Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1095 (2005) 189–192
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Separation and determination of phthalates by micellar electrokinetic chromatography

Bao-Yuan Guo, Bei Wen, Xiao-Quan Shan*, Shu-Zheng Zhang, Jin-Ming Lin

State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, No. 18 Shuangqing Road, P.O. Box 2871, Beijing 100085, China

Received 21 July 2005; received in revised form 27 September 2005; accepted 28 September 2005
Available online 12 October 2005

Abstract

A method has been developed for the separation and determination of dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), di-(2-ethylhexyl) phthalate (DEHP) and di-*n*-octyl phthalate (DnOP) by micellar electrokinetic chromatography (MEKC). The baseline separation of phthalates was achieved by using a buffer of 100 mM sodium cholate, 50 mM borate and 15% methanol (pH 8.5). The optimized MEKC method was used to quantify the concentrations of phthalates in 11 soil samples from different regions of China. The contents of DEP, DBP and DEHP in soils were ranged 0–0.42, 0–1.43, and 0.24–2.35 mg/kg, respectively, and no DMP and DnOP was detected. The limits of detection for DMP, DEP, DBP, DEHP, and DnOP were found to be 0.050, 0.051, 0.052, 0.054, and 0.063 mg/kg, respectively. The results obtained by the MEKC method were compared with those obtained by gas chromatography with flame ionization detector (GC-FID), and a good agreement was achieved.

Keywords: Phthalate; Micellar electrokinetic chromatography; Sodium cholate; Soil

1. Introduction

Phthalates including dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), di-(2-ethylhexyl) phthalate (DEHP) and di-*n*-octyl phthalate (DnOP) are well-known plasticizers, and are omnipresent in the environment. The toxicological studies reveal their possible toxic, carcinogenic, mutagenic, and teratogenic effects on animals although it is still doubtful in the case of human beings [1,2]. Recent reports on their endocrine disrupting properties concern their long-term hazardous effects on the environment from various aspects, including their multiform threats to human reproductive health [3]. Increasing exposure to phthalates might be partially responsible for the recent decline in the male ratio [4], the premature breast development [5], and the development of breast cancer [6]. The continuous release of large quantities of phthalates to the environment made the concentrations and exposures remained substantial.

The gas chromatography with electron capture detector (GC-ECD) was the most common method for the determination of phthalates in the environment [7,8]. The GC with flame ionization detector (GC-FID) was also used when phthalates were in polar solvents [9]. Kato et al. [10] also used the high performance liquid chromatography (HPLC) to determine phthalates in human urine.

Because capillary electrophoresis (CE) provides the advantages of rapidity, high efficiency and high sensitivity, CE is gradually adopted as a part of environmental analyses [11]. Takeda applied micellar electrokinetic chromatography (MEKC) for the separation of phthalates using sodium dodecyl sulphate (SDS) [12], but no effective separation was achieved due to their high octanol–water partition coefficients (Pow) of DnOP and DEHP [1], leading to their migration with micelle simultaneously [12].

To obtain a satisfactory separation of the phthalates, sodium cholate (SC) MEKC was selected in the present work. SC, as a bile salt, is of chiral structures and has many advantages over alkyl surfactants. SC micelle has moderate ability to retain hydrophobic analytes and has high separating selectivity of the highly hydrophobic compounds [13], which was

* Corresponding author. Tel.: +86 10 62923560; fax: +86 10 62923563.
E-mail address: xiaoquan@mail.rcees.ac.cn (X.-Q. Shan).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.09.075

commonly used in the MEKC separation of chirals [14], biosamples [15,16] and pharmaceuticals [17].

The aim of the present study was to develop a SC MEKC method for the separation and determination of phthalates in soils from different regions of China. A comparison was also made between the concentration levels obtained by the recommended method and by GC-FID.

2. Experiment

2.1. Equipment and chemicals

MEKC separation was performed on a Beckmann P/ACE MDQ capillary electrophoresis system equipped with a UV detector (Beckman, Fullerton, CA, USA). A fused silica capillary (Ruifeng, Yongnian, China), 75 μm I.D., 375 μm O.D., total length of 60 cm (50 cm to detector) was used. The pH of buffer was measured by a DELTA 320 pH meter (Mettler-Toledo, Shanghai, China).

An Agilent 6890 gas chromatograph equipped with flame ionization detector and a HP-5 fused silica capillary column (0.32 μm film, 250 μm I.D., length 30 m) (Agilent, Palo Alto, CA, USA) was used. A portion of 1 μl of soil extract was injected to GC system for separation and determination of phthalates.

