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Capillary liquid chromatography–mass spectrometry and micellar electrokinetic chromatography as complementary techniques in environmental analysis

William C. Brumley*[,a], Cynthia M. Brownrigg[a,1], Andrew H. Grange[b]

[a]*US Environmental Protection Agency, Environmental Monitoring Systems Laboratory, P.O. Box 93478, Las Vegas, NV 89193-3478, USA*

[b]*Lockheed Environmental Sciences and Technology Company, 980 Kelly Johnson Drive, Las Vegas, NV 89119, USA*

Abstract

Applications of capillary electrophoresis (CE) and capillary liquid chromatography (LC) to environmental analysis have been limited. In this work we present applications of micellar electrokinetic chromatography (MEKC) to the analysis of environmental matrices for synthetic dyes. Separations obtained by capillary LC are compared with those obtained under MEKC for seven selected dyes. Both techniques are capable of resolving the subject compounds at high efficiency. Recovery data for spiked water and soil matrices were obtained for four dyes using solid-phase extraction cartridges and disks with determination by MEKC–UV detection. Both pH adjustment via acid and ion-pairing via a cationic surfactant were investigated for isolating dyes. Capillary LC detection was by continuous-flow liquid secondary ion mass spectrometry (CF-LSI-MS) whereas MEKC used UV detection (214 nm). Application of peak-profiling at high mass resolution is illustrated with the capillary LC–MS technique. Interfacing capillary LC under CF-LSI-MS using the coaxial arrangement is easier than interfacing CE with this arrangement. MEKC provides a powerful screening and determinative technique, while capillary LC–MS provides a confirmatory tool.

1. Introduction

Very polar and ionic analytes (non-volatiles) such as synthetic dyes and herbicides are of current interest in environmental analysis because they are beyond the reach of capillary gas chromatography. They have become analytically accessible through the development of reversed-phase (RP) high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS) interfaces [1–10]. The Environmental Monitoring Systems Laboratory in Las Vegas has a long history of interest in LC–MS methods including applications of thermospray [11] and particle-beam [12] interfaces. The US Environmental Protection Agency (EPA) now has several HPLC and LC–MS methods approved for general use including Method 553 [13] for benzidines and Method 8321 [14] for phenoxy acid herbicides.

However, applications of capillary LC (cLC) and micellar electrokinetic chromatography (MEKC) to environmental analysis have been much more limited relative to pharmaceutical

* Corresponding author.

[1] Present address: University of Nevada-Las Vegas, Las Vegas, NV 89119, USA.

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and biochemical applications as revealed in a recent review [15]. Compounds such as dyes, sulfonyl ureas, benzidines, aromatic acids, phenols, polychlorinated biphenyls and phthalates have been studied [16–23].

The high efficiency of cLC and MEKC, together with their low solvent consumption, make them ideally suited to environmental analysis. Efficiency provides narrow peaks of high mass sensitivity and aids in achieving separations in a minimal amount of time. Solvent waste reduction achieved through the low flow-rates of cLC is now a major consideration in methods development. Solid-phase extraction (SPE) techniques are also important in eliminating large volumes of chlorinated and other organic solvents.

In this work we compare cLC with MEKC for the separation of synthetic dyes. Four dyes were selected for detailed studies of the extraction/cleanup of the dyes from water and soil matrices. Mass peak-profiling [24] of cLC peaks demonstrates the applicability of this technique to the determination of accurate mass and, therefore, elemental composition. The selectivity and sensitivity of laser-induced fluorescence (LIF) detection was illustrated with the determination of a fluorescent dye recovered from spiked soil.

2. Experimental

2.1. Chemicals

All chemicals were obtained from Aldrich (Milwaukee, WI, USA) unless otherwise indicated. Compounds were purified by dissolution in acetone and filtering to remove inorganic salts. The structures of the seven dyes are given in Fig. 1. The following compounds are numbered for convenience and use in the tables: **1** = cresol red; **2** = acid blue 40; **3** = acid orange 8; **4** = tropaeolin O. The other three dyes shown in Fig. 1 are nuclear fast red, orange II and acid red 151. Two internal standards were used as migration time markers but only the first was used for quantitations: 4-hydroxyphenylacetic acid (IS1) and anthraquinone-2,6-disulfonic acid, sodium salt (IS2). An aqueous solution containing **1–4** was made up at concentrations of 0.096 mg/ml **1**, 0.100 mg/ml **2**, 0.96 mg/ml **3** and 0.095 mg/ml **4**. IS1 was made up at a concentration of 0.209 mg/ml with IS2 at 0.442 mg/ml.

