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Electrophoretic behavior of aromatic-containing organic acids and the determination of selected compounds in water and soil by capillary electrophoresis

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ABSTRACT

The behavior of 56 aromatic-containing organic acids (ACOAs) was obtained under free zone electrophoresis and under micellar electrokinetic chromatography using cholic acid as the micellar agent. The compound classes encompass phenoxy acid herbicides, phenylalkanoic acids, aromatic carboxylic acids, aromatic sulfonic acids, azo and other dyes, and ACOAs containing nitrogen. Seven compounds were studied with respect to extraction and cleanup from spiked water and soil at levels of 1.0 and 0.1 μg/g and 100 and 20 μg/g, respectively. A general scheme of isolation and cleanup was developed that used extraction disks and solid-phase extraction cartridges. Average recoveries for three determinations ranged from 26.5 to 98.2% with relative standard deviations ranging from 6.7 to 55%.

INTRODUCTION

Aromatic-containing organic acids (ACOAs) are important compounds in commerce, pharmaceutics, biochemistry and the environment. Typical examples of ACOAs from these respective areas include phthalic acid, a precursor to phthalate esters; acetylsalicylic acid, a drug; nicotinic acid, a vitamin; and 2,4-dichlorophenoxyacetic acid, a herbicide. Analytical interest in the determination of ACOAs in various matrices may be concerned with waste streams, drinking water, biological activity, quality control of drugs, and hazardous waste, among others.

Our focus is the environmental occurrence and determination of specific ACOAs. Azo dyes are an important subset of such compounds because of the large amounts produced and the potential for the production of toxic by-products. The dyes are essential to the textile industry and are used in food and cosmetics as well, where concern has been raised about their potential carcinogenicity. One dye, trypan blue, is listed as an Appendix VIII RCRA analyte [1]. The dyes themselves or their breakdown products, which may be toxic, have been the subject of studies assessing their environmental distribution, occurrence and fate [2]. Herbicides also constitute an important subset of ACOAs that are of intense analytical interest [3,4]. The levels of such compounds as 2,4-dichlorophenoxyacetic acid in hazardous waste, for example, are of special concern under RCRA [1].

Even relatively simple ACOAs can present chromatographic problems for techniques such as capillary gas chromatography. It is, therefore, not surprising that such compounds have not received as thorough a treatment in existing US Environmental Protection Agency methodology such as the SW-846 manual [5] as have volatile and semivolatile analytes. Recent updates plan-

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ned for SW-846 include methods for non-volatile compounds such as aromatic sulfonic acids (Method 8350) [6].

Because ACOAs are often highly polar and may occur as salts, rendering them non-volatile, liquid chromatography (LC) is an obvious choice for effecting their separation. Gradient elutions in reversed-phase (RP) LC are possible if ionization can be suppressed by appropriate buffers [4]. Alternatively, RP separations using ion-pairing reagents can be carried out [7]. Azo dyes are sufficiently retained in C_{18} RPLC to allow separation with methanol–water gradients [2]. Ion chromatography has often been the technique of choice for ACOAs [8], but even supercritical fluid chromatography has been used [9,10].

Hyphenated techniques of LC with mass spectrometry (MS) are a powerful means to the identification and quantitation of ACOAs in various contexts [11]. Usually, LC–MS is carried out with relatively large bore columns (> 2 mm I.D.) using particle-beam MS [4] and thermospray MS [12]. Problems with decomposition using particle-beam interfaces have been addressed [4]. Applications of spray ionization techniques are highly relevant to ACOAs [13]. These compounds may also be determined by open tubular capillary LC [14] and packed column capillary LC [15].

Thin-layer chromatography (TLC) is also highly applicable to the determination of ACOAs. Several reviews have been published and the review by Sherma [16] may be consulted. Recent TLC applications involving ACOAs include separations involving phenylglyoxylic acids [17], dihydroxybenzoic acids [18,19], and arylsulfonyl alkanoic and arylsulfonylcycloalkanecarboxylic acids [20].

