Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1022 (2004) 9–16
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Comparison of gas chromatography–mass spectrometry and capillary electrophoresis in analysis of phenolic compounds extracted from solid matrices with pressurized hot water

Juhani Kronholm[a], Patricia Revilla-Ruiz[b], Simo P. Porras[a], Kari Hartonen[a], Rita Carabias-Martínez[b], Marja-Liisa Riekkola[a,*]

[a] *Department of Chemistry, Laboratory of Analytical Chemistry, University of Helsinki, P.O. Box 55, FIN-00014, Helsinki, Finland*
[b] *Department of Analytical Chemistry, F. Ciencias Químicas, University of Salamanca, Plaza de la Merced s/n, 37008 Salamanca, Spain*

Received 2 June 2003; received in revised form 25 August 2003; accepted 23 September 2003

Abstract

Self-constructed pressurized hot water extraction (PHWE) equipment was used in dynamic mode to extract spiked phenolic compounds (phenol, 3-methylphenol, 4-chloro-3-methylphenol and 3,4-dichlorophenol) from sea sand and soil. Phenols were analyzed by both gas chromatography–mass spectrometry (GC–MS) and capillary zone electrophoresis (CZE) to compare the techniques and to find out if CZE is a suitable tool for analysis of phenols extracted from environmental matrix. Good recoveries of phenols spiked in sea sand were achieved at all PHWE temperatures (50, 100, 200, 300 °C). GC–MS studies showed that phenols were selectively extracted from soil at 50 °C but various other compounds (e.g. polyaromatic hydrocarbons) were extracted along with the phenols at 300 °C. In the case of CZE, phenols extracted from the soil, at 300 °C were separated with good resolution at pH 9.7, and co-extracted compounds did not interfere with the analysis. The analytical values obtained by GC–MS and CZE were generally of similar magnitude.

Keywords: Pressurized hot water extraction; Phenolic compounds

1. Introduction

Water is non-flammable, non-toxic, readily available and cheap and as solvent environmentally benign. The dramatic change in its physico-chemical properties at elevated temperatures and pressures enhances its usefulness. Pressurized hot water (PHW) has been used to replace conventional organic solvents in a variety of extraction processes (pressurized hot water extraction (PHWE)). Temperatures below the critical value of water ($T_c = 374\,°C$) but usually above 100 °C are employed. In work in liquid phase, pressure must be high enough to prevent the water from vaporizing. In studies carried out in vapor phase, some pressure is generally needed for effective transportation of the water. Above the critical temperature and critical pressure, the technique is called supercritical fluid extraction (SFE).

Relative permittivity (ε_r) is the key parameter in determining solute-solvent interactions. At elevated temperatures and pressures, ε_r of water is decreased significantly [1,2]. At room temperature, ε_r of water is high (−78 at 25 °C), but at 300 °C ($P = 23$ MPa) it is only −21. For comparison, ε_r of acetone at 25 °C is 20.7 [3]. The dramatic change in the ε_r value can partly be explained by the decrease in hydrogen bonding at elevated temperatures and the weaker intermolecular forces between water molecules. Ionic and very polar compounds can be extracted quantitatively near room temperature, but non-polar compounds require significantly higher temperatures. Selective extraction can thus be achieved by temperature tuning.

Although high PHWE temperature decreases the solubility of polar compounds in water, it may also have some positive effects on the extraction result. High temperature increases the initial desorption of the compounds from the sample particles, and the properties of the matrix may be altered, making the analytes more accessible to PHW. In addition, fast diffusion, low viscosity and low surface tension

* Corresponding author. Tel.: +358-919150268; fax: +358-919150253.
E-mail address: liisa.riekkola@helsinki.fi (M.-L. Riekkola).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.09.052

are achieved at high temperatures. On the negative side, thermally labile compounds may be destroyed and the amount of co-extracted compounds (other than analytes) may be greater than at lower temperature.

Hot water at atmospheric pressure has long been used in the extraction of relatively polar compounds from solid matrices [4]. Hawthorne et al. [5] were among the first fully to exploit the altered physico-chemical properties of pressurized hot water in extraction. PHWE has already been applied, for example, to the extraction of phenols [5,6], alkanes [6,7], PAHs [6–9], PCBs [10,11], pesticides [12,13], essential oils [14,15] and flavor compounds [16,17] from solid matrices. Phenols are relatively polar compounds and it can be assumed that moderate temperatures are sufficient for good PHWE recoveries. For example, Hawthorne et al. [5] obtained 99–100% recoveries for 2,4-dichlorophenol, 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol at 150 °C from spiked sand, and at 50 °C, the recoveries were only slightly lower. Determination of phenolic compounds has usually been carried out by gas chromatography (GC) or liquid chromatography (LC) [18]. A mass spectrometer (MS) has often been connected on-line to GC to provide reliable analysis of phenols in environmental samples, and, in many cases, a relatively non-polar capillary column has been the choice in GC separation [5,6,19,20]. With this type of column, the separation of the analytes is based mainly on the vapor pressures of the compounds.

