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Journal of Chromatography A, 697 (1995) 357–362

JOURNAL OF CHROMATOGRAPHY A

Simultaneous determination of 27 phenols and herbicides in water by high-performance liquid chromatography with multi-electrode electrochemical detection

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Abstract

A sensitive and simple method for the simultaneous evaluation of phenol, 26 substituted phenols and herbicides was developed using HPLC and electrochemical detection. After extraction from the samples on solid-phase cartridges, the compounds were separated on a reversed-phase column by using a combined gradient of organic modifier and counter-ion. The total analysis time was less than 63 minutes. Identification of the compounds was based on retention time comparison with authentic standards. In addition, further confirmation of peak identities and of their purity was determined by comparison of the ratio of the peak's height of each compound across the electrode array, with the similar ratio of the authentic standard. The detection limit was found to be much lower than that indicated by the European Community: for the least sensitive compound (a herbicide, Linuron) it was less than 0.0005 μg/l at signal-to-noise of 3. The method was used to examine the residual levels of phenylurea herbicides, phenol, chloro- and nitro-phenols in tap water, mineral water and spring water from different sources.

1. Introduction

Phenol and substituted phenols are common products of many industrial processes, while substituted phenylureas are selective herbicides used often in agriculture. The leaking of these substances from the soil into local ground water is a common phenomenon. Under environmental conditions phenols and phenylureas can persist at the mg/l level in ground water [1], for a number of days or weeks depending on temperature and pH. Therefore, if such ground waters are to be used as sources of drinking water, it is necessary to screen them for contamination by these organic pollutants, given the high mammalian toxicity of these substances. The high standards for drinking water purity laid down by the European Community give 0.1 μg/l as the admissible concentrations for any individual pesticide, with a limit of up to 0.5 μg/l for the total content of pesticides. The local legislation in Italy (DPR 236/88) allows up to 0.5 μg/l of phenols. As a result, an analytical method which offers high selectivity and sensitivity for both the identification and the quantitation of these substances is needed. This paper describes a procedure for the simultaneous determination of phenols and phenylureas using HPLC and elec-

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SSDI 0021-9673(94)00791-8

trochemical detection. After preconcentration of the samples on solid-phase cartridges, the compounds were separated on a reversed-phase column by using a combined gradient of organic modifier and counter-ion.

2. Experimental

2.1. Chemicals

The mobile phases used in the gradient runs were provided by ESA (Bedford, MA, USA). Mobile phase A was composed of 34.7 μM sodium dodecyl sulphate (SDS)–0.1 M monobasic sodium phosphate–50 nM nitrilotriacetic acid (pH 3.45), while mobile phase B was composed of 173 μM SDS–0.1 M monobasic sodium phosphate–50 nM nitrilotriacetic acid–50% aqueous methanol (pH 3.45).

Solutions A and B were filtered through 0.2 μm PTFE lyophilic filters (Millipore, Bedford, MA, USA) and degassed by sonication under vacuum for 10 min prior to use.

The water used for dilution of the standards and of the samples was purified with a Milli-Q R/O water purification system (Millipore).

2.2. Apparatus

A Coulochem Electrode Array System (CEAS) obtained from ESA was used. The instrument consisted of a refrigerated autosampler which is capable of variable volume injections with a 100-μl loop. A circulating bath was used to maintain sample vials between 0°C to 4°C prior to sample injection. Gradient operation was provided by two HPLC pumps capable of operating from 0.05 to 10 ml/min. The output of the pumps was connected to a dynamic gradient mixer. The analytical column (80 × 4.6 mm I.D.) used was a stainless-steel column packed with 3 μm particles of silica-based C_{18} materials (HR 80, ESA). The detection system consisted of four coulometric array cell modules, each containing four electrochemical detector cells (cat. no. 55-0685 A). The detectors, porous graphite working sensors with palladium reference and counter electrodes, were arranged in series after the analytical column. The detector, the column and a pulse damper were housed in a thermal chamber maintained at 37°C. Two additional pulse dampers were placed before the column and cell compartment. The autosampler, pumps, detectors, temperature controlled box and all associated electronic circuitry were monitored and controlled by CEAS software installed on a Model 386 computer equipped with a 32 Mb hard disk and a 1.2 Mb floppy disk drive. The computer was coupled with a high resolution colour monitor with a "touch screen" interface and to a matrix graphic printer. The computer system also performed data storage, analysis and report generation. An appropriate software package was used for summary reports of the final data (Lotus 1-2-3, Lotus Corp., Cambridge, MA, USA).