SC was purchased from Sigma (St. Louis, MO, USA). Phthalates reference standards (DMP, DEP, DBP, DEHP, DnOP), benzyl benzoate (BB) and dicyclohexyl phthalate (DCHP) were purchased from AccuStandard (New Haven, CT, USA). Polyethylene glycol 400 (PEG 400) was of chemical grade from Beijing Regents (Beijing, China). The laboratory glassware was cleaned with detergent and acid (50% HNO_3), rinsed with distilled water and acetone thoroughly, and then heated to 400 °C overnight prior to use. All organic solvents were of pesticide grade.

2.2. Soil pretreatment and extraction of phthalates

Field arable surface soil (0–5 cm) samples were collected from 11 sites in China using a vertical corer, and then packed in aluminum foil and deep frozen for storage. Before analysis, soil samples were air-dried in a clean laboratory and prepared by grinding and screening through 2 mm sieve. Our previous extraction method [7] was used. Briefly, a portion of 10 g soil sample was placed into a thimble filter and extracted with 100 ml mixture of hexane and dichloromethane (1:1) for 24 h at 5–6 min cycle^{-1}. The extract was concentrated to about 10 ml by rotary evaporator. The concentrated extract was transferred to a Kuderna–Danish (KD) concentrator (25 ml) and carefully concentrated to 0.5 ml. After concentrating, 10 ml methanol was added to the extract. Then the mixture was carefully concentrated to 0.5 ml again prior to the determination by MEKC and GC-FID.

2.3. Quality control

To assure merit of the proposed analytical method, blank, mixed standards (50 mg/l of each phthalates including BB and DCHP) and soil extracts were successively analyzed in each analytical run. The blank values of the analytical procedure were determined by extracting an empty cellulose thimble by the same method as the real soil samples. Baseline separated peaks of phthalates in soil extract obtained by MEKC and GC methods were identified by comparing their migration time or retention time with that of authentic standards. BB (50 μg/ml) was used as an internal standard in the extracted samples. To monitor the performance of the extraction, cleanup, analytical system and the effectiveness of the method, each sample and blank were spiked with 2.5 mg/kg of surrogate standard, DCHP. Therefore, under the optimized separation condition, a spiked sample containing DMP, DEP, DBP, BB, DCHP, DEHP and DnOP (50 μg/ml each) in methanol was used.

2.4. MEKC determination

Prior to use the capillaries were sequentially rinsed with 0.1 M sodium hydroxide for 10 min, distilled water for 10 min and running buffer for 5 min. Before each determination the capillary was flushed with the running buffer for 3 min.

The micellar buffer was composed of 100 mM SC, 50 mM borate and 15% methanol (pH 8.5). The soil extract was hydrodynamically introduced by applying pressure of 3.5 kPa (0.5 psi) for 3 s; UV detector was at 214 nm; separation voltage was 20 kV; the capillary was thermo-stated at 25 °C.

2.5. GC determination conditions

GC-FID method was employed. Column temperature increased from 40 to 70 °C at a rate of 30 °C/min, and then programmed to a final temperature of 280 °C at 5 °C/min, and held for 1 min. The injector and detector temperatures were kept at 250 and 300 °C, respectively. The flow rates of nitrogen as carrier and makeup gas were 2.0 and 58 ml/min, respectively.

3. Results and discussion

3.1. Optimization of sodium cholate concentration

To optimize the separation conditions, SC concentration increased from 50 to 150 mM in 50 mM borate (pH 8.5) and EOF deceased with the increase of SC concentration. The resolution between DEHP and DnOP increased with the increase of SC from 50 to 100 mM, while peaks of phthalates were broadened when SC was from 100 to 150 mM due to the influence of Joule heat. Therefore, 100 mM SC was used in the following study (Fig. 1A). Further improvement was still needed for the baseline separation of DEHP and DnOP.

3.2. Addition of PEG 400

PEG could modify the micellar phase, change the viscosity of the buffer and interact with analytes by hydrogen bonding or lipophilic interactions in MEKC [18]. When PEG 400 was increased from 0 to 5% in the buffer of 100 mM SC, 50 mM borate (pH 8.5), the mobility of EOF and phthalates decreased

