonitrile) over 12 min, and subsequently detected using an electrochemical detector equipped with a glassy carbon working electrode, operated at a potential of +1150 mV. This approach resulted in minimal sample interferences and practical quantitation limits in the low μg/l range. The LC–ED method offers a distinct advantage over the established GC and GC–MS phenol methods in that no sample extraction is required, hence avoiding the losses which occur during the extraction, concentration and solvent exchange steps required for the GC-based analyses.

The method was evaluated through participation in a round robin testing program, involving determination of the eleven priority pollutant phenols in environmental water matrices. The results and recoveries obtained using the LC–ED method compared well with the overall consensus results, with recoveries ranging from 75% up to 118% for the eleven phenols. The between-laboratory reproducibility data indicated a high level of collective proficiency, with the overall average coefficients of variation for the eleven analytes being 18%, 18% and 13%, for spiked oil refinery effluent, paper mill effluent and reagent water samples, respectively.

Keywords: Electrochemical detector; Water analysis; Environmental analysis; Phenols

1. Introduction

The analysis of phenolic compounds is of importance to environmental regulatory agencies as these compounds pose significant human and environmental hazards, even at μg/l levels. The US EPA lists eleven phenols as priority pollutants, which include phenol itself, 2,4-dimethylphenol and a number of nitro- and chloro-substituted phenols [1]. Phenols have commonly been quantitated using methods that provide a measurement of the 'total phenol' concentration of the sample. Typically, a colorimetric reaction based upon a 4-amino antipyrine derivatisation provides a total phenol result, which is determined relative to the response of phenol itself [2]. This method of determination is suited to the analysis of samples from sources in which the constituent phenols are already known and a rapid test for monitoring purposes is required. The method is limited to this purpose as the degree of derivatisation

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varies with the degree and type of substitution of the phenol ring, hence it only yields a least possible phenol concentration. It is not suitable for toxicity determinations of unknown samples, as phenol toxicity is dependent on the phenolic species [3].

Gas chromatography (GC) allows for speciation of the various substituted phenols after liquid–liquid extraction. The US EPA Method 604 for phenols involves the serial extraction of an acidified sample with dichloromethane [4]. The extract is dried and exchanged to 2-propanol, typically using Kuderna–Danish glassware, after which the phenols are analysed by GC using a flame-ionisation detector. This method also provides for a pentafluorobenzyl bromide derivatisation and column chromatographic clean-up, followed by separation and electron-capture detection, to aid in the elimination of interferences [5]. Alternatively, US EPA Method 625 for extractable base/neutrals and acids can be used for phenols determination [6]. This method involves serial extraction with dichloromethane at a pH > 11, then at a pH < 2. After the extracts have been dried the phenols are analysed by gas chromatography–mass spectrometry. The major limitation of these GC methods is the extraction procedure, where losses can occur during extraction, concentration and solvent exchange steps. The extraction is also very labour intensive and requires specialised glassware as well as the use of chlorinated solvents.

As with GC, liquid chromatography (LC) also permits speciation of the various substituted phenols. The primary advantages of using LC are that there is no requirement for precolumn derivatisation and the need for sample pretreatment is minimal. Liquid chromatography with electrochemical detection (ED) has been widely applied to the measurement of phenols in environmental samples, such as pulp mill effluents and receiving waters [7–9]. A number of separation options have been utilised for phenol analysis, however, reversed-phase chromatography using either ion pairing agents or pH control is the most common approach. The eleven priority phenols have been separated previously using a Waters Resolve column with a mobile phase of 40% methanol, 15% ethanol and an ion pairing agent of 5 m*M* low-UV Pic A [10]; however, the chromatographic run time was over 30 min, leading to poor detection limits for the later eluting peaks. Also, the resolution of the early eluting peaks proved to be inadequate for wastewater analysis, due to chromatographic interferences. Butler and Dal Pont recently reported the isocratic LC separation of a number of chlorophenols using a Waters Phenyl column with an eluent of acetate buffer–acetonitrile (ACN) (60:40) at pH 4.15 [9]; however, these conditions did not provide complete resolution of the eleven priority pollutant phenols.