In the past few years, capillary electromigration techniques (CE) have been widely applied to the determination of phenolic compounds, affording excellent separation efficiency. Good separation of phenols has been achieved by both capillary zone electrophoresis (CZE) [21–24] and micellar electrokinetic capillary chromatography (MECC) [25,26]. In these approaches, selectivity control is achieved by adjusting the pH of the separation buffer or by adding anionic [25] or neutral surfactants [26] in concentrations higher than the critical micelle concentration. Recently, non-aqueous capillary electrophoresis has been applied to the separation of phenols [27–30]. In the present work, the ionization constants of the phenols in water (Table 1) were clearly different from each other and the simplest capillary electromigration technique, CZE, was sufficient for their investigation.

The primary aim of the study was to compare GC–MS and CZE techniques in the analysis of phenol and three substituted phenols extracted with PHW from sea sand and soil. Especially, CZE analysis of phenols extracted from environmental matrix was of great interest, because this type of matrix contains often various compounds possibly having effect on the reliability of the CZE analysis. Different PHWE conditions were studied to determine the most suitable conditions for effective extraction of the compounds.

2. Experimental

2.1. Solid matrix

Two types of solid matrix were employed: acid-washed, calcined sea sand (Riedel-de Haën, Seelze, Germany, grain size 0.1–0.3 mm) and soil mixed with the sea sand in a ratio of 1:6. The soil, kindly supplied by Dr. Bert van Bavel (MTM Research Centre, Örebro University, Sweden), was from a decomissioned coal gasification plant in Husarviken, Stockholm (Sweden). The moisture content of the soil was 21 and 29% of the dry mass was organic carbon. The pH of the soil was 6.2. The soil was sifted through a 4 mm sieve, homogenised, and air-dried for three days. The dry soil was ground to a fine powder in a ball mill.

2.2. Reagents

The model pollutants were phenol (>99.5%), obtained from E. Merck Darmstadt, Germany, and 3-methylphenol (>98%), 4-chloro-3-methylphenol (99%) and 3,4-dichlorophenol (>97%) from Fluka AG, Buchs SG, Switzerland. All four compounds were used without further purification. Aqueous phenolic solutions were prepared by weighing predetermined amounts of the analytes (c = ca. 1.0 mg/ml) into distilled deionized water (UHQ water from Millipore Milli-Q system, Molsheim, France) and mixing the solutions in an ultrasonic bath for ca. 5 min. All the phenolics were soluble in water at working concentrations (Table 1). Dichloromethane (HPLC grade, Mallinckrodt

Table 1
Physicochemical properties of the investigated phenols, obtained from reference [34], and fragment ions used in the qualification and quantitation of the phenols in GC–MS analysis

	Quantitative ion (m/z)	Qualitative ion (m/z)	pK_a dissosciation constant	Solubility in H_2O (g/l)	Vapour P (mm Hg)
Phenol	94	66	9.99	82.8	0.35
3-Methylphenol	108	107	10.1	22.7	0.11
4-Chloro-3-methylphenol	107	142	9.55	3.8	0.05[a]
3,4-Dichlorophenol	162	164	8.63[b]	9.26	0.017
4-Bromophenol (ISTD)	172	65	9.17	14.0	0.012

[a] T = 20 °C.
[b] T not stated.

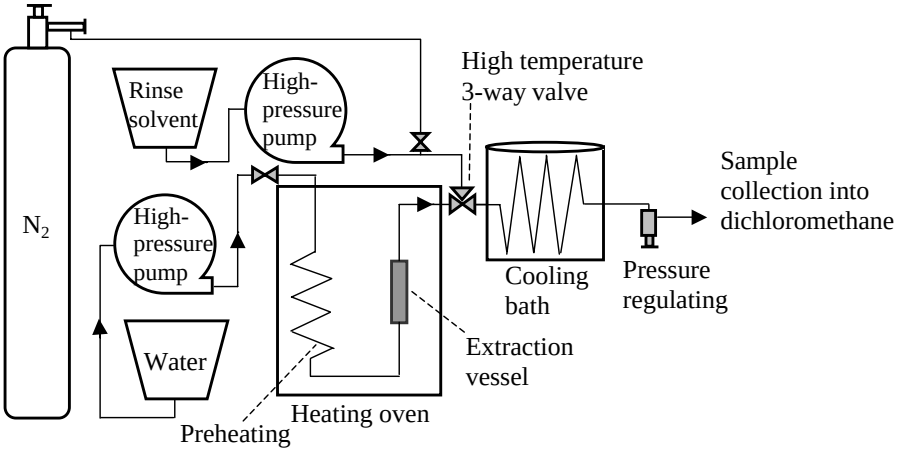

Fig. 1. PHWE equipment used in the experiments.