2.3. Chromatographic method

A method capable of completely separating the 27 compounds chosen was developed. It consisted of a gradient where the organic modifier and counter-ion were modified during the run. The gradient used in the separation, expressed by percentage of phase B was: 6% isocratic for 4 min, then it reached 100% of phase B at 14 min after the injection; it was isocratic till 54 min after the injection, then the phase B was returned to the initial value of 6%. Nine minutes were allowed for column reequilibration. The flow-rate was 0.8 ml/min and the cell potentials constituted an increasing array: 0 mV at electrode 1, 80 mV at electrode 2, with increments of 80 mV at each subsequent electrode until a value of 1200 mV was reached at electrode 16. The indicated potentials are referred to the solid state palladium reference electrode built in the coulometric cell; their absolute value is about 250 mV lower than the corresponding potential measured by using an Ag/AgCl reference electrode. At the end of each analysis, all cell potentials were increased to 1200 mV for 60 s to prevent long term adsorption of material to the electrode surface. The elec-

trodes were then allowed to stabilize for 9 min before the next injection.

2.4. Standard and sample preparation

The pollutants chosen to be studied were the most ubiquitous species of the aminophenols, nitrophenols, chlorophenols, cresols, phenols (residues of many industrial processes) and Phenylurea herbicides (widely used in agriculture). In fact numerous studies have investigated these particular molecules [2–6].

All standards (Table 1) were purchased from Sigma (St. Louis, MO, USA). The primary stock standard solutions were made by dissolving 10 mg of the component in 10 ml of methanol. These concentrates were then subdivided into 1-ml portions. They were stored at −30°C and thawed when necessary at 4°C. Individual secondary working stock standard solutions were made by diluting each component of the primary solutions with methanol in order to give a concentration of 500 ng/ml (with the exception of phenol and 2-nitrophenol whose concentrations were 250 ng/ml). Injection of these single components was done for the characterization of the chromatographic and electrochemical behaviour of each molecule. A 27-component working standard solution was prepared by combining and diluting with methanol the aliquot of each of

Table 1
Chromatographic and electrochemical characteristics of the 27 external standards