In this paper, the development of optimised gradient elution conditions for the LC–ED determination of the eleven priority pollutant phenols in environmental waters is discussed. The use of gradient elution allowed complete separation within 20 min and resulted in good peak shape, hence low detection limits, for the eleven phenols. The method was evaluated through participation in a round robin testing program, involving the determination of the eleven phenols in five (duplicate) environmental water matrices.

2. Experimental

2.1. Instrumentation

The liquid chromatograph consisted of a Waters Corporation (Milford, MA, USA) Model 600 quaternary solvent delivery system fitted with column heater, 717+ autosampler, 464 electrochemical detector with a glassy carbon working electrode operated at +1150 mV and a Millennium 2010 Chromatography Management System. Data were collected at 1 point per second. A number of reversed-phase columns were used for this work; a Chromspher Pesticides C_{18} (100×3.0 mm I.D.), Spherisorb C_8 (250×4.6 mm I.D.), Zorbax C_{18} (250×4.6 mm I.D.), Zorbax Phenyl (250×4.6 mm I.D.), Waters Nova-Pak C_8 (150×3.9 mm I.D.) and a Waters Nova-Pak Phenyl (150×3.9 mm I.D.).

2.2. Reagents and procedures

Millipore (Bedford, MA, USA) Milli-Q 18

megohm water was used for all mobile phase, sample and standard preparation. Ammonium acetate (analytical-reagent grade) was obtained from Ajax Chemicals (Sydney, Australia), as were Unichrom HPLC grade acetic acid and acetonitrile. Phenol standards (analytical-reagent grade) were also obtained from Ajax Chemicals, while the phenol reference material used for spiking was obtained from ChemService (West Chester, PA, USA) through Rhone-Poulenc Laboratory Products (Sydney, Australia). Mixed phenol standards were prepared from a concentrated stock solution containing 100 μg/ml of phenol, 4-nitrophenol (4NP), 2-chlorophenol (2CP), 2-nitrophenol (2NP), 2,4-dimethylphenol (2,4DMP), 4-chloro-3-methylphenol (4C3MP), 2,4-dichlorophenol (2,4DCP), 2,4,6-trichlorophenol, (2,4,6TCP) and pentachlorophenol (PCP); and 1000 μg/ml of both 2,4-dinitrophenol (2,4DNP) and 2-methyl-4,6-dinitrophenol (2M4,6DNP). The round robin samples were provided in hexane-washed, 500-ml amber glass bottles and, except the spiked reagent water, were preserved with 1.0 ml concentrated sulfuric acid/500 ml sample. Mobile phases containing 20 m*M* ammonium acetate were prepared from a 2.0 *M* ammonium acetate stock solution which had been passed through a 1.0 cm I.D. glass chromatography column containing 4.0 g of Chelex 100 resin (Bio-Rad, Richmond, CA, USA). Mobile phases were prepared daily, filtered through a Millipore 0.45-μm HV filter and degassed in an ultrasonic bath before use. Complete details of the method procedures are provided in Ref. [11].

3. Results and discussion

3.1. Optimisation of chromatographic conditions

The development of an optimised LC separation of the eleven EPA phenols is complicated by the wide range of polarities of the compounds. In addition, the acidity of the phenols increases with increasing chloro-substitution, from a pK_a value of 10.1 for phenol to 4.9 for pentachlorophenol [12]. Several separation options have been investigated for phenol analysis, however, reversed-phase chromatography using either ion pairing agents or pH control appears the most common approach. The separation of compounds with widely differing polarities by reversed-phase chromatography is typically carried out with gradient elution [13], however, electrochemical detection is not frequently used with this elution mode as the background is 'visualised' by the detector. This, in turn, leads to a significant baseline shift when the detector is operated at high sensitivity. Step flow-rate changes have previously been used in conjunction with the electrochemical detection of phenols as a means of reducing overall run time while attempting to minimise the baseline shift [14]. In this work, the separation of the priority pollutant phenols is investigated using a number of seemingly appropriate columns in conjunction with a variety of elution conditions, i.e. isocratic, step and continuous mobile-phase gradients.