Baker B.V., Deventer, Holland) was applied as solvent in the liquid–liquid extraction of the organics from the PHWE effluent. The internal standard (ISTD) used in sample analysis was 4-bromophenol (99%, $c = 1.0$ mg/ml in UHQ water) obtained from Fluka AG, Buchs SG, Switzerland. The buffer solution used for the CZE analysis was based on 2-(*N*-cyclohexylamino)-ethanesulphonic acid, 30 mmol/l CHES (Sigma Chemical Co., St. Louis, Mo, USA), in water. The pH of the buffer solution was adjusted to 9.7 with sodium hydroxide (>99 %, Fluka Chemie AG, Buchs, Switzerland). In the following, background electrolyte (BGE) means the pH-adjusted CHES buffer solution.

2.3. PHWE equipment

Fig. 1 shows a simplified view of the PHWE equipment used in dynamic mode in the experiments. One high-pressure pump (Jasco PU-980 HPLC pump, Tokyo, Japan) was employed to deliver water to the laboratory-constructed stainless steel extraction vessel ($V = 2.8$ ml, 37 mm × 10 mm i.d.) described in detail elsewhere [31]. Tubing was connected to a three-way valve (HIP 30-15-HF4-HT, High Pressure Equipment Co., Erie, PA, USA) to allow the capillary to be flushed to the sample collection with dichloromethane using another similar pump. Also two on/off valves (HIP 15-11AF1, High Pressure Equipment Co., Erie, PA, USA) were employed in the system. All the tubes were made from a stainless steel capillary with an inner diameter of 0.5 mm (1/16 in. o.d.), except for the capillary that connected the extraction vessel to the high pressure three-way valve, which had an inner diameter of 0.75 mm. The pre-heating coil leading to the extraction vessel was 3.0 m long and the cooling coil, which was inserted in an ice bath, was 1.0 m long. The PHWE oven was a Fractovap series 2150 oven (Carlo Erba Strumentazione, Milan, Italy). A pressure regulator (stainless steel, micrometering valve, Jasco, Japan) was used for pressure adjustment.

2.4. PHWE procedure

Four experiments ($n = 4$) were carried out under similar conditions, and relative standard deviation (%R.S.D.) was used as a measure of repeatability. Three gram of sea sand or mixture of soil and sea sand (1:6, m:m) was weighed into the extraction vessel and 90 μl of each aqueous phenolic solution was injected to the solid matrix. After 20 h, the vessel was inserted to the equipment and the PHWE was started. Direction of the water flow was from the bottom to the top of the vessel.

In the PHWE with spiked sea sand, temperatures 50, 100, 200 and 300 °C and extraction times 20 and 40 min were employed. With the soil, the temperatures were 50 and 300 °C and the extraction time was 20 min. The heating time to the selected temperature (ca. 1–7 min depending on the temperature) was not included in the nominal extraction time. Flow rate was 1.0 ml/min and pressure (8–18 MPa) was high enough to keep the water in liquid state.

For sample collection, the exit capillary from the pressure regulator was inserted in a flask containing 10 ml of dichloromethane. After PHWE the tube from the three-way valve to the sample collection was quickly flushed with nitrogen and then for 4 min with dichloromethane at 2.0 ml/min. After this, ISTD ($V = 100$ μl) was added to the sample. The separated aqueous sample was further extracted with dichloromethane (2 ml × 3.0 ml), and the organic fractions were combined.

The dichloromethane solution was divided into two aliquots, one for GC–MS and the other for CZE analysis. For GC–MS analysis, the sample was concentrated to a final amount of 1.5 ml by gentle nitrogen evaporation using a Pierce Reaction-Therm heating module (Rockford, IL, USA) ($T = 30$ °C). For CZE analysis, the sample was carefully evaporated to dryness and reconstituted with 1.5 ml of the BGE.

Reference samples were prepared by adding 90 μl of each phenolic solution to a flask containing 20 ml of distilled

deionized water and 10 ml dichloromethane. The analytes were extracted and concentrated, as just described, to determine the 100% recoveries of phenols obtained by PHWE. One PHWE experiment with soil not spiked with phenols was carried out to determine if any phenols were naturally present in the sample.