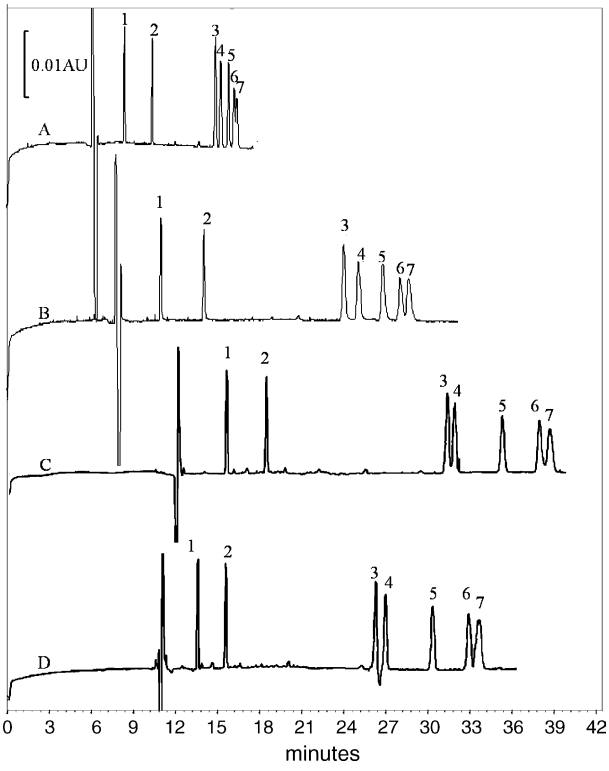

Fig. 1. Electropherograms of phthalates (50 μg/ml each) in 100 mM sodium cholate, 50 mM borate (pH 8.5) with or without additives, separation voltage 20 kV, UV detection at 214 nm, temperature of 25 °C: (A) without any additive; (B) with 2% PEG 400; (C) with 15% methanol; (D) with 30% acetonitrile: (1) DMP, (2) DEP, (3) BB, (4) DBP, (5) DCHP, (6) DEHP, (7) DnOP.

and the analytical time increased from 17 to about 45 min, resulting in the increased resolution of DEHP and DnOP. When 2% PEG 400 was used, baseline separation of all phthalates was obtained (Fig. 1B).

3.3. Addition of methanol and acetonitrile

In this study, methanol and acetonitrile were used as organic modifiers to optimize the separation of phthalates. With the increase of the solvents fraction in the buffer, EOF decreased, and methanol became more effective in depressing EOF than acetonitrile; the resolution of DEHP and DnOP was improved with increasing methanol or acetonitrile in the buffer. A baseline separation of DEHP and DnOP was achieved when 15% methanol or 30% acetonitrile was appended to the buffer of 100 sodium cholate and 50 mM borate (pH 8.5) (Fig. 1C and D).

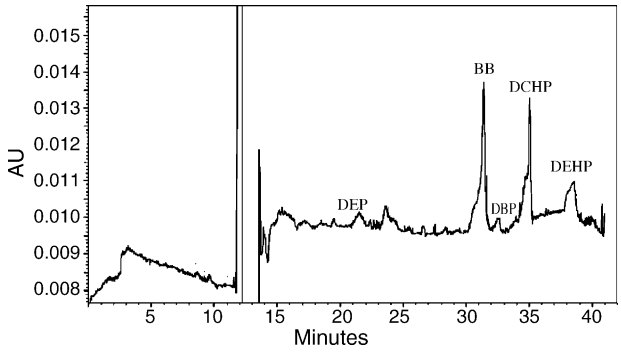

Fig. 2. Electropherogram of soil (Hangzhou) extract by MEKC in 100 mM sodium cholate, 50 mM borate (pH 8.5) and 15% methanol. Other conditions were same as those of Fig. 1.

3.4. Soil analysis

Considering the soil extract, was finally transferred to methanol, therefore, the buffer of 100 mM SC, 50 mM borate (pH 8.5) and 15% methanol was finally chosen for real sample analysis.

Limits of detection of DMP, DEP, DBP, DEHP and DnOP were 0.050, 0.051, 0.052, 0.054, and 0.063 mg/kg, respectively, and in the concentration range of 0.15–5 mg/kg phthalates, the detector responses were linear ($R^2 > 0.99$). The electropherogram of Hangzhou soil is shown in Fig. 2. The phthalates contents determined by the MEKC were compared to those obtained by GC-FID (Table 1), and a good agreement was obtained between these two methods. The concentrations of DEP, DBP and DEHP in soils were found to be in the range of 0–0.42, 0–1.43, and 0.24–2.35 mg/kg, respectively,

Table 1
Comparison of determined phthalates by MEKC and GC-FID methods ($n = 4$)

