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Capillary zone electrophoresis/electrospray mass spectrometry of priority phenols

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Abstract

Eleven priority phenols were separated by capillary zone electrophoresis (CZE) and detected on-line with electrospray ionization mass spectrometry (ESI-MS) in the negative-ion mode. Parameters critical to the coupling of CZE and MS were studied. The best result was obtained with 2-[N-cyclohexylamino]ethanesulfonic acid (CHES) as the running buffer and a solution of water–2-propanol (20:80) containing 0.5% ammonia as the sheath liquid. With the use of field amplification as the preconcentration technique and the mass spectrometer operated in the selected ion monitoring mode, the detection limit of most phenols was found to be in the range of 50 ppb. This method has been successfully applied to the determination of pentachlorophenol in a paper clay sample.

Keywords: Mass spectrometry; Buffer composition; Phenols; Priority phenols

1. Introduction

Many phenolic compounds are of great environmental concern because of their toxicity. Eleven phenols including phenol, 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 2,4-dimethylphenol (2,4-DMP), 4-chloro-3-methylphenol (4-C-3-MP), 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) were listed by the United States Environmental Protection Agency as priority pollutants. Conventional methods based on gas chromatography (GC) [1] or high-performance liquid chromatography (HPLC) [2–4] have been routinely used for the monitoring of these pollutants. Recently, mainly due to its superior separation efficiency, several groups have explored the potentials of analyzing phenols by capillary electrophoresis (CE) [5–8].

The primary detection method of CE is UV absorption [9–11], although, electrochemical and fluorescence detection have also been reported [12–15]. The sensitivity of these techniques is, in general, adequate for many applications, however, the information provided by these techniques is often not enough for a high confidence identification. Furthermore, while the retention times are often used for compound identification, the retention times of CE are not as reproducible as in GC and HPLC and thus there is a greater risk in the identification of analytes based on retention time.

Because of its low detection limit, high specificity and more importantly, abundant structural informa-

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tion, mass spectrometry (MS) has been considered as one of the ideal devices for chromatographic detection. The merits of using MS as the chromatographic detector is best demonstrated with the highly successful GC–MS. The coupling of HPLC and CE with MS is, in general, much more difficult than the interfacing of GC with MS, however, the recent developments in MS such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) makes the coupling of HPLC and CE with MS [16–20] much easier.

CE is a modern technique known for its superior separation efficiency per unit time. The much smaller loading capacity and higher separation efficiency of CE make fraction collection more difficult compared to HPLC. In this report, we described the on-line coupling of capillary zone electrophoresis (CZE) with ESI-MS in the analysis of priority phenols.

2. Experimental

2.1. Chemicals

Phenol was obtain from Aldrich (Milwaukee, WI, USA). 2,4-Dinitrophenol (2,4-DNP) was obtain from Janssen (Belgium). 2-Nitrophenol (2-NP), 4-nitrophenol (4-NP), 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 2,4-dimethylphenol (2,4-DMP), 4-chloro-3-methylphenol (4-C-3-MP), 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) were purchased from Fluka (Switzerland). 2-[N-Cyclohexylamino]ethanesulfonic acid] (CHES) was purchased from Sigma (St, Louis, MO, USA). Sodium tetraborate anhydrous and ammonium hydroxide were purchased from Janssen (Belgium). Deionized (18 MΩ) water (Milli-Q water system, Millipore, Bedford, MA, USA) was used in the preparation of the samples and buffer solution. 2-Propanol was HPLC grade from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Apparatus

The CZE system was made in-house using CZE 1000R (Spellman, Plainview, NY, USA) high-voltage power supply. CE columns were fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) 50-μm I.D., 375-μm O.D. and 100 cm length (90 cm to detector). A small area of the polyimide coating was burned off to form a window for UV detection. The detector (UV-C, Rainin, Emeryville, CA, USA) wavelength was set at 210 nm. Before use, the new capillary column was washed with 1 *M* NaOH followed by water and running buffer. The capillary was then equilibrated with the running buffer under an electric field 200 V cm^{-1} for 20 min. To avoid absorption, the capillary column needs to be rinsed with sodium hydroxide between analysis. In normal operation, the sample was injected electrokinetically at 10 kV for 10 s. The injection volume was calculated to be near 10 nl. In field amplification for improving the sensitivity of this approach, phenols prepared in water were introduced hydrodynamically into the column (15 cm; 7 min). High voltage with reversed polarity (−20 kV) was then applied to the column to push water out while retaining the analytes on the column. When the current reaches about 95% of the original value (about 45 s) the polarity of the electrodes was switched back to normal configuration (20 kV).