Mobile phases based on ammonium acetate buffer and acetonitrile (ACN) were used, as this had previously been shown to allow sensitive electrochemical detection of phenol compounds [9]. Stock acetate buffer was passed through a Chelex-100 clean-up column in order to reduce metal impurities in the eluent, resulting in a lower background current and improved detector performance, in terms of drift and noise [9]. In addition, the use of ACN was found to be preferable to methanol as a mobile phase modifier in terms of reducing the background current and detector performance. Sparging with helium also reduced the background current and consequently baseline noise. Careful attention was also paid to the glassy carbon electrode surface; mechanical polishing, as detailed in the detector manual, was found to give the best results [15]. This procedure was carried out every three days in order to maintain optimum detector sensitivity.

Of the columns investigated, neither the Zorbax C_{18}, Waters C_8 nor Zorbax Phenyl columns provided adequate resolution of the phenols, while the Chromspher C_{18} proved inappropriate due to poor peak shape. The eleven phenols could be separated on the Spherisorb C_8 column using a linear gradient, although a run time of 35 min was required, leading to poor sensitivity for the later eluting compounds. The Waters Nova-Pak Phenyl column was found to give

the best overall selectivity of the columns tested. The use of the Phenyl column and acetate buffer with an acetonitrile step gradient from 20 m*M* ammonium acetate (18% ACN) to 20 m*M* ammonium acetate (38% ACN) at 6 min (hold for 6 min before returning to initial conditions) allowed all the phenols to be resolved in under 20 min, resulting in good peak shape and sensitivity for the later eluting 2,4,6TCP and PCP peaks. At moderate sensitivity, the detector baseline appeared relatively stable; however, at higher detection sensitivity the 'step' baseline shift made integration, hence quantitation, of the last four phenols difficult.

In order to determine the phenols at low levels, continuous acetonitrile gradient elution was used, as this allowed more reproducible quantitation than the step gradient elution approach. Fig. 1a shows a chromatogram of a 75 μg/l phenol standard obtained using the Nova-Pak Phenyl column with concave gradient elution from 20 m*M* ammonium acetate (19% ACN) to 20 m*M* ammonium acetate (54% ACN) over 12 min. When operating at a detector range of 100 nA response=1000 mV output, a baseline 'shift' of ca. 1000 mV was evident as a result of the gradient. Despite the shift, all the phenols were well resolved and could be reproducibly quantitated by the chromatography management software. In fact, the shape of the baseline profile was so reproducible, that it allowed automated baseline subtraction to be routinely used, as shown in Fig. 1b.

Linear calibration curves were obtained for all the phenols, from the detection limit up to the mid μg/l range, e.g. the correlation coefficient (R^2) for 4NP was 0.9994 over the 5–250 μg/l range for a 50-μl injection. The calibration curves became non-linear for a number of phenols, e.g. phenol, 2NP, 2CP, 2,4DNP and 4NP, at concentrations around 1 mg/l. The retention time repeatability, determined from ten replicate injections of an eleven phenol mixed standard at the 30 μg/l level (300 μg/l for 2,4DNP and 2M4,6DNP), is shown in Table 1. The peak area repeatability, determined from the same ten replicate injections is also shown in Table 1, along with method detection limits and the suggested practical quantitation limits for each of the eleven phenols. The retention time repeatability was excellent, indicating good gradient reproducibility. The peak area repeatability at the 30 μg/l level was somewhat high, particularly for a number of the more retained solutes, although the repeatability at the 100 μg/l level improved to be in the order of 1–3% C.V. for the same solutes.