Acetone and methanol were obtained from Burdick & Jackson (Muskegon, MI, USA). Deionized water (ASTM Type II) was produced (Barnstead/Thermolyne, Dubuque, IA, USA) for all aqueous solutions.

2.2. HPCE

A Beckman P/ACE 2100 instrument was used for electrophoretic separations and fitted with a 57 cm × 0.050 mm I.D. fused-silica capillary (50 cm to the detector). Buffer consisted of 50 m*M* boric acid/sodium borate (pH 8.35), 100 m*M* sodium cholate and 10% acetone. Voltage was 25 kV with UV detection at 214 nm with IS1 as the internal standard for migration time corrections.

A Beckman P/ACE 5000 was used for obtaining separations by free zone electrophoresis (CZE) or by MEKC when LIF detection was used. Capillary was a 57 cm × 0.075 mm I.D. (50 cm to the detector). Buffers consisted of 50 m*M* boric acid/sodium borate (CZE) or 50 m*M* boric acid/sodium borate, 100 m*M* sodium cholate and 10% acetone (MEKC). Voltage was 25 kV under MEKC and 30 kV under CZE. LIF detection used an excitation wavelength of 488 nm (Ar ion laser), and sodium fluorescein was used as internal standard.

2.3. CF-LSI-MS–MS

MS used a Fisons/VG 70SE operated in the negative ion mode at −8 kV accelerating voltage with Cs ion gun operated at 17 kV. The cLC column was a 35 cm × 0.075 mm I.D. fused-silica column (Polymicro Technologies, Phoenix, AZ, USA) packed with C_{18}-bonded 5-μm diameter silica beads (ODS-Hypersil; Shandon Southern Products, Cheshire, UK). The column was connected to the instrument in the coaxial arrangement [25] via the standard VG CF-LSI-MS probe. The instrument was operated in the selected ion recording (SIR) mode at 1000 or 10 000 mass resolution.

2.4. Water extraction

Extraction disks (Empore Disks from Varian, Sunnyvale, CA, USA) were prepared according to the manufacturer's directions. Briefly, the disks were soaked in 10 ml of methanol at least 3 min before pulling 10 ml of water through. Water to be extracted was adjusted to pH 1, or solid cetyldiethylmethylammonium bromide (CEMA) was added to the spiked water (pH 6) to a concentration of 5 m*M* for ion-pairing extraction; adsorbed compounds were eluted from the disk with three 6-ml portions of methanol (pH 8–9). CEMA was removed from the extractant via an SCX cartridge (Supelco, Bellefonte, PA, USA) activated with sodium sulfate [16].

2.5. Soil extraction

Soils (local soil predominantly clay with organic potting soil added) were subjected to sonication extraction [26] with 30–50 ml methanol–water (75:25) solvent, filtered, and diluted to 250 ml with deionized water. The diluted extract (pH 1) was subjected to SPE with extraction disk and compounds isolated as for spiked water. The additional C_{18} SPE cartridge cleanup for 3-ppm (w/w) spiked soil levels used a concentrated 2-ml methanolic sample isolate from extraction disk. The concentrate was diluted to 12 ml with deionized water (i.e., now a 17% methanol solution), adjusted to pH 3, and applied to the cartridge. The dyes were eluted with 3 ml of methanol–water (60:40) at pH 3.

3. Results and discussion

3.1. cLC–MS

The seven monosulfonated dyes (structures in Fig. 1) encompass structures that include azo, diazo, triarylmethane and anthraquinone moieties. They are representative of the great variety of synthetic dyes and serve as prototypes of other organic compounds that are not amenable to separation techniques based on gas chromatography. cLC is capable of exhibiting high efficiency with a low flow-rate (0.5 to 3 μl/min) and in resolving such compounds as illustrated in Fig. 1. The low flow-rate is consistent with direct interfacing to MS and has the added benefit of reducing solvent waste.