2.5. GC–MS analysis

A Hewlett-Packard model 5890 gas chromatograph connected to a model 5989 A quadrupole mass spectrometer (USA) was used in the GC–MS analysis. All MS analyses were carried out in SCAN mode (mass range 50–550 amu) by electron impact ionization (EI, 70 V). The temperature of the GC–MS interface was 300 °C, that of the ion source 220 °C and that of the analyser 120 °C. Samples were injected ($V_{inj} = 2.0$ μl) in on-column mode with a Hewlett-Packard 7673 auto sampler using oven tracking for the injector temperature. The analytical column of the gas chromatograph was a 25.0 m HP-5 (Hewlett-Packard, USA) with 0.2 mm i.d. and 0.11 μm film thickness. A 2.0–3.0 m retention gap (BGB Analytik AG, Rothenfluh, Switzerland) of 0.53 mm i.d. with DPTMDS (1,2-diphenyl-1,1,3,3-tetramethyldisilazane) deactivation was connected to the analytical column with a press-fit connector (BGB Analytik AG, Rothenfluh, Switzerland). The inlet pressure of the carrier gas (He 4.6, Oy Aka Ab, Espoo, Finland) was 100 kPa and the temperature program of the GC oven 30 °C (2 min)–10 °C/min–300 °C (10 min).

The ions used in quantification and identification of the phenols are listed in Table 1. The software used in the computer was Hewlett-Packard ChemStation (G1034C version C 03.00). The software included a mass spectral library (Wiley), which was used in identifying organic compounds (also other than the four phenols) extracted from the soil. The calibration for quantitative analysis of phenols by GC–MS was carried out with a dilution series of phenols spiked in dichloromethane.

2.6. CZE analysis

Capillary zone electrophoresis experiments were carried out with a HP ^{3D}CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode-array detector. Instrument control and data analysis were performed with HP Chem Station software (Revision A.06.03). Uncoated fused-silica capillaries (Composite Metal Service, Hallow, UK) were 50 μm i.d. and 375 μm o.d., with effective length of 50.0 cm and total length of 58.5 cm. The capillary cassette temperature was maintained at 25.0 °C with an air-cooling system. Ultra violet (UV) detection of the analytes was carried out at 220 nm.

All new capillaries were conditioned before use. They were pretreated sequentially for 10 min with sodium hydroxide ($c = 0.1$ mol/l), 5 min with UHQ water and 15 min with the BGE. This same procedure was applied in daily start-up. The capillary was rinsed with the BGE for 3 min between runs. The BGE solution in the vials was replaced after every third run. After use, the capillary was rinsed with UHQ water and dried with air.

Samples were introduced to the capillary under pressure (5 KPa) fixed time period (3 s) and the analyses were performed applying a constant voltage of +20 kV. The calibration for quantitative analysis of phenols by CZE was carried out with a dilution series of phenols spiked in the BGE solution.

3. Results and discussion

3.1. Preliminary studies

In the development of the pressurised hot water extraction, the GC–MS and CZE methods were initially tested separately. First, the separation of phenols by CZE was studied. The acid–base properties of the analytes in CZE depend mainly on the pH. Thus, the mobility differences of the analytes can be maximized and high resolution can be achieved by selecting the correct pH of the buffer. After preliminary investigations on several buffers, CHES was chosen as the background buffer for the separation. Study was then made of the influence of the buffer pH on the migration time and resolution of the phenols. pH values in the range of 9.5–10.0 were tested, and the most appropriate separation, concerning selectivity and analysis time, was obtained at pH 9.7. The pK_a values of the phenols are listed in Table 1.

The results of earlier experiments with phenols were utilized in finding suitable parameters for the GC separation and MS detection. The conditions have been described in Section 2.

The detection limits (S/N = 3) for the phenolic compounds were determined by both GC–MS and CZE (with UV detection). They were as follows (S/N = 3): 67 ng/ml (GC–MS) and 410 ng/ml (CZE) for phenol; 107 ng/ml (GC–MS) and 270 ng/ml (CZE) for 3-methylphenol; 176 ng/ml (GC–MS) and 300 ng/ml (CZE) for 4-chloro-3-methylphenol; 282 ng/ml (GC–MS) and 380 ng/ml (CZE) for 3,4-dichlorophenol. As can be seen, detection limits for the compounds, especially for compounds with low vapour pressure, were only a little lower with GC–MS than with CZE technique. Although selected ion monitoring (SIM) mode would have produced significantly lower detection limits for GC–MS, SCAN mode was used in this work because other compounds than phenols extracted from the soil were of interest as well. Linearity (r^2 value) for GC–MS method was calculated from the calibration curve and it was 0.997 for phenol and 3-methylphenol, 0.998 for 4-chloro-3-methylphenol and 0.999 for 3,4-dichlorophenol (concentration range ca. 1.5–50 μg/ml). With CZE, the r^2 values were 0.999 for all compounds.