A VG Platform single quadrupole mass spectrometer (Fisons Instruments/VG Bio Tech, Altrincham, UK) equipped with a CE interface was used for this study. The interface (Fig. 1) utilizes a triaxial flow arrangement whereby the CZE eluent is mixed with a suitable make-up solution (sheath liquid) at the probe tip and then nebulized using N_2 gas. In the negative-ion mode, a potential about −3.5 kV was applied to the probe tip. When the high voltage used for the CZE separation was maintained at +20 kV, the overall potential across the separation capillary was about 23.5 kV. A syringe pump (Model H-74900-00, Cole-Parmer, Niles, IL, USA) was used to deliver the sheath flow at a flow-rate of 10 μl/min. Nitrogen gas at a flow-rate of 0.5 l/min was used as the nebulizing gas. The warm (80°C) bath gas (nitrogen) at a flow-rate of 1.6 to 2.5 l/min aided dissolution of the electrospray droplets. Mass spectral data were collected at either full scan or selected-ion monitoring (SIM) mode (0.2 s dwell time, 0.2 mass unit span).

3. Results and discussion

Both continuous flow fast atom bombardment (CF-FAB) and ESI have been successfully coupled

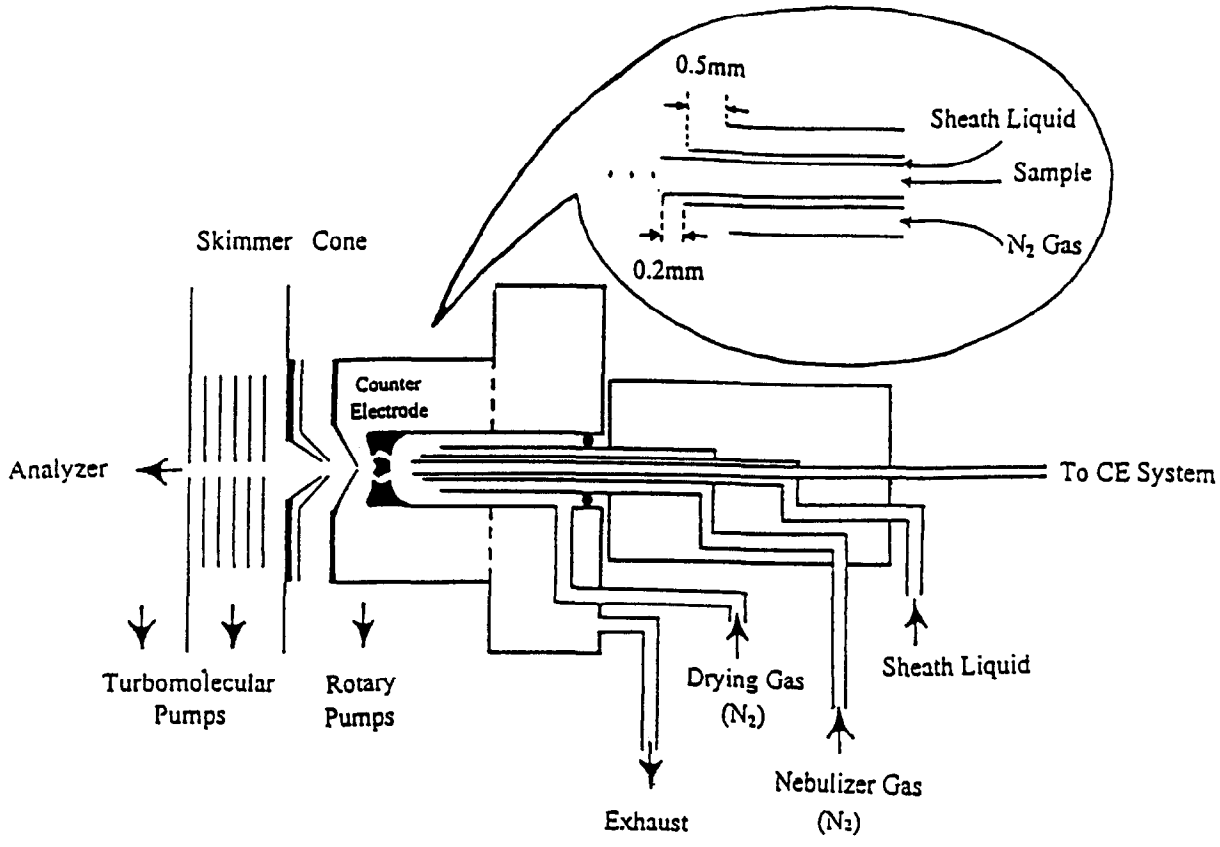

Fig. 1. The triaxial CZE–ESI interface.

with CE [18–22]. In comparison with FAB, ESI has the advantage of operating at atmospheric pressure so that hydrodynamic flow due to the vacuum in the separation capillary does not occur. Although CF-FAB and ESI can provide comparable sensitivity for different types of analytes, the coupling of CZE to ESI has gained considerable popularity in view of the ease of operation and simplicity of the interface design.

CZE and micellar electrokinetic chromatography (MEKC) are the two most popular CE techniques. CZE is amenable to broad compound classes with applications limited only by the necessity for solubility in the buffer and a non zero net electrophoretic mobility. Separation is based on the differential rate of migration of ionic species in an electric field [23–25]. In contrast to CZE, MEKC is often used for the separation of water insoluble and