The peak widths of some of the compounds resolved by cLC in Fig. 1 suggest the need for short MS scan cycle times. The problem becomes more acute if accurate mass measurements are desired. We have developed a technique that provides mass peak-profiling on capillary width chromatographic peaks without loss of resolution or chromatographic sacrifice. Fig. 2 illustrates the ability of mass peak-profiling to accurately determine mass while faithfully reproducing cLC peaks (0.8 s cycle time). Mass peak-profiling is a potentially useful technique for helping to determine elemental composition of unknowns. Its extension to cLC–MS suggests its potential in qualitative analysis by identifying unknowns that are non-volatile. This application makes cLC–MS complementary to MEKC where identity can only be based on migration times. On the other hand, MEKC with UV detection provides a technique that readily quantitates analytes.

3.2. MEKC

The seven dyes can be separated by MEKC as illustrated in Fig. 3. Again, high efficiency and selectivity are illustrated in the separation which is complementary to that obtained by cLC–MS. The order of elution in comparing the two techniques of RP cLC and MEKC has changed completely for compounds cresol red and tropaeolin O. Changes in elution order for nuclear fast red and acid red 151 are observed. Orange II, acid blue 40 and acid orange 8 are grouped at the center of the cLC chromatogram and the MEKC electropherogram. Thus, cLC and MEKC are complementary in their separation mechanisms.

3.3. Recoveries of dyes from water

Four dyes were selected for recovery studies from water and soil. Table 1 compares extraction disk recoveries based on pH adjustment and on