Recently, high-performance capillary electrophoresis (HPCE) has emerged as a powerful technique for the separation of many types of compounds sufficiently soluble in aqueous buffer solutions. A comparison of ion chromatography with CE revealed the greater efficiency of CE [21]. A comprehensive review by Kuhr and Monnig [22] may be consulted for further references. The coupling of CE to MS has been described [23]. Aromatic sulfonic acids were studied under free zone capillary electrophoresis (CZE) conditions [24] as were isomers of benzoic acid [25,26]. Sulfonated azo dyes were subjected to CE–MS [2]. The application of micelles in HPCE has been described by Terabe [27] and Nishi *et al.* [28], and has resulted in increased selectivity in many separations.

In this work we present a scheme for the isolation of ACOAs from water and soil based on extraction disks and solid-phase extraction (SPE) cartridges. The electrophoretic behavior of 56 ACOAs is tabulated under CZE and micellar electrokinetic chromatography (MEKC) conditions. Seven compounds are studied in detail with regard to recovery and determination in water and soil by MEKC.

EXPERIMENTAL

Chemicals

Compounds were used as received, and came from Aldrich (Milwaukee, WI, USA) unless otherwise noted. Trypan blue was obtained from Pfalz & Bauer (Stamford, CT, USA). Dodecylbenzenesulfonic acid, cumenesulfonic acid, xylenesulfonic acid and toluenesulfonic acid were obtained from Chem Service (West Chester, PA, USA). Analytical standards of seven compounds (**1–7**, Fig. 1) were made up in water–methanol solution at a concentration of about 0.2 to 0.4 mg/ml in a mixed standard referred to as the 7-STD. This solution was used for matrix spiking and for response factor determinations. An internal standard solution of 4-hydroxyphenylacetic acid (IS1) and anthraquinone-2,6-disulfonic acid (IS2) was prepared at a concentration of about 0.2 mg/ml. A 1-ml volume each of the 7-STD and of the internal standard solution was combined with buffer and diluent in the 4-ml sample vials. This constituted the center of the working concentration range for these analytes (*i.e.*, about 0.05–0.1 mg/ml in the sample vials).

Deionized water was produced (Barnstead/Thermolyne, Dubuque, IA, USA) for all aqueous solutions. The organic solvents, methanol and acetone, were distilled in glass (Burdick & Jackson, Muskegon, MI, USA). Boric acid–sodium borate buffer was made to a nominal concentration of 50 m*M* by titrating 100 m*M*

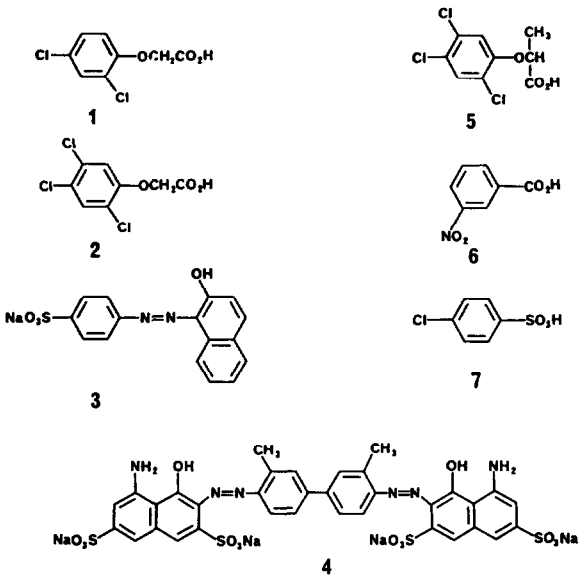

Fig. 1. Structures of seven compounds used in recovery studies: **1** = 2,4-dichlorophenoxyacetic acid; **2** = 2,4,5-trichlorophenoxyacetic acid; **3** = orange II; **4** = trypan blue; **5** = 2,4,5-trichlorophenoxypropionic acid; **6** = 3-nitrobenzoic acid; **7** = 4-chlorobenzenesulfonic acid.

solutions to a pH 8.3 endpoint. The micellar solution was made up of 0.1722 g of cholic acid sodium salt monohydrate in each 4-ml sample vial, giving a solution approximately 100 m*M* in the cholic acid (total volume 4.4 ml) [28]. Solutions in all vials were filtered through 0.2-μm pore size disposable nylon filters (Alltech, Deerfield, IL, USA). The 6 *M* HCl and 6 *M* NaOH were made from concentrated HCl (Mallinckrodt, Paris, KY, USA) and sodium hydroxide pellets (Fisher Scientific, Fair Lawn, NJ, USA).