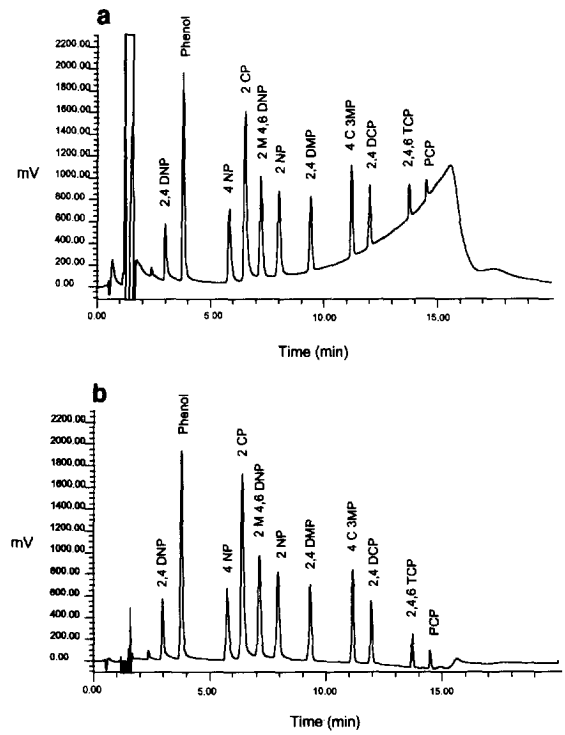

Fig. 1. Separation of μg/l level priority pollutant phenols using a Waters Phenyl column and continuous (concave) gradient elution, without (a) and with (b) automated baseline subtraction. Conditions: column, Waters Nova-Pak Phenyl; mobile phases, (A) 20 m*M* ammonium acetate with 20% acetonitrile and (B) 20 m*M* ammonium acetate with 60% acetonitrile. Continuous gradient profile: time 0.0 min, 97% A and 3% B; time 12 min, 0% A and 100% B (curve 7 as produced by the Millenium software and the Model 600 gradient pump); time 12.1 min, 97% A and 3% B, curve 11; flow-rate, 1.0 ml/min; injection volume, 50 μl; detection, +1150 mV at glassy carbon; detector output, 100 nA response=1000 mV output; column temperature, 35°C; He sparge rate, 15 ml/min; solutes, 75 μg/l phenols, except 750 μg/l of 2,4DNP and 2M4,6DNP.

3.2. Round robin quantitative results

Having developed optimised LC conditions for the

Table 1
Retention time and peak area repeatability (%C.V.); method detection limits (MDL) and practical quantitation limits (PQL) (μg/l) for the eleven priority pollutant phenols

Analyte	Retention time (%C.V.)	Peak area (%C.V.)	MDL (μg/l)	PQL (μg/l)
2,4DNP	0.3	13	2.0	6.0
Phenol	0.1	4.0	0.2	0.5
4NP	0.1	3.5	0.2	0.5
2CP	0.2	7.3	0.1	0.3
2M4,6DNP	0.2	7.1	0.5	1.3
2NP	0.2	9.8	0.03	0.1
2,4DMP	0.2	12	0.03	0.1
4C3MP	0.1	20	0.04	0.2
2,4DCP	0.1	7.1	0.06	0.2
2,4,6TCP	0.2	20	0.6	2.0
PCP	0.2	25	0.7	2.0