Identif. number	Name[a]	Retention time (min)	Recovery (%)	Detection limit (μg/l)	Within-run R.S.D. (%)	Between-run R.S.D. (%)	Dominant potential (mV)
1	Pyrogallol[5]	4.07	98	0.00008	1.8	2.2	80
2	4-Hydroxy-aniline[1]	5.03	96	0.00045	1.9	2.4	80
3	Benzocatechine[5]	9.73	97	0.00022	2.2	2.9	160
4	2-Hydroxy-aniline[1]	12.58	102	0.00032	2.5	3.5	160
5	Phenol[5]	13.93	105	0.00003	1.2	2.0	640
6	1,2-Phenylendiamine[1]	14.98	98	0.00036	3.1	4.8	160
7	4-Nitrophenol[2]	16.90	101	0.00018	2.8	5.2	880
8	2,4-Dinitrophenol[2]	17.69	96	0.00022	3.5	5.3	1040
9	*o*-Cresol[4]	18.33	97	0.00015	2.9	4.3	560
10	2-Nitrophenol[2]	18.77	100	0.00008	2.8	4.2	880
11	Metoxuron[6]	19.43	96	0.00021	2.6	3.9	560
12	3-Methyl-2-nitrophenol[2]	20.23	102	0.00038	2.2	4.2	720
13	Monuron[6]	20.99	96	0.00030	3.2	4.1	800
14	2,6 Dichlorophenol[3]	23.42	100	0.00031	2.8	3.8	640
15	4,6-Dinitrocresol[4]	23.47	101	0.00035	3.4	4.2	960
16	5-Methylphenol[5]	23.83	98	0.00030	2.5	3.5	640
17	Monolinuron[6]	23.88	96	0.00041	3.3	5.3	880
18	4-Methyl-2-nitrophenol[2]	24.75	98	0.00032	2.7	4.8	720
19	Methobromuron[6]	25.86	102	0.00040	3.2	5.2	960
20	Chlortholuron[6]	26.15	104	0.00043	3.2	4.9	800
21	4-chloro-3-methylphenol[3]	26.50	98	0.00038	2.2	3.2	640
22	Buturon[6]	28.02	96	0.00043	3.5	5.5	800
23	2,4-Dichlorophenol[3]	28.27	98	0.00029	2.9	4.2	720
24	Isoproturon[6]	28.88	102	0.00032	2.1	4.1	640
25	Diuron[6]	29.51	97	0.00028	3.2	5.0	880
26	Linuron[6]	34.92	98	0.00049	3.3	4.9	960
27	2,4,5-Trichlorophenol[3]	46.69	98	0.00042	2.8	3.9	640

[a] The reference made to each compound refers to their classification: 1 = Amino-phenols, 2 = Nitro-phenols, 3 = Chloro-phenols, 4 = Cresols, 5 = Phenols, 6 = Phenylurea herbicides.

the primary stock standard solutions to the concentration of 500 ng/ml (with the exception of phenol and 2-nitrophenol whose concentrations were 250 ng/ml). For recovery studies this 27-component working standard solution was prepared by diluting with water the primary standard solutions to appropriate concentrations. Prior to the injection all standard solutions were filtered through a 0.22-μm membrane (Millipore).

This method was used to measure the compounds in three different samples of water: tap water from an aqueduct of Piacenza (a city in the middle of Val Padana, Italy), water from a spring at 1600 m over sea level and commercial mineral water (Levissima). The samples of water were collected in 1.5-l polyethylene bottles and stored at 4°C prior to extraction.

To increase the sensitivity of the method all the water samples were concentrated by a solid-phase extraction. This was achieved by filtering 1000 ml of sample through the Sep-Pak cartridges C-18, 5 mm (Millipore) at a flow-rate of about 16 ml/min. The percolate was discarded and the cartridge was eluted with 1 ml of methanol.

Of this organic phase 10 μl were injected into the CEAS.

2.5. Assay performance

Assay linearity and detection limit were examined by analysing in triplicate the 27-component standard solution with increasing concentrations of the components. Recovery was examined by analysing the 27-component standard solutions in water treated in the same manner as the samples. The concentration ranges studied were from 10 000 μg/l to 0.1 μg/l with seven concentrations of each analyte. Each sample was analyzed in triplicate. To examine the within-run variability of the assay, 10 replicates of the 27-component standard solution in water (containing 5 ng of each component) were analyzed after having been treated as a sample. Variability between runs was studied by analysing two replicates of the same solution used for the within-run study. This was done on 10 separate days.

3. Results

The 27 standards are listed according to their retention times. Their concentration is 500 μg/l with the exception of phenol and 2-nitrophenol whose concentrations were 250 μg/l (corresponding to 5 and 2.5 ng/10 μl, respectively). The recovery, the detection limit, the within- and between-run precision and their dominant potentials are also reported (the dominant potential is that of the electrode potential where the maximum signal occurs). The peak confirmation was achieved by comparing the matching ratio (R) between a standard and the actual sample (R is the dominant channel/subdominant channel ratio) [7].

Fig. 1 shows the chromatogram of a 10-μl sample containing the 27 components as external standard at the concentration reported above. The total analysis time was 63 min. Retention time reproducibility reported during the precision study (carried out over a 10-day span) of each individual standard was found to be very good (R.S.D. $< 2\%$).