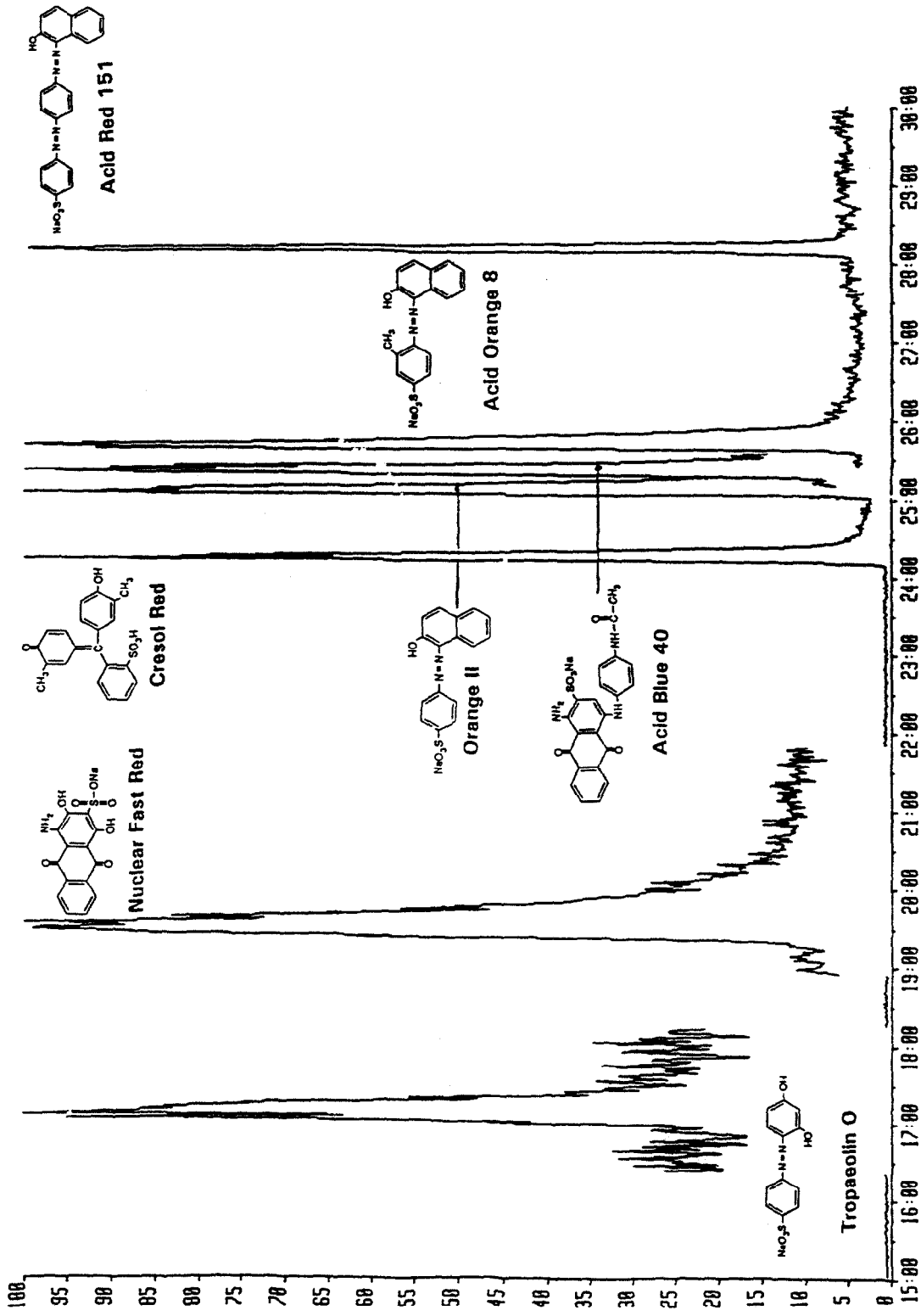

Fig. 1. Separation of seven dyes by cLC–MS (CF-LSI-MS) illustrated by superimposing seven ion chromatograms; 2-s injection and 1 step program of mobile phase: 3 min 100% 0.05 *M* ammonium acetate; 3–30 min 100% methanol. Vertical axis normalized to 100% relative abundance for each ion chromatogram that represents the $(M-Na)^-$ ion of each dye.

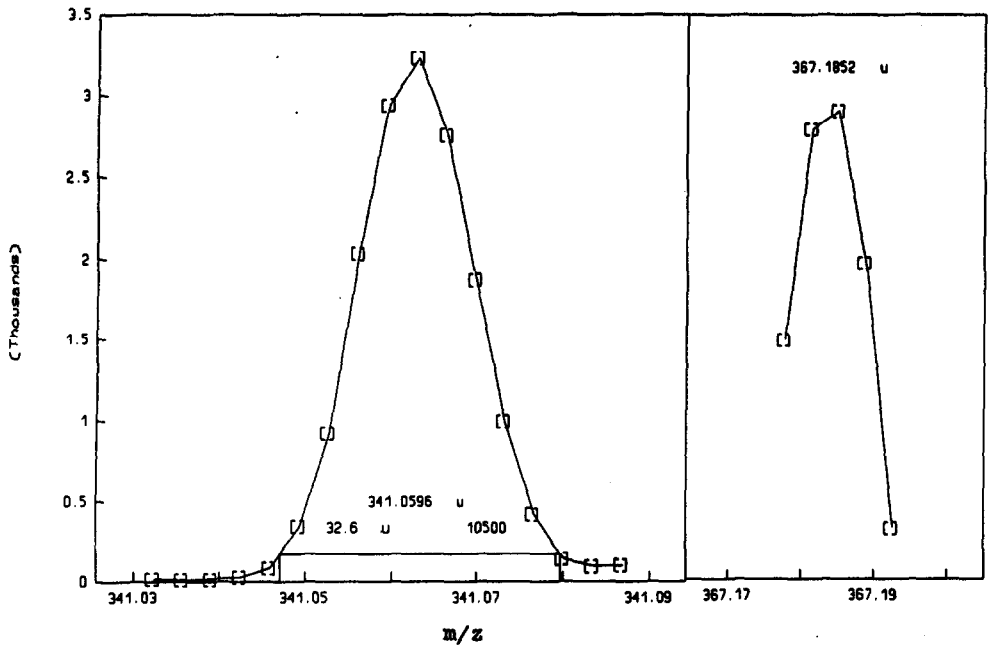

Fig. 2. Mass peak-profiling at 10 000 resolution on cLC–MS chromatographic peaks: m/z 341.0596 of orange 8 and m/z 367.1816 of glycerol matrix as the lock mass.