noncharged compounds. Both CZE and MEKC have been successfully used in the separation of priority phenols [5–8]. In this work, CZE instead of MEKC was chosen as the separation technique because, most likely due to the high concentration of surfactants, the sensitivity of CZE/ESI was much better than that of MEKC/ESI.

Sodium tetraborate at pH 9.9 has been used by Whang et al. [8] as the running buffer in the CZE analysis of phenols with indirect fluorescence detection. Although the system produced a baseline resolved electropherogram, borate buffer was not a good choice for the CZE/ESI–MS operation. Both sensitivity and ion current stability were not suitable for the CZE/ESI–MS operation. Moreover, white solid deposit was observed in the source due to the poor volatility of borate buffer. This solid deposit slowly blocked the orifice of the mass analyzer and thus reduced the sensitivity. A more volatile buffer, CHES, was then tested. The long term sensitivity and ion current stability are superior to borate buffer.

With the use of CHES as the running buffer, the

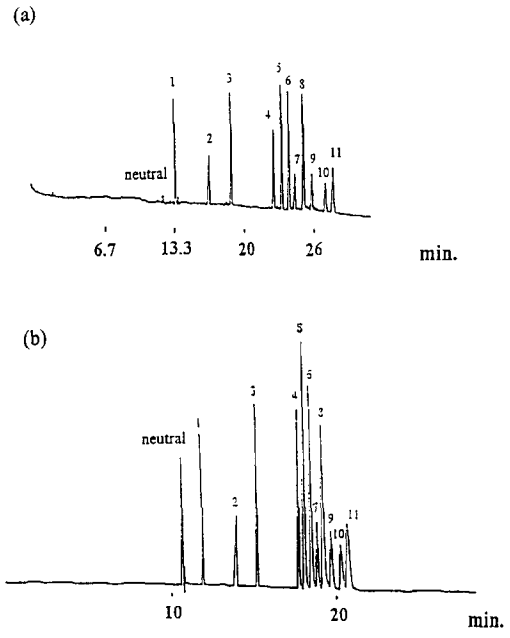

Fig. 2. Electropherograms of the eleven priority phenols. (a) sodium tetraborate buffer (pH 10.0). (b) CHES buffer (pH 10.0). Peaks were assigned as 1=2,4-DMP; 2=phenol; 3=4-C-3-MP; 4=PCP; 5=2,4,6-TCP; 6=2,4-DCP; 7=2-M-4,6-DNP; 8=2-CP; 9=2,4-DNP; 10= 4-NP; 11=2-NP.

resolution is not as good as with the borate as the running buffer (Fig. 2a), however, all eleven phenols are still baseline resolved (Fig. 2b). The elution order of the phenols was found to be the same as with the borate buffer. The resolution and retention time may be affected by pH of the running buffer. Fig. 3 shows the pH dependence of migration time for phenols between pH 9 to pH 10.5 with 20 m*M* CHES buffer. With pH above 9, all eleven phenols could be separated with CZE. The migration times increased as pH of the buffer increased. It is interesting to see that when pH of the solution decreased to below 9.75, the elution order of 2-CP and 2-M-4,6-DNP was reversed in comparison with higher pH.