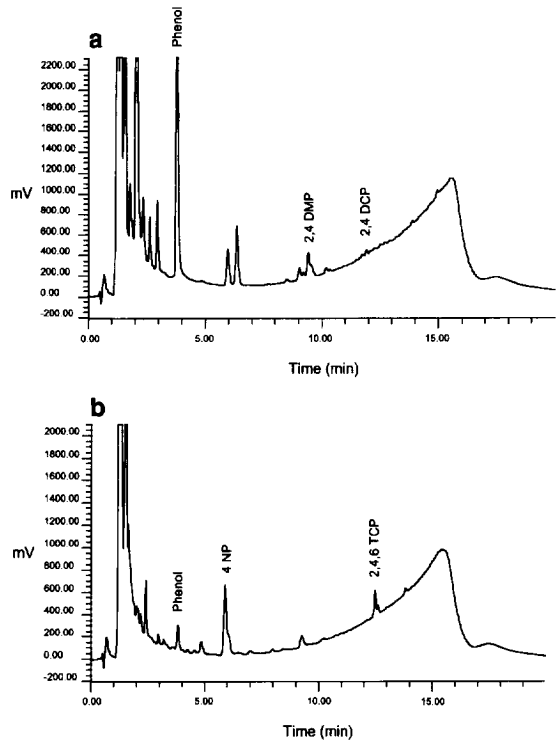

Fig. 2. Example LC–ED chromatograms obtained for the saline oil refinery effluent (a) and paper mill effluent (b) samples. Conditions as for Fig. 1a, except: solute concentrations, as shown in Table 2 and Table 3.

eleven priority pollutant phenols, the method was then evaluated for the determination of phenols in five 'blind' environmental water matrices. These samples were supplied (in duplicate) as part of a round robin testing program, based on that outlined by Dennis [16], involving nine laboratories. Complete details of the round robin testing program protocol are provided in Ref. [17]. The samples, a saline oil refinery effluent, spiked refinery effluent, a paper mill effluent, spiked paper mill effluent and a spiked reagent grade water, were analysed using either GC or LC methodology by the nine laboratories. The LC–ED procedure developed for this work required no sample pretreatment, other than filtration through a Millipore Millex 0.45-μm HA filter prior to injection. Fig. 2a and Fig. 2b give examples of the chromatograms obtained for the saline oil refinery effluent and paper mill effluent samples, before baseline subtraction, using the same conditions as for Fig. 1a.

The LC–ED sample chromatograms were virtually free of sample interferences, with the exception of two interfering peaks which eluted in the region near the 4NP and 2CP peaks. The reported LC–ED results for the oil refinery effluent, paper mill effluent and reagent water samples are shown in Table 2, Table 3 and Table 4, respectively. Acceptable recoveries were obtained for most of the analytes in the spiked samples. A low 4NP recovery occurred for both the oil refinery and paper mill spiked samples due to integration difficulties caused by partial co-elution of 4NP with an interfering compound. Overall consensus values, also shown in Tables 2–4, were calculated for analytes where at least five results were available. The round robin testing program provided for the identification of unsatisfactory results using Cochran's and Dixon's tests [16] and only statistically acceptable results were used in the calculation of the overall consensus results. The results given in Tables 2–4 indicate that the LC–ED results and recoveries generally compared well with the overall consensus results obtained from the nine laboratories [17]. In addition,

Table 2
LC–ED method phenol results (duplicate, μg/l) obtained for the oil refinery effluent sample, in addition to the overall consensus results obtained from the round robin testing program

Analyte	LC–ED oil refinery effluent results			Oil refinery effluent concensus results				
	Effluent (μg/l)	Spiked effluent (μg/l)	Recovery (%)	Effluent $\bar{x}$ (μg/l)	Effluent S.D. (μg/l)	Spiked effluent $\bar{x}$ (μg/l)	Spiked effluent S.D. (μg/l)	Recovery $\bar{x}$ (%)
2,4DNP	<6	520, 540	98			525	108	97
Phenol	110, 94	180	118	99	25	164	37	98
4NP	<0.5	46, 45	75			61	25	99
2CP	<0.3	60	98			59	9.2	97
2M4,6DNP	<1.3	450, 490	89			536	55	101
2NP	<0.1	61, 63	91			60	7.2	86
2,4DMP	15, 14	65, 73	88	17	6.8	75	16	96
4C3MP	<0.2	57, 58	89			57	9.8	87
2,4DCP	2.6, 2.0	63, 65	101	7.3	4.0	61	13	96
2,4,6TCP	<2	58, 57	89			57	15	87
PCP	<2	58, 59	96			60	16	97

the reproducibility (between-laboratory) results were quite reasonable considering the small sample number involved. The reproducibility ranged from a coefficient of variation of 3.8% up to 35% (average of 18%) for the analytes in the spiked oil refinery sample; 4.6% up to 57% (average of 18%) for the analytes in the spiked paper mill sample; and 6.1% up to 23% (average of 13%) in the spiked reagent water sample.