After optimization of the separations, PHWE conditions (extraction temperature and time) were studied to determine

Table 2
Relative recoveries in % and R.S.D. in % (within parenthesis) of phenols extracted with PHW at 50, 100 and 200 °C ($t = 20$ min) from sea sand and analysed by GC–MS and CZE (with UV detection)

	$T = 200$ °C, $t = 20$ min, $P_{AV} = 17$ MPa		$T = 100$ °C, $t = 20$ min, $P_{AV} = 13$ MPa		$T = 50$ °C, $t = 20$ min, $P_{AV} = 12$ MPa	
	GC–MS	CZE	GC–MS	CZE	GC–MS	CZE
Phenol	96.4 (8.2)	101.0 (5.0)	96.5 (5.2)	98.1 (5.7)	98.2 (3.0)	100.6 (0.9)
3-Methylphenol	89.4 (11.5)	94.7 (4.2)	90.8 (6.6)	92.2 (6.6)	89.0 (0.3)	92.9 (0.9)
4-Chloro-3-methylphenol	96.5 (4.8)	96.8 (3.2)	91.6 (3.9)	87.1 (5.0)	93.3 (0.5)	94.6 (5.7)
3,4-Dichloro-phenol	93.4 (1.7)	86.6 (1.7)	87.9 (7.7)	82.2 (5.0)	88.2 (3.9)	89.8 (5.4)

the most suitable conditions for effective extraction of the compounds. Since pressure has been reported to have only a minor effect on PHWE efficiency [5], the effect of pressure on the recoveries was not studied in detail. Relative percentage PHWE recovery of the phenolic compounds was obtained by comparing the amounts of phenols determined in the PHWE-treated sample and in the reference sample (see PHWE procedure).

3.2. Spiked sea sand

Good recoveries for phenolic compounds spiked in the sea sand were achieved at all PHWE temperatures (Tables 2 and 3). Phenols are relatively polar compounds and soluble in water at room temperature (Table 1). Although the polarity of water decreases with increase in temperature from 50 to 300 °C, diffusion and transportation properties of the water and desorption of the analytes from the matrix are enhanced at high temperatures and the overall extraction efficiencies are good. The recoveries of the compounds with lowest water solubility at 25 °C (4-chloro-3-methylphenol and 3,4-dichlorophenol) were generally slightly lower at 50 and 100 °C than at higher temperatures. In addition to the thermal effects explained above, this result can be explained by enhanced water solubility of the phenols with the lowest polarity.

The recoveries obtained for the separate phenolic compounds from sea-sand by CZE at the studied extraction temperatures were compared using analysis of variance (ANOVA). In each case the level of significance was determined; when this value was greater than 0.05, which was chosen as the minimum level of significance, the null hypothesis was accepted. The null hypothesis means that there were no significant differences among the results. The levels of significance corresponding to this test for phenol, 3-methylphenol, 4-chloro-3-methylphenol and 3,4-dichlorophenol were 0.83, 0.85, 0.04 and 0.05, respectively. Thus, the analysis of the results obtained by ANOVA showed that there were no significant differences among the results (except the values for 4-chloro-3-methylphenol and 3,4-dichlorophenol, which were close to 0.05), so the extraction temperature in the studied range was concluded not to have a significant effect on the recoveries of the compounds.

GC–MS analysis showed that, at both 20 and 40 min extraction times, a little benzeneamine and some organic compounds other than phenols (not positively identified) were present in trace amounts in the samples obtained by PHWE at 300 °C. The compounds were not detected in PHWE at 50, 100 or 200 °C or in the reference samples, which suggests that phenols (or perhaps only one phenolic compound; not studied in detail) were decomposed to some extent at 300 °C. By way of comparison, Windal et al. [32] found that dioxins were degraded much more rapidly in pressurized hot water at 300 °C than at 250 °C, while no decomposition occurred at 200 °C.

Extraction was studied at 300 °C with extraction times of 20 and 40 min (Table 3). Application of the unpaired two-tailed *t*-test for each of the phenols showed that there were no significant differences among the CZE results except for phenol (significance level 0.01). For other compounds the levels of significance were higher than 0.05. Thus, it can be concluded that extraction time (20 or 40 min) did not have a significant effect on the recoveries of most of the compounds. Application of the *t*-test to the relative recovery values obtained by GC–MS at the two extraction

Table 3
Relative recoveries in % and R.S.D. in % (within parenthesis) of phenols extracted with PHW at 300 °C ($t = 20$ and 40 min) from sea sand and analysed by GC–MS and CZE (with UV detection)

	$T = 300$ °C, $t = 20$ min, $P_{AV} = 18$ MPa		$T = 300$ °C, $t = 40$ min, $P_{AV} = 17$ MPa	
	GC–MS	CZE	GC–MS	CZE
Phenol	98.2 (9.9)	98.4 (6.8)	92.1 (8.7)	77.7 (10.7)
3-Methylphenol	94.6 (4.2)	94.1 (3.6)	93.9 (4.7)	89.4 (2.2)
4-Chloro-3-methylphenol	97.1 (8.8)	93.8 (3.1)	97.6 (6.7)	96.1 (4.0)
3,4-Dichlorophenol	95.2 (8.4)	90.5 (4.4)	97.8 (4.1)	93.8 (1.9)

Table 4
Relative recoveries in % and R.S.D. in % (within parenthesis) of phenols extracted from contaminated soil by PHW at 50 and 300 °C ($t = 20$ min) and analysed by GC–MS and CZE (with UV detection)