HPCE

A P/ACE Model 2100 instrument (Beckman, Fullerton, CA, USA) was used for all CE experiments. A 57 cm (50 cm to the detector) × 50 μm I.D. capillary was used at 25 kV for MEKC separations and 30 kV for CZE separations. Injections were by pressure for 5 to 10 s at the anode (positively charged) end. Detection was by UV at 214 nm.

Spiking/extraction studies

Water. Deionized water of 250- and 1000-ml volumes was spiked at nominal concentrations of 1.0 and 0.1 μg/g with the 7-STD. The water was adjusted to pH 2 and extracted by means of extraction disks (Varian version of Empore, 3M, St. Paul, MN, USA). We followed the manufacturer's recommendation of adding 5 ml of methanol per 1000 ml water to be extracted and of using a drip rate no greater than 5 min per 1000 ml of water (usually 10 min per 250 ml). The disks were prepared according to the manufacturer's instructions that accompany the disks. This consisted of rinsing with 10 ml of methanol, 10 ml of water, and 10 ml of water–methanol (95:5, v/v). The first fraction of retained analytes (OR fraction) was eluted with two 7-ml portions of basic methanol (pH 8–9); the eluate comprised analytes **1**–**6**. Compounds that were not retained under these acidic conditions were isolated by readjusting the pH to 6 and adding 0.050 g of cetyldiethylmethylammonium bromide (CEMA) per 250 ml of aqueous sample. A new disk extraction was carried out with the disk activated as above but with an additional 10 ml of water–methanol (95:%) containing CEMA at 0.025 g per 100 ml. The second fraction of retained analytes was eluted twice with two 10-ml volumes of methanol and contained analytes **6** and **7** (CL fraction). The OR fraction was readjusted to pH 6 and then the OR and CL fractions were each passed through an SCX (Bond Elut; Varian, Harbor City, CA, USA) cartridge followed by a 1-ml methanol rinse. The SCX cartridge was activated with 3 ml of water, 3 ml of 0.1 *M* sodium sulfate, 1 ml of water, and finally 2 ml of methanol. A drip rate of about 3 ml per 15 s was used. The eluates were concentrated as appropriate under a nitrogen stream and subjected to MEKC.

Soil. A 10-g portion of soil was spiked by pipet with the 7-STD, mixed, and allowed to sit overnight. The spiked soil was extracted by either sonication or Soxhlet methods [5] using a water–methanol (25:75, v/v) solvent mixture. The extract was concentrated to about 25 ml and diluted with deionized water to 250 ml, and then treated exactly as the spiked water sample.

Additional cleanup

TLC. Silica gel 60 plates (E. Merck, Darmstadt, Germany) were spotted and then developed with the solvent mixture isopropanol–

tetrahydrofuran–ammonia–water (100:50:4:1, v/v). Typical R_F values were as follows: **1–3** and **5–7** were found in the range 0.30–0.55; **4** was essentially unmoved at 0.02; polar neutrals were found at 0.80 or greater.

Ion-pairing on C_{18} SPE cartridges. The cartridges were activated with 5 ml of methanol followed by 3 ml of water–methanol (95:5) containing 0.025 g CEMA per 100 ml. The CL fraction was diluted to 50 ml with deionized water, 0.010 g of CEMA was added, and this solution was passed through the cartridge. The cartridge was washed with 3 ml of water–methanol (95:5) containing 0.025 g CEMA per 100 ml and then eluted with methanol–water (75:25) containing 0.025 g CEMA per 100 ml.

RESULTS AND DISCUSSION

Migration times

Tables I–VI tabulate the CZE and MEKC migration times (MT_e and MT_c, respectively) for 56 ACOAs at pH 8.3 using the boric acid–sodium borate buffer. This system was chosen as a generally applicable system for the determination of ACOAs. The basic pH conditions avoid, to a large extent, activity and adsorption effects than can affect peak shape when acidic pH values are used. As such, these conditions are likely a good compromise for multiresidue environmental analysis and analytes such as dyes.