Make-up solution (sheath liquid) performs two functions, one is to supplement the CZE flow to a level suitable for ESI operation and the other is to make electrical contact between the CZE eluent and probe tip. Sheath liquid was reported critical to the performance of the CZE/ESI interface [19]. Sheath liquids of different composition have been tested for sensitivity and stability under negative ESI conditions. In the experiment, a 10 ppm 4-NP solution was electroosmotically infused through the silica capillary, and then mixed with sheath liquid (10 μl/min) at the tip of the CE probe. The results showed that the addition of ammonia improved the sensitivity of phenols and 2-propanol produced a more stable signal than methanol and ethanol (Fig. 4). The best result was obtained with a solution of 20:80 water–2-propanol containing 0.5% ammonia.

One major function of the sheath liquid is to supplement the CZE flow to a level suitable for electrospray operation. With the use of a sheath

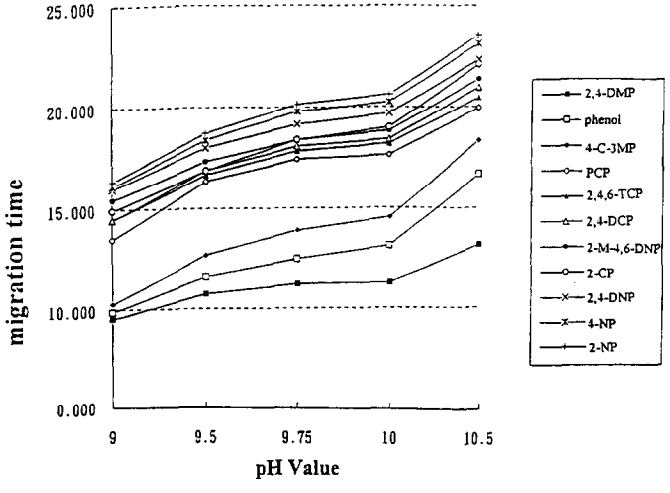

Fig. 3. Effect of pH on elution time and order.

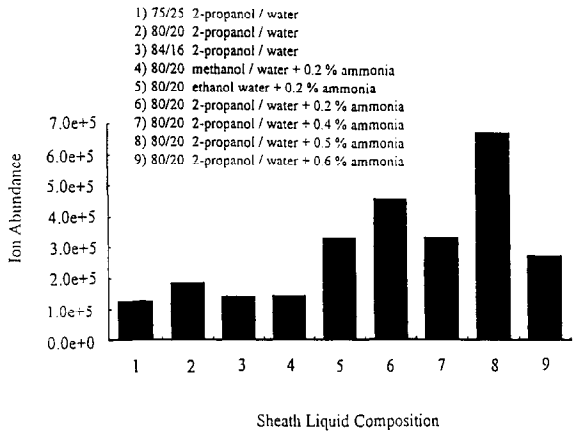

Fig. 4. Ion abundance as a function of ESI sheath liquid composition. The 4-nitrophenol (10 ppm) was electroosmotically infused (20 kV) through the silica capillary.

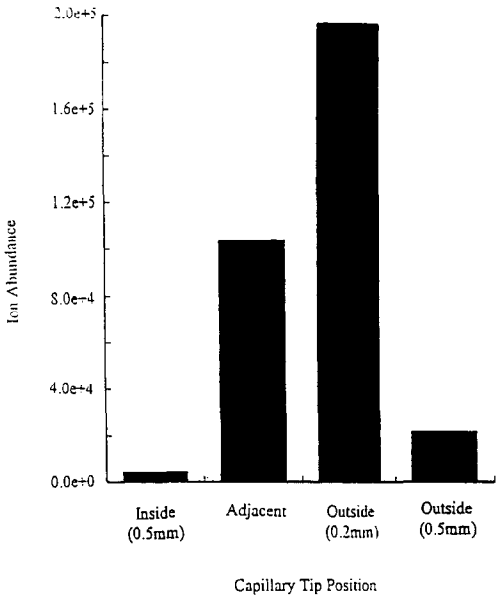

Fig. 5. Ion abundance as a function of capillary tip position. The 4-nitrophenol (10 ppm) was electroosmotically infused (20 kV) through the silica capillary. The flow-rate of sheath liquid [water–2-propanol (20:80) containing 0.5% ammonia] was 10 μl/min.