	$T = 300$ °C, $t = 20$ min, $P_{AV} = 16$ MPa		$T = 50$ °C, $t = 20$ min, $P_{AV} = 8$ MPa	
	GC–MS	CZE	GC–MS	CZE
Phenol	98.2 (2.2)	105.2 (12.7)	93.3 (7.6)	97.4 (9.2)
3-Methylphenol	92.0 (2.5)	93.0 (12.3)	85.2 (2.9)	88.8 (11.1)
4-Chloro-3-methylphenol	89.6 (12.3)	81.6 (7.1)	86.3 (7.0)	85.8 (23.6)
3,4-Dichlorophenol	92.7 (4.9)	85.9 (5.0)	79.4 (6.5)	77.7 (21.6)

times revealed no significant differences among the results: a level of significance higher than 0.05 was obtained for all phenolic compounds. Problems in data processing could not have been responsible for the minor differences found for phenol working with CZE, because the peaks recorded for this analyte with both analytical techniques were near the gaussian shape. No evident reason was found for the low relative recovery of phenol with 40 min extraction time ($T = 300$ °C) obtained by CZE (Table 3).

The PHW extraction study of the phenolic compounds spiked in sea sand demonstrated that the analytes can be extracted with good recoveries even at low temperatures. The differences between the recoveries obtained by GC–MS and CZE analysis were relatively small and the R.S.D. were low (0.3–11.5%).

3.3. Spiked soil

The recoveries of phenolic compounds, especially 4-chloro-3-methylphenol and 3,4-dichlorophenol, spiked in the soil (mixture of soil and sea sand) were generally a little lower than those of phenolic compounds spiked in the sea sand (Table 4). The structure of the matrix and its chemical properties may have affected the results. Probably the compounds were more tightly bound to the soil than to the sea sand, even though the PHWE experiments were carried out only 20 h after spiking. Presumably native analytes would be even harder to extract. Organic compounds other than phenols present in the soil may also have interfered with the analysis through interacting with the phenols.

Yang et al. [6] found that the recoveries of phenolic compounds extracted with PHW from petroleum waste sludge were increased somewhat when the temperature was raised from 100 to 250 °C ($P = 50$ atm). In our study, increase in the extraction temperature from 50 to 300 °C provided a slight increase in the relative recovery of the least polar analyte, 3,4 dichlorophenol. The relative recovery values obtained for this analyte by GC were compared using an unpaired two-tailed *t*-test, and the level of significance was 0.04. The value is close to 0.05 and can be considered acceptable. We conclude, that the extraction temperature does not have a significant effect on the recoveries.

Application of the *t*-test to compare the recoveries obtained by GC–MS and CZE analysis from real soil showed that there were no significant differences among the results: levels of significance were higher than 0.05. In the light of these observations, it can be concluded that the two methods afford comparable results. As expected, the

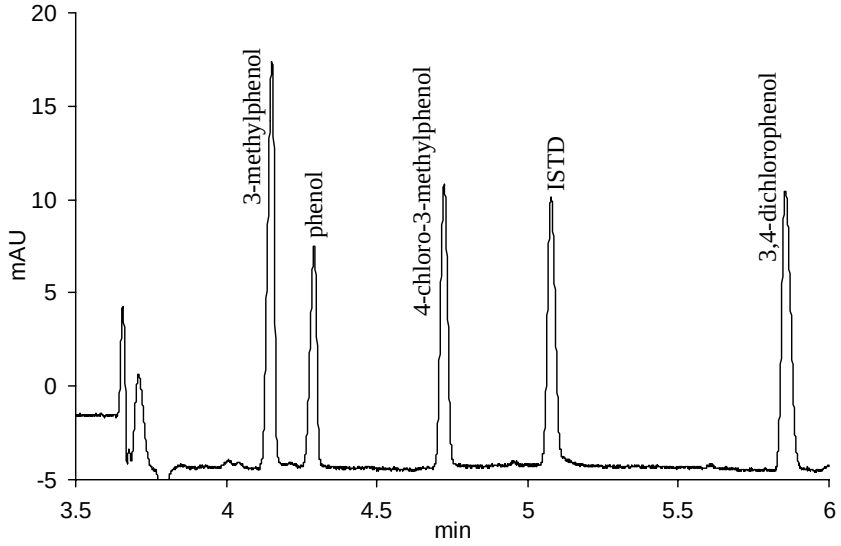

Fig. 2. Electropherogram of phenols spiked in the soil and extracted by PHWE ($T = 300$ °C, $t = 20$ min). BGE: 30 mmol/l CHES, pH 9.7.