This was due to the strict control of the detector and column temperature, mobile phase composition and gradient profile. Assay linearity was found to be good in all the intervals analyzed. In fact with least-squares regression analysis, detector response was directly proportional to standard concentration (10-μl sample injection) and calibration curves were linear in the tested range from 10 000 μg/l to 0.1 μg/l. The detection limit (signal-to-noise ratio = 3) is very low for all the components; for the less sensitive compound (linuron) it is < 0.0005 μg/l which is much lower than the limit laid down by the European Community (0.1 μg/l or 0.1 ppb). The analytical recovery is high for all the components, ranging from 96% to 105%. As a result, the use of an internal standard was not taken into consideration. The within-run concentration variability (R.S.D.) ranged from 1.2%

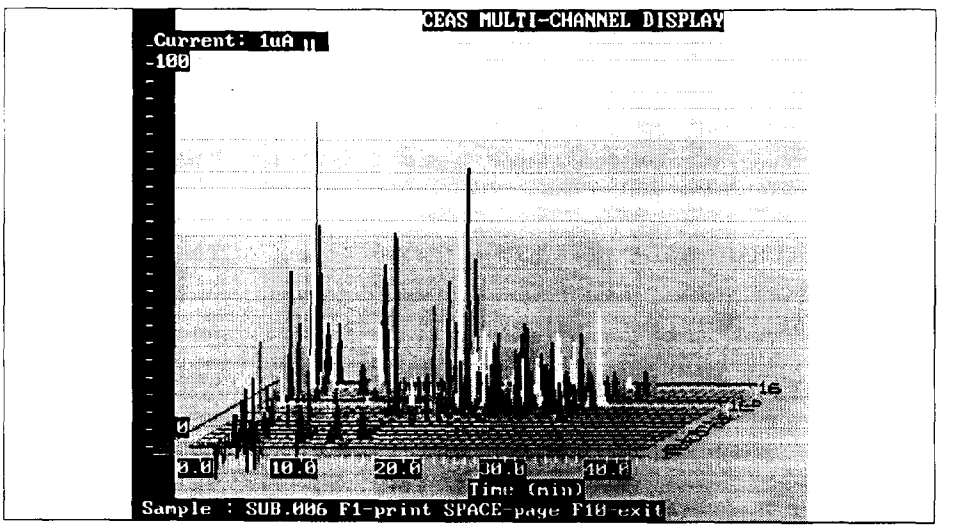

Fig. 1. 16-channel chromatogram of 10 μl of extracted standard mixture containing the 27 compounds.

to 3.5%; the between-run concentration variability ranged from 2.0% to 5.5%.

This method was used to examine the level of 27 different molecules in three different types of water: tap water from an aqueduct of Piacenza, a rural area of the flat Padana region with intensive cultivation; spring water collected at Pian delle Betulle (Alps) at 1600 m above sea level, and an oligomineral water sold commercially in Italy (Levissima). The concentration of the various components found in these samples are reported in Table 2, sorted according their retention time. The 16-channel chromatogram for one of the water samples (Mineral water Levissima) is shown in Fig. 2.

4. Discussion and conclusion

The use of the CEAS for the determination of neurochemicals in tissues and biological fluids has already been reported [7–11]. Recently, it has been used for the determination of 36 phenolic constituents in natural beverages and plant extracts [12]. The coulometric efficiency of each element of the array allows a complete

Table 2
Concentrations of the compounds identified in water samples from the 3 different sources.