4. Conclusions

The determination of phenols at low μg/l levels

Table 3
LC–ED method phenol results (duplicate, μg/l) obtained for the paper mill effluent sample, in addition to the overall consensus results obtained from the round robin testing program

Analyte	LC–ED paper mill effluent results			Paper mill effluent concensus results				
	Effluent (μg/l)	Spiked effluent (μg/l)	Recovery (%)	Effluent $\bar{x}$ (μg/l)	Effluent S.D. (μg/l)	Spiked effluent $\bar{x}$ (μg/l)	Spiked effluent S.D (μg/l)	Recovery $\bar{x}$ (%)
2,4DNP	<6	510, 500	94			443	188	82
Phenol	5.7, 5.8	65, 66	91	12	8.5	64	12	81
4NP	7.8	53, 54	75	37	40	78	42	106
2CP	<0.3	68, 66	105			61	9.3	99
2M4,6DNP	<1.3	520	98			557	51	105
2NP	<0.1	70, 71	101			61	9.0	89
2,4DMP	<0.1	57, 58	93			54	13	87
4C3MP	<0.2	63, 62	95			60	13	93
2,4DCP	<0.2	63, 61	102			62	8.2	97
2,4,6TCP	20, 18	79, 85	97	13	3.7	69	23	91
PCP	<2	62, 61	101			70	24	113

Table 4
LC–ED method phenol results (duplicate, μg/l) obtained for the spiked reagent water sample, in addition to the overall consensus results obtained from the round robin testing program

Analyte	LC–ED spiked reagent water results		Spiked reagent water concensus results		
	Spiked water (μg/l)	Recovery (%)	Spiked effluent $\bar{x}$ (μg/l)	Spiked effluent S.D. (μg/l)	Recovery $\bar{x}$ (%)
2,4DNP	540	100	523	84	97
Phenol	62, 63	95	59	9.2	79
4NP	62, 57	98	63	16	103
2CP	66, 64	107	66	21	102
2M4,6DNP	530, 530	100	557	54	105
2NP	68, 65	98	61	6.4	90
2,4DMP	59, 58	94	53	14	85
4C3MP	63, 62	96	62	12	95
2,4DCP	59	97	55	4.3	80
2,4,6TCP	64, 66	100	65	9.4	100
PCP	60, 59	98	58	17	95

in environmental waters can be achieved by liquid chromatography using a gradient reversed-phase separation and electrochemical detection. The use of gradient elution allowed the eleven priority pollutant phenols to be resolved in under 20 min, resulting in good peak shape and sensitivity, with minimal sample interferences. The sensitivity of electrochemical detection negates the requirement for sample extraction and concentration as used in the established GC and GC–MS phenol methods. This avoids the losses associated with these sample preparation steps.

The results and recoveries obtained for five unknown environmental water matrices using the LC–ED method compared well with the overall consensus results from a round robin testing program for the determination of the eleven priority pollutant phenols. The LC–ED method recoveries ranged from 75% up to 118% for the eleven phenols. The between-laboratory reproducibility indicated a high level of collective proficiency, with overall average coefficients of variation for the eleven analytes of 18%, 18% and 13% in the oil refinery, paper mill and reagent water spiked samples, respectively. Further collaborative studies are planned to provide better estimates of repeatability, reproducibility and method (LC and GC) compatibility for phenols in environmental waters. Studies based on solid matrices, such as soils and sludges, are also planned.

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