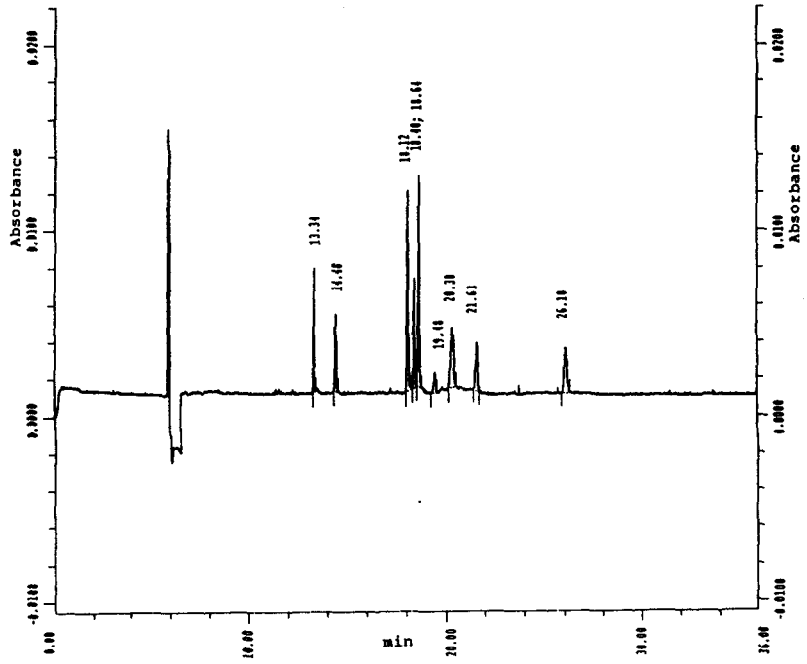

Fig. 3. Electropherogram of seven dyes under MEKC (10% acetone–cholate–borate buffer). Peaks: migration time (t_m) 13.34 min = IS1; t_m 14.48 min = cresol red; t_m 18.12 min = orange II; t_m 18.40 min = acid blue 40; t_m 18.64 min = acid orange 8; t_m 19.48 min = nuclear fast red; t_m 20.38 min = acid red 151; t_m 21.63 min = tropaeolin O; t_m 26.10 min = IS2.

Table 1
Extraction disk recoveries of four dyes at 1 ppm in water (250-ml volume) by MEKC

Compound	Recovery (%)	Average (%)	R.S.D. (%)
pH 1			
1	77.0, 94.8, 89.8	87.2	11
2	111, 100, 115	109	7.1
3	109, 107, 117	111	4.8
4	76.5, 73.2, 70.2	73.3	4.3
Ion-pairing at pH 6			
1	85.8, 84.8, 85.1	85.2	0.6
2	74.4, 74.8, 65.0	71.4	7.8
3	90.3, 79.3, 79.0	82.9	7.8
4	46.7, 42.7, 41.6	43.7	6.1

ion pairing. Recoveries at 1 μg/g (ppm) were relatively good using either method. Compound **4** was clearly less quantitatively recovered by ion pairing. This compound also proved to be a difficult analyte to recover from soils.

The use of pH adjustment is the most direct approach for isolating the dyes. Ion pairing, however, offers an alternative approach for compounds that are sensitive to strongly acidic conditions. The generality of ion-pairing approaches using C_{18} extraction disks has not been fully explored.

Table 2 tabulates additional recovery data for the four dyes at 0.1- and 0.025-ppm levels using pH adjustment. From the data it is apparent that disk extraction of large volumes of water can be effective in recovering low contaminant levels of these highly water soluble compounds. An electropherogram of the recovered dyes at 0.1 ppm is illustrated in Fig. 4.

3.4. Recoveries from soil

Table 3 tabulates recoveries of the four dyes from spiked soil at two spiking levels. At 10-ppm spiking level, isolation from the extraction disk results in a finished extract that can be run directly by MEKC. At 3 ppm, additional cleanup

Table 2
Extraction disk recoveries of four dyes at 0.1 ppm (1-l volume) and 0.025 ppm (4-l volume) determined by MEKC

Compound	Recovery (%)	Average (%)	R.S.D. (%)
0.1 ppm			
1	93.4, 93.2, 101	95.9	4.6
2	39.9, 58.7, 75.5	58.0	31
3	78.4, 67.2, 98.1	81.2	19
4	120, 137, 129	129	6.6
0.025 ppm			
1	96.6, 94.5, 106	99.0	6.2
2	95.0, 101, 102	99.3	3.8
3	102, 108, 111	107	4.3
4	39.5, 38.7, 49.2	42.5	14