Identif. number	Name	Concentration in μg/l		
		Tap water	Spring water	Mineral water
5	Phenol	0.580	0.051	0.161
7	4-Nitrophenol			0.002
8	2,4-Dinitrophenol			0.001
17	Monolinuron	0.009		0.051
18	4-Methyl-2-nitrophenol		0.016	0.087
20	Chlortholuron	0.177		
25	Diuron			0.005

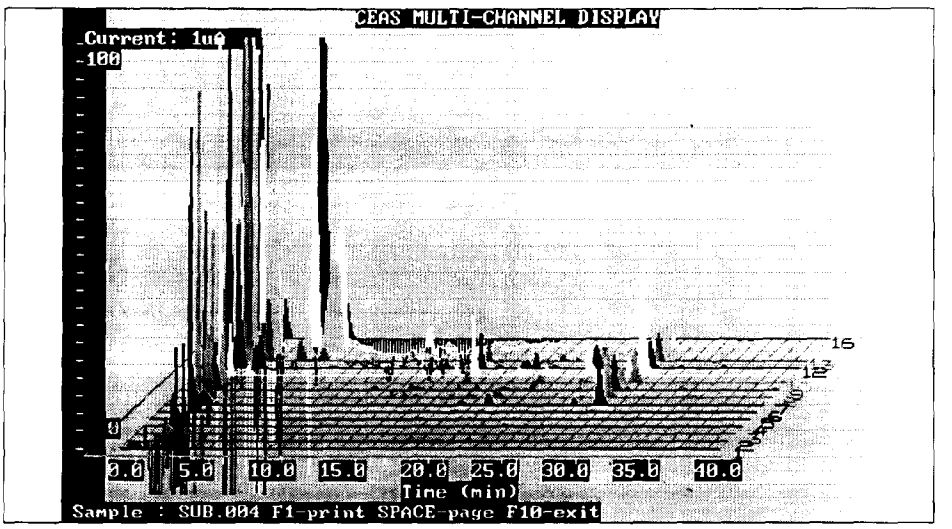

Fig. 2. 16-channel chromatogram of 10 μl of extracted sample of Piacenza tap water.

voltammetric resolution of analytes as a function of their reaction potential. Some peaks can be resolved by the detector even if they are unresolved when they leave the chromatographic column. In this study we have demonstrated that this technique can also be applied to phenols, substituted phenols as well as the phenylurea herbicides found in water. We separated 27 compounds in less than 63 min with this method. The reproducibility of the retention time coupled with the selectivity inherent to this detector allows measurements with high precision of a variety of different compound families in a single sample. For the samples analyzed here, we were able to measure 3 compounds in the tap water of the flat Padana region, 2 in the spring water of the Alps, and 6 in the oligomineral water. Of the three samples of water considered in this study only that from the Alps is below the limits of standards of purity. The tap water contains phenols and chlortholuron, and even the mineral water contains phenols above the limit set by the European Community.

References

[1] M.E. Leon-Gonzalez and A. Townshend, *J. Chromatogr.*, 539 (1991) 47.
[2] A. Di Corcia and M. Marchetti, *J. Chromatogr.*, 541 (1991) 365.
[3] A. Balinova, *J. Chromatogr.*, 643 (1993) 203.
[4] R.B. Geerding, A.M.B. Graumans and J. Viveen, *J. Chromatogr.*, 547 (1991) 478.
[5] Y. Ishli, K. Asakura and T. Sakamoto, *Nippon Noyaku Gakkaishi*, 15 (1990) 435.
[6] S. Yao, A. Meyer and G. Henze, *Fresenius' J. Anal. Chem.*, 339 (1991) 207.
[7] V. Rizzo, G. Melzi d'Eril, G. Achilli and G.P. Cellerino, *J. Chromatogr.*, 536 (1991) 229.
[8] K.J. Swartz and W.R. Matson, *Anal. Biochem.*, 185 (1990) 363.
[9] C.N. Svendsen and E.D. Bird, *Neurosci. Lett. Suppl.*, 35 (1989) 49.
[10] W.R. Matson, P.G. Gamache, M.F. Beal and E.D. Bird, *Life Sci.*, 41 (1987) 905.
[11] P.H. Gamache, M.L. Kingery and I.N. Acworth, *Clin. Chem.*, 39/9 (1993) 1825.
[12] G. Achilli, G.P. Cellerino, P. Gamache and G. Melzi d'Eril, *J. Chromatogr.*, 632 (1993) 111.