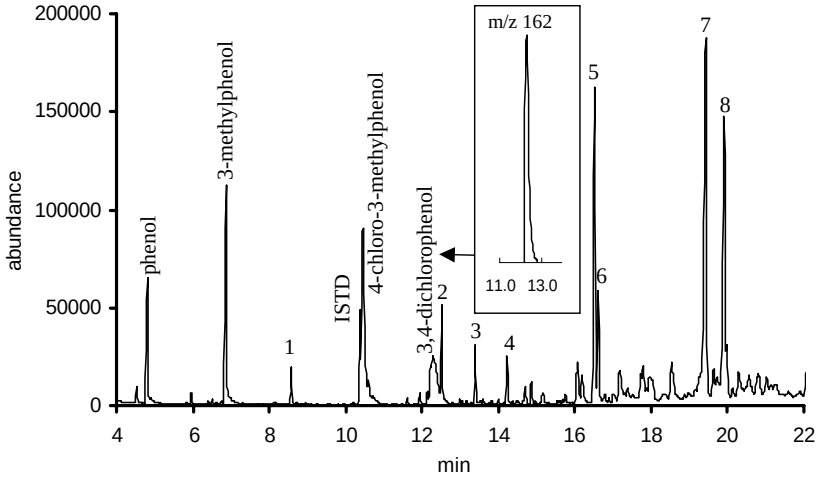

Fig. 3. Total ion chromatogram of phenols spiked in the soil and extracted by PHWE ($T = 300\,^{\circ}C$, $t = 20$ min). Explanations: (1) naphthalene; (2) acenaphthylene; (3) dibenzofuran; (4) fluorene; (5) phenanthracene; (6) anthracene; (7) fluoranthene; (8) pyrene. The peak of the quantitation ion (*m*/*z*, 162) for 3,4-dichlorophenol is presented separately in the small window.

R.S.D. of the recoveries were generally higher with soil than with sea sand, especially the R.S.D. of the recoveries of 4-chloro-3-methylphenol and 3,4-dichlorophenol at extraction temperature 50 °C obtained by CZE. A possible explanation of the higher R.S.D. for these last two phenols is that they are less polar and less soluble in water at 50 °C than are phenol and 3-methylphenol.

Furthermore, it can be noted that the shapes of the peaks obtained for spiked soil by CZE (PHWE at 50 and 300 °C) were good (Fig. 2). In GC–MS analysis (total ion chromatogram), the shape of the 3,4-dichlorophenol peak was poor (PHWE of spiked soil at 300 °C, Fig. 3). No such problems existed with spiked sea sand under any conditions, nor with PHWE of soil at 50 °C. The sample matrix may contain some non-volatile compounds that adsorb on the GC pre-column and disturb the formation of the flooded solvent zone, negatively affecting the shape of the 3,4-dichlorophenol peak. The recovery calculations were based on the peak area, not on the peak height, so the influence of poor peak shape on the recovery value was less. Furthermore, the shape of the peak of the quantitative ion *m*/*z* 162 (see small window in Fig. 3) representing 3,4-dichlorophenol and used for quantitative analysis was good.

A PHWE experiment carried out with soil not spiked with phenols ($T = 300\,^{\circ}C$) showed that minute amounts of phenol and 3-methylphenol were naturally present (not spiked) in the soil. The amount of phenol corresponded to 5.2% of the relative recovery (with respect to the spiked amount of phenol) obtained by GC–MS and 8.6% of the relative recovery obtained by CZE. Similarly, the amount of 3-methylphenol naturally present corresponded to 8.6% (GC–MS) and 4.4% (CZE) of the relative recovery. R.S.D. are not available for these results because only one experiment was carried out with the soil not spiked with phenolic compounds.

GC–MS studies showed that phenols were selectively extracted from soil at 50 °C. Various other compounds (e.g. several polyaromatic hydrocarbons (PAHs), dibenzofuran and 1,1′-biphenyl) were found in large amounts at extraction temperature of 300 °C (Fig. 3). These other compounds are of non-polar nature and higher temperatures are required for their efficient extraction. Quantitative information about the recoveries of PAHs from the Husarviken soil used in this study can be found in our previous paper [33].

The other compounds co-extracted with phenols from the soil at 300 °C were not separated by CZE (Fig. 2) because the co-extracted compounds were not present in the BGE as ions. Peaks of the phenols in the electropherograms were similar to those obtained from the spiked sea sand and no interference from the matrix was detected. Thus, good separation of phenols can be achieved even from a complex matrix with an appropriate selection of the pH.

4. Conclusions

Comparable results were obtained by the GC–MS and CZE methods. CZE was a reliable analytical tool for the analysis of PHW-extracted phenols: the compounds co-extracted from the matrix of the soil sample did not interfere with the CZE analysis of the phenols, and the resolution was very good thanks to an appropriate selection of the BGE (pH 9.7). PHWE recoveries of phenolic compounds were a little lower from the soil than from the sea sand, showing that interactions between the analyte and matrix may affect the extraction efficiency. PHWE selectivity can be adjusted with temperature: GC–MS studies showed that phenols were almost exclusively extracted from the soil at 50 °C, but also other compounds (e.g. PAHs) were extracted in great amount at 300 °C.