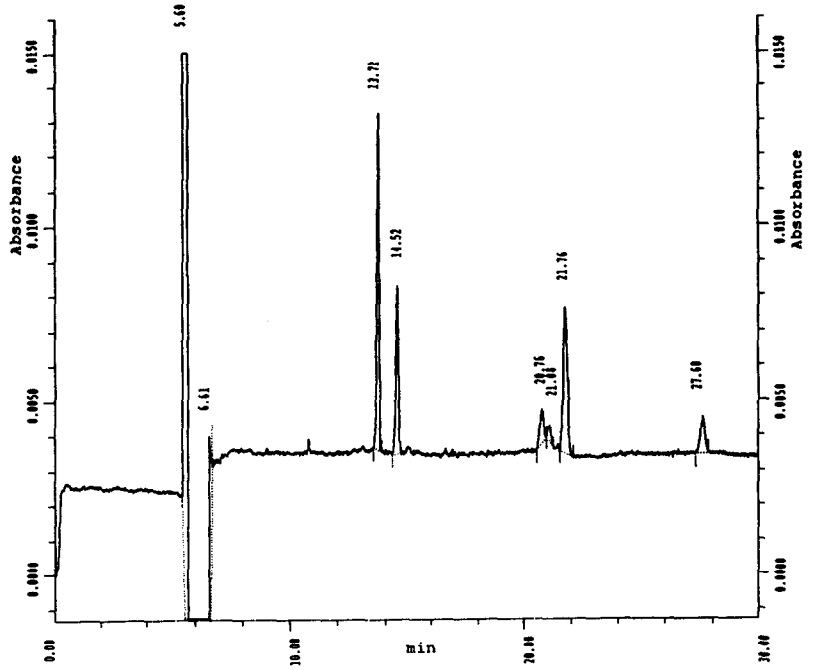

Fig. 4. Electropherogram of four dyes recovered from 0.1-ppm spiked water under MEKC (10% acetone–cholate–borate buffer). Peaks: t_m 13.71 min = IS1; t_m 14.52 min = cresol red; t_m 20.76 min = acid blue 40; t_m 21.76 min = acid orange 8; t_m 27.60 min = tropaeolin O.

by C_{18} SPE cartridge was needed. Fig. 5 illustrates an electropherogram of a sample extract from a 3-ppm spiked soil. There was still evidence of coextractives even after cleanup of the sample with a C_{18} SPE cartridge using pH adjustment to help eliminate aromatic carboxylic acids and other coextractives more highly retained by C_{18} adsorbent.

Compound **4** was poorly recovered from soil, especially at the 3-ppm spiking level. Actually, it

Table 3
Recoveries of four dyes from soil as determined by MEKC

Compound	Recovery (%)	Average (%)	R.S.D. (%)
10 ppm			
1	90.7, 78.9, 77.2	82.3	8.9
2	97.0, 86.8, 80.1	88.0	9.7
3	114, 80.5, 73.7	89.4	24
4	28.3, 18.4, 6.9	17.9	60
3 ppm			
1	124, 105, 86.5	105	18
2	69.0, 70.9, 55.5	65.1	13
3	89.6, 79.2, 66.7	78.5	15
4	22.6, 18.8, 13.3	18.2	26

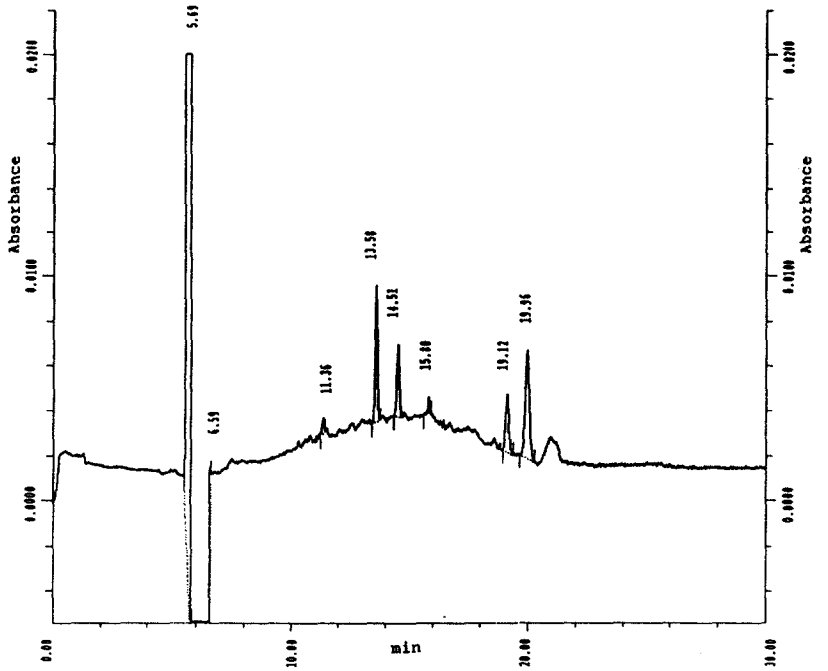

Fig. 5. Electropherogram of four dyes recovered from 3-ppm spiked soil under MEKC; additional cleanup by C_{18} cartridge (pH 3) after extraction disk isolation of diluted sonication extraction solvent. Peaks: t_m 13.58 min = IS1; t_m 14.51 min = cresol red; t_m 19.12 min = acid blue 40; t_m 19.96 min = acid orange 8; tropaeolin O not detected.

is not surprising that dyes could be difficult to recover from soils since they are by design a staining (dyeing) agent.