liquid of 20:80 water–2-propanol containing 0.5% ammonia, a flow of 8 to 12 μl/min was found to be best suited for ESI operation.

Sheath liquid was chosen because of the optimization in sensitivity and stability, therefore, sheath liquid would be an ideal choice as the running buffer in CZE. Unfortunately, good separation could not be obtained with the sheath liquid (20/80 water/2-propanol containing 0.5% ammonia) as the running buffer.

Sheath liquid was passed between the separation and the stainless capillary column (Fig. 1) and then mixed with the running buffer at the tip of the probe. The relative position between these two columns affected the sensitivity of the CZE/ESI interface. The best sensitivity was obtained with the separation column protruding slightly (about 0.2 mm) beyond the tip of the stainless capillary column (Fig. 5). If the silica tubing protruded too far to make a good electrical contact, the sensitivity dropped very quickly.

Phenols showed very little fragmentation under negative ESI conditions. The negative ESI mass spectra of phenols are characterized with molecular ion and background ions from buffer and solvent (Fig. 6a and b). For the following reasons, the mass spectrometer was operated at the SIM mode in the CZE/MS analysis of low level phenols. First, the sensitivity of SIM was shown to be about 10 times better than full scan. Secondly, background subtracting routines, which were used to remove background ions from the spectra of high concentration of phenols (Fig. 6c and d), were not quite successful for low levels (ppm–ppb) of phenols. The small variation in background ion current, not a serious problem for high concentration samples, appeared to affect low level phenols greatly. Finally, as mentioned earlier, there is little fragmentation in the spectra, the lack of fragment ions makes little difference between full scan and SIM.

The on-line CZE/MS analysis of a 10 ppm mixture of phenols is given in Fig. 7. All phenols gave single ion electropherograms with good peak shape and signal-to-noise ratio. Although the same amount of samples were injected, phenol, 2-CP, 2-NP, and 4-C-3-MP appeared to have poorer S/N ratio (Fig. 7). Direct injection of phenols into the ESI showed that the responses of phenol, 2-CP, 2-NP, and 4-C-3-MP were poorer than others.

The separation efficiency, represented by the number of theoretical plates, N, was calculated from the peak half-width for each phenol. For CZE–UV, the range was from 50 900 for phenol to 182 000 for 2-M-4,6-DNP. The efficiency of CZE–MS was not