Acknowledgements

We thank Dr. Bert van Bavel (MTM Research Centre, Örebro University, Sweden) for submitting the soil sample. Financial support from the Academy of Finland is greatly appreciated (project 52746).

References

[1] K. Heger, M. Uematsu, E.U. Franck, Ber. Bunsenges Phys. Chem. 84 (1980) 758.
[2] M. Uematsu, E.U. Franck, J. Phys. Chem. Ref. Data 9 (1980) 1291.
[3] R.C. Weast, M.J. Astle, W.H. Beyer (Eds.), CRC Handbook of Chemistry and Physics, 67th ed., CRC Press, Florida, USA, 1987.
[4] M.U. Beer, P.J. Wood, J. Weisz, Cereal. Chem. 74 (1997) 476.
[5] S.B. Hawthorne, Y. Yang, D.J. Miller, Anal. Chem. 66 (1994) 2912.
[6] Y. Yang, S.B. Hawthorne, D.J. Miller, Environ. Sci. Technol. 31 (1997) 430.
[7] K. Hartonen, G. Meissner, T. Kesälä, M.-L. Riekkola, J. Microcolumn Sep. 12 (2000) 412.
[8] S.B. Hawthorne, C.B. Grabanski, E. Martin, D.J. Miller, J. Chromatogr. A 892 (2000) 421.
[9] A.J.M. Lagadec, D.J. Miller, A.V. Lilke, S.B. Hawthorne, Environ. Sci. Technol. 34 (2000) 1542.
[10] Y. Yang, S. Bøwadt, S.B. Hawthorne, D.J. Miller, Anal. Chem. 67 (1995) 4571.
[11] S.B. Hawthorne, C.B. Grabanski, K.J. Hageman, D.J. Miller, J. Chromatogr. A 814 (1998) 151.
[12] M.M. Jiménez-Carmona, J.J. Manclús, A. Montoya, M.D. Luque de Castro, J. Chromatogr. A 785 (1997) 329.
[13] A.E. McGowin, K.K. Adom, A.K. Obubuafo, Chemosphere 45 (2001) 857.
[14] R.S. Ayala, M.D. Luque de Castro, Food Chem. 75 (2001) 109.
[15] M.M. Jiménez-Carmona, M.D. Luque de Castro, Chromatographia 50 (1999) 578.
[16] S. Rovio, K. Hartonen, Y. Holm, R. Hiltunen, M.-L. Riekkola, Flavour Fragr. J. 14 (1999) 399.
[17] A. Basile, M.M. Jiménez-Carmona, A.A. Clifford, J. Agric. Food Chem. 46 (1998) 5205.
[18] D. Puig, D. Barceló, Trends Anal. Chem. 68 (1996) 362.
[19] M. Möder, S. Schrader, U. Franck, P. Popp, Fresenius J. Anal. Chem. 357 (1997) 326.
[20] R. Li, P.E. Savage, D. Szmukler, AIChE J. 39 (1993) 178.
[21] D. Martínez, E. Pocurull, R.M. Marcé, F. Borrull, M. Calull, J. Chromatogr. A 734 (1996) 367.
[22] C.-Y. Tsai, G.-R. Her, J. Chromatogr. A 743 (1996) 315.
[23] D. Martínez, F. Borrull, M. Calull, J. Chromatogr. A 788 (1997) 185.
[24] I. Canals, E. Bosch, M. Rosés, Anal. Chim. Acta 458 (2002) 355.
[25] C.P. Ong, C.L. Ng, N.C. Chong, H.K. Lee, S.F.Y. Li, J. Chromatogr. 516 (1990) 263.
[26] G. Li, D.C. Locke, J. Chromatogr. A 734 (1996) 357.
[27] S. Morales, R. Cela, J. Chromatogr. A 846 (1999) 401.
[28] J.L. Miller, D. Shea, M.G. Khaledi, J. Chromatogr. A 888 (2000) 251.
[29] S.P. Porras, R. Kuldvee, S. Palonen, M.-L. Riekkola, J. Chromatogr. A 990 (2003) 35.
[30] S. Morales, R. Cela, J. Chromatogr. A 896 (2000) 95.
[31] T. Andersson, K. Hartonen, T. Hyötyläinen, M.-L. Riekkola, Anal. Chim. Acta 466 (2002) 93.
[32] I. Windal, S. Hawthorne, E. de Pauw, Organohalogen Compd. 40 (1999) 591.
[33] J. Kronholm, J. Kalpala, K. Hartonen, M.-L. Riekkola, J. Supercrit. Fluids 23 (2002) 123.
[34] Interactive PhysProp Database Demo, Syracuse Research Corporation, USA: http://esc.syrres.com/interkow/physdemo.htm.