3.5. LIF detection of a dye from spiked soil

To investigate the considerable potential of LIF detection in environmental analysis, a soil spiked at 2.29 ppm with erythrosin B was extracted but not subjected to cleanup (final dilution volume 47 ml) or further isolation. An impurity in the standard (also a fluorophore, 17% relative response) was thus spiked in at 0.389 ppm (based on relative response). Fig. 6 shows the electropherogram of this extract under CZE with the internal standard sodium fluorescein. Recovery was calculated as 18.8% resulting in a quantitation of 0.389 ppm (0.073 ppm for the impurity) of recovered dye. Low recoveries of dyes that are often used as biological stains are not surprising.

Erythrosin B is not optimally detected at 488 nm, but better at 514.5 nm (Ar ion laser) or 543.5 (helium–neon laser) [27]. Nevertheless, actual spiking and recovery levels for the dye and impurity (factor of 10 below the 3-ppm level) with no cleanup and no concentration steps suggests the relative sensitivity and selectivity of LIF detection. Strategies based on taking advantage of LIF in environmental analysis are therefore an important research goal for future work.

4. Conclusions

cLC appears to be an excellent separation technique for mass spectral identification of nonvolatile analytes of environmental significance. High mass resolution capability via mass peak-profiling is a potentially useful tool in the identi-

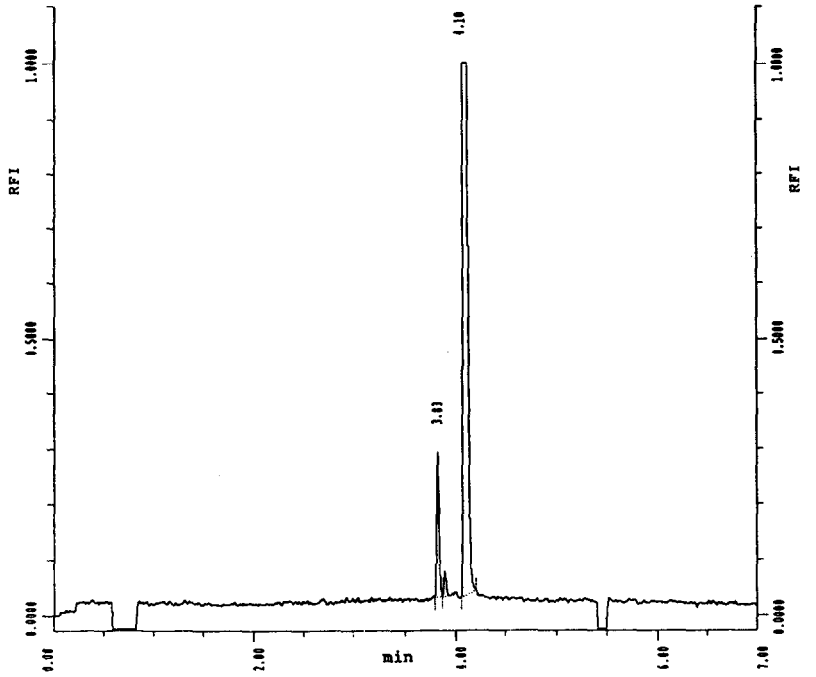

Fig. 6. Electropherogram of laser-induced fluorescence detection of erythrosin B from 2-ppm (0.3-ppm, isomer) spiked soil (18% recovery) under CZE (borate buffer). No disc isolation and no cleanup. Peaks: t_m 3.83 min = erythrosin B; t_m 3.91 min = impurity in erythrosin B; t_m 4.10 min = sodium fluorescein.

fication of unknowns. MEKC provides excellent separation and quantitation of synthetic dyes as demonstrated in this work. The complementary relationship of MEKC and reversed-phase cLC is evident in the separations reported here, and in their ability to quantitate and identify analytes, respectively. Multidimensional chromatography involving collection of peaks under CE conditions for subsequent qualitative determination by cLC–MS is under investigation. The selectivity and sensitivity of LIF detection suggests the potential application of derivatization using dyes in the environmental analysis of selected compounds.

Notice

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