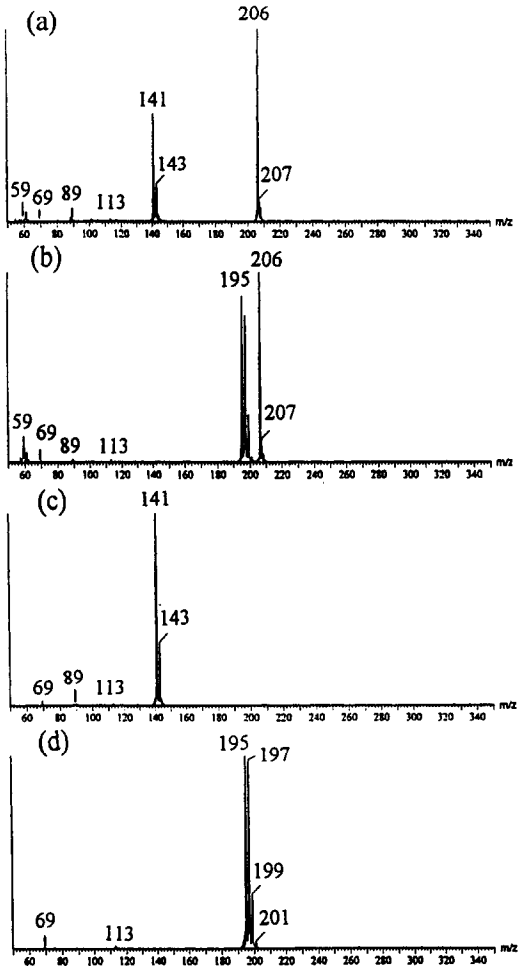

Fig. 6. Electrospray mass spectrum of (a) 4-C-3-MP and (b) 2,4,6-TCP obtained in the full scan mode under CZE–MS operation. The ions at m/z 141 and 195 are the $[M-H]^-$ ion of 4-C-3-MP and 2,4,6-TCP. The m/z 206 ion is the $[M-H]^-$ ion of CHES buffer. (c) and (d) were obtained after background subtraction.

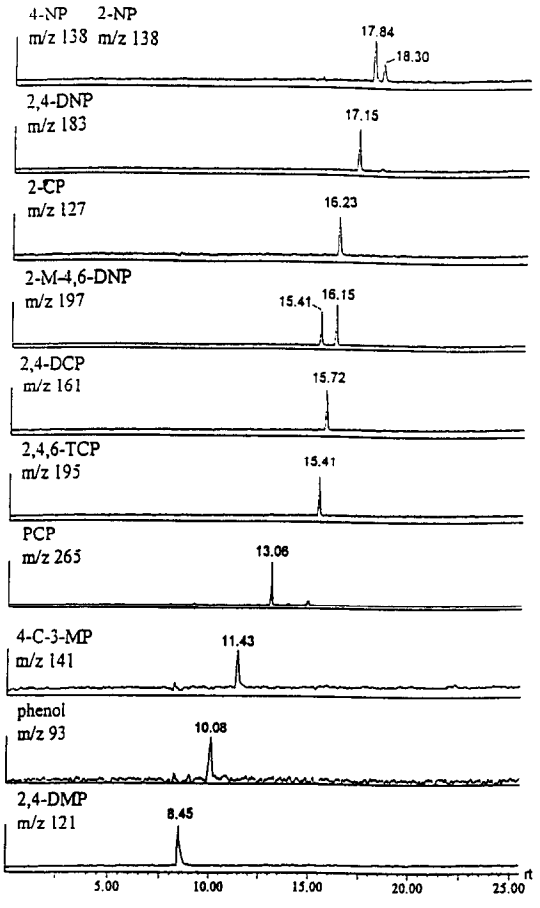

Fig. 7. Selected ion electropherograms of the eleven priority phenols (10 ppm concentration). The peak at 15.41 min of the m/z 197 mass electropherogram is the [M+2] isotope peak of 2,4,6-TCP.

as good as in CZE–UV. The number of theoretical plates drooped to 22 200 for phenol to 100 339 for 2-M-4,6-DNP. The loss of separation efficiency is most likely due to the dead volume of the CZE–MS interface. The repeatability in migration time and signal area were found to be 4% and 17% respectively.

The method was used to the determination of priority phenols in a paper clay sample. The sample was extracted with acidified methanol and then analyzed directly by CZE–MS. Only one peak was observed in the total ion current electropherogram (Fig. 8). This peak was assigned to pentachlorophenol based on the molecular weight and isotopic ratio of the peak. After the identification, the compound was quantified by CZE–MS and CZE–UV. Our results showed that while the dynamic range of these two approaches were similar (two orders), the calibration curve from CZE–UV had a better linear correlation than CZE–MS (r=0.999 vs. 0.984). The concentration of pentachlorophenol in the paper clay sample was determined to be 0.3 mg/g and 0.34 mg/g by CZE–UV and CZE–MS respectively.

The detection limit of this CZE–MS technique is in the low ppm range. This sensitivity is not adequate for the detection of many environmental

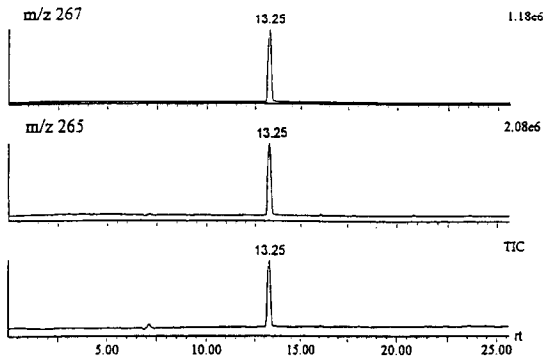

Fig. 8. Total and selected mass electropherograms of the paper clay sample.

samples without preconcentration. In order to improve the sensitivity of this approach, the technique of field amplification was adopted. Phenols in water were injected hydrodynamically (15 cm, 7 min) and then stacked under reversed polarity (−20 kV) for 45 s. Except for 2,4-DMP, phenol and 4-C-3-MP, the detection limit of this modified approach was found to be in the range of 50 ppb.