Sample location	DEP (mg/kg)		DBP (mg/kg)		DEHP (mg/kg)	
	MEKC[a]	GC-FID[b]	MEKC	GC-FID	MEKC	GC-FID
Heilongjiang	0.33 ± 0.09	0.38 ± 0.05	0.22 ± 0.06	0.25 ± 0.07	0.24 ± 0.03	0.31 ± 0.00
Yunnan	ND	ND	0.17 ± 0.03	0.22 ± 0.02	0.50 ± 0.11	0.55 ± 0.17
Dongguan	ND	ND	1.34 ± 0.15	1.30 ± 0.07	2.32 ± 0.86	2.30 ± 0.27
Xuzhou	0.27 ± 0.05	0.20 ± 0.03	ND	0.05 ± 0.02	1.50 ± 0.09	1.45 ± 0.15
Yingtan	ND	ND	ND	ND	0.50 ± 0.09	0.61 ± 0.07
Wuhan	ND	ND	0.10 ± 0.03	0.15 ± 0.03	0.31 ± 0.05	0.37 ± 0.09
Kisamusze	0.42 ± 0.07	0.38 ± 0.03	ND	ND	2.41 ± 0.41	2.82 ± 0.58
Chaozhou	ND	ND	ND	ND	2.81 ± 0.38	3.12 ± 0.19
Hangzhou	0.30 ± 0.06	0.35 ± 0.04	0.41 ± 0.12	0.47 ± 0.04	3.20 ± 0.22	3.27 ± 0.28
Shanghai	ND	ND	1.26 ± 0.37	1.40 ± 0.25	1.25 ± 0.33	1.35 ± 0.55
Beijing	ND	ND	0.30 ± 0.08	0.35 ± 0.02	0.27 ± 0.08	0.29 ± 0.05

ND: not detected.
[a] Electropherographic conditions were described in Section 3.4 in detail.
[b] GC-FID conditions were described in Section 2.2 in detail.

while no DnOP and DMP were detected in all the soils. The reason for this is that DMP and DnOP are seldom used as plasticizers, and it is seldom detected in the soil environment. The recoveries of surrogate standard DCHP varied from 81% to 98%. The results indicated that the contaminations of phthalates were different from site to site in soils in China and the phthalate contaminations in arable soils in China are already serious to cause a long-term ecotoxicological problem.

Acknowledgement

We gratefully acknowledge a research grant from the National Natural Science Foundation of China (Grant No. 20237010).

References

[1] C.A. Staples, D.R. Peterson, T.F. Parkerton, W.J. Adams, Chemosphere 35 (1997) 667.
[2] A.M. Api, Food Chem. Toxicol. 39 (2001) 97.
[3] G. Latini, C.D. Felice, A. Verrotti, Reprod. Toxicol. 19 (2004) 27.
[4] T. Vartiainen, L. Kartovaara, J. Tuomisto, Environ. Health Perspect. 107 (1999) 813.
[5] I. Colón, D. Caro, C.J. Bourdony, O. Rosario, Environ. Health Perspect. 108 (2000) 895.
[6] D. Feldman, Endocrinol 138 (1997) 1777.
[7] X.Y. Hu, B. Wen, X.-Q. Shan, J. Environ. Monit. 5 (2003) 649.
[8] X. Hu, B. Wen, S. Zhang, X.-Q. Shan, Ecotoxicol. Environ. Saf. 62 (2005) 26.
[9] M.A. Gotardo, M. Monteiro, J. Pharm. Biomed. Anal. 38 (2005) 709.
[10] K. Kato, S. Shoda, M. Takahashi, N. Doi, Y. Yoshimura, H. Nakazawa, J. Chromatogr. B 788 (2003) 407.
[11] F. Regan, A. Moran, B. Fogarty, E. Dempsey, J. Chromatogr. A 1014 (2003) 141.
[12] S. Takeda, S. Wakida, M. Yamane, A. Kawahara, K. Higashi, Anal. Chem. 65 (1993) 2489.
[13] R. Kuhn, S. Hoffstetter-Kuhn, Capillary Electrophoresis Principles and Practice, Springer, Berlin, 1993 (Chapter 5).
[14] A.L.C. Navazo, M.L. Marina, M.J. Gonzalez, Electrophoresis 19 (1998) 2113.
[15] L. Zhang, S.N. Krylov, S. Hu, N.J. Dovichi, J. Chromatogr. A 894 (2000) 129.
[16] B.-Y. Guo, B. Wen, X.-Q. Shan, S.-Z. Zhang, J.-M. Lin, J. Chromatogr. A 1047 (2005) 205.
[17] S.W. Sun, A.C. Wu, J. Chromatogr. A 814 (1998) 223.
[18] P. Bednář, P. Barták, P. Adamovský, A. Gavenda, J. Ševčík, Z. Stránský, J. Chromatogr. A 917 (2001) 319.