4. Conclusion

The combination of CZE and MS provided an excellent analytical tool for the separation and identification of priority phenols. This study showed that the position of the probe tip, the composition of running buffer and sheath liquid were all critical to the performance of the CZE–ESI interface. Although it is possible to obtain useful mass electropherogram with 50 ppb level of most phenols, further improvement in sensitivity is needed for the detection of low ppb level of phenols.

Acknowledgments

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References

[1] R.A. Booth and J.N. Lester, J. Chromatogr. Sci., 32 (1994) 259.
[2] N.G. Buckman, J.O. Hill, R.J. Magee and M.J. McCormick, J. Chromatogr., 284 (1984) 441.
[3] F.P. Bigley and R.L. Grob, J. Chromatogr., 350 (1985) 407.
[4] H.K. Lee, S.F.Y. Li and Y.H. Tay, J. Chromatogr., 438 (1988) 429.
[5] K. Otska, S. Terabe and T. Ando, J. Chromatogr., 348 (1985) 39.
[6] C.D. Gaitonde and P.V. Pathak, J. Chromatogr., 514 (1990) 389.
[7] C.P. Ong, C.L. Ng, N.C. Chong, H.K. Lee and S.F.Y. Li, J. Chromatogr., 516 (1990) 263.
[8] Y.C. Chao and C.W. Whang, J. Chromatogr. A, 663 (1994) 229.
[9] F.E.P. Mikkers, F.M. Everaerts and Th.P.E.M. Verheggen, J. Chromatogr., 169 (1979) 11.
[10] S. Terabe, K. Otsuka K. Ichikawa, A. Tsuchiya and T. Ando, Anal. Chem., 56 (1984) 111.
[11] K. Ganzler, K.S. Greve, A.S. Cohen, B.L. Karger, A. Guttman and N.C. Cooke, Anal. Chem., 64 (1992) 2665.
[12] Y.E. Yik and S.F.Y. Li, Trends Anal. Chem., 11 (1992) 325.
[13] R.A. Wallingford and A.G. Ewing, Anal. Chem., 59 (1987) 1762.
[14] S. Nie, R. Dadoo and R.N. Zare, Anal. Chem., 65 (1993) 3571.
[15] E.S. Yeung, D. Wang, W. Li and R.W. Giese, J. Chromatogr., 608 (1992) 73.
[16] G. Hopfgartner, T. Wachs, K. Bean and J. Henion, Anal. Chem., 65 (1993) 439.
[17] S. Jun, U. Yoshihisa, K. Akihiro and K. Ikunoshin, Anal. Biochem., 207 (1992) 1.
[18] R.D. Smith, J.A. Olivares, N.T. Nguyen and H.R. Udseth, Anal. Chem., 60 (1988) 426.
[19] M.A. Moseley, J.W. Jorgenson, J. Shabanowitz, D.F. Hunt and K.B. Tomer, J. Am. Soc. Mass Spectrom., 3 (1992) 289.
[20] S. Pleasance, P. Thibault and J. Kelly, J. Chromatogr., 591 (1992) 325.
[21] M.A. Moseley, L.J. Deterding, K.B. Tomer and J.W. Jorgenson, Anal. Chem., 63 (1991) 109.
[22] M.A. Moseley, L.J. Deterding, K.B. Tomer and J.W. Jorgenson, J. Chromatogr., 516 (1990) 167.
[23] H.H. Lauer and D. McManigill, Anal. Chem., 58 (1986) 166.
[24] J.W. Jorgenson and K.D. Lukacs, Anal. Chem., 53 (1981) 1298.
[25] J.S. Green and J.W. Jorgenson, J. Chromatogr., 352 (1986) 337.