TABLE I

CORRECTED MIGRATION TIMES OF PHENOXY ACIDS UNDER MEKC AND CZE CONDITIONS

Buffer, borate pH 8.3; MEKC with cholic acid.

Phenoxy acids	MT_c	MT_e
2,4-Dichlorophenoxyacetic acid	8.87	3.46
2-Methyl-4-chlorophenoxyacetic acid	9.03	3.45
2,3-Dichlorophenoxyacetic acid	9.08	3.50
3,4-Dichlorophenoxyacetic acid	9.21	3.48
2,4,5-Trichlorophenoxypropionic acid	9.23	3.29
2,4,5-Trichlorophenoxyacetic acid	9.37	3.40
2,4-Dichlorophenoxybutyric acid	9.93	3.28
2-Methyl-4-chlorophenoxybutyric acid	9.93	3.46

TABLE II

CORRECTED MIGRATION TIMES OF AROMATIC CARBOXYLIC ACIDS UNDER MEKC AND CZE CONDITIONS

Buffer, borate pH 8.3; MEKC with cholic acid.

Aromatic carboxylic acids	MT_c	MT_e
9-Anthracenecarboxylic acid	8.65	3.41
4-Hydroxybenzoic acid	8.95	3.73
3-Hydroxybenzoic acid	9.17	3.77
4-Aminobenzoic acid	9.19	3.78
2-Chlorobenzoic acid	9.46	3.77
4-Cyanobenzoic acid	9.54	4.24
2-Hydroxybenzoic acid	9.57	3.81
4-Nitrobenzoic acid	9.64	3.78
4-Chlorobenzoic acid	9.66	3.75
3-Nitrobenzoic acid	9.70	3.89

The tables group the compounds according to a common structural feature, which is indicated in the titles. Within the tables, compounds are sorted by increasing MT_c. The MT_e and MT_c values are corrected to a standard set of conditions to facilitate comparison among the many compounds using data accumulated over several months; a short discussion of a convenient approach to the correction will be given (see *Corrections to migration times*).

Of the 56 compounds, more than 80% of them have an MT_e that falls between 3.0 and 4.0 min. We estimate that under these conditions, we could reasonably expect to resolve about 10 compounds based on observed peak widths. Therefore, the contention that CZE is a technique of high efficiency but limited selectivity is supported [29] within this context. In contrast, examination of the MT_c reveals considerably more selectivity at pH 8.3. As pointed out by Terabe *et al.* [30], the optimum pH for separation of acids is in the vicinity of their pK_a. Therefore, the implications of our comparison of CZE and MEKC is limited in view of the one pH value used.

MEKC also exhibits wider applicability to complex ions such as dyes because of improved peak shape. We estimate that of the nearly 40

TABLE III

CORRECTED MIGRATION TIMES OF AROMATIC SULFONIC ACIDS UNDER MEKC AND CZE CONDITIONS

Buffer, borate pH 8.3; MEKC with cholic acid.

Aromatic sulfonic acids	MT_c	MT_e
Cumenesulfonic acid	9.04	3.57
Anthraquinone-2-sulfonic acid	9.37	3.52
Xylenesulfonic acid	9.55, 9.24, 9.45, 9.07	3.75, 3.30, 3.56, 3.70
Naphthalenesulfonic acid	9.70	3.75
4-Hydroxybenzenesulfonic acid	9.99	3.74
Toluenesulfonic acid	10.51	3.90
4-Chlorobenzenesulfonic acid	10.51	3.95
3-Nitrobenzenesulfonic acid	10.65	3.98
Dodecylbenzenesulfonic acid	11.34 (center)	3.13 (center)
Anthraquinone-2,6-disulfonic acid	14.92	5.31
1,2-Benzenedisulfonic acid	28.14	7.24

compounds (about 70% of the total) with MT_c in the range of 8.0 to 10.0 min, nearly half could be separated. About 30% of the compounds have MT_c greater than 10.0. Therefore, HPCE under these conditions is a technique of high efficiency and relatively high selectivity. In the context of a given separation, the additional flexibility of choosing the surfactant compound for micellar formation enhances the separation power of the technique. Further modifications based on the use of additives such as ion-pair agents and organic modifiers are discussed by Terabe and co-workers [27,28].

Corrections to migration times

A convenient approach was used to correct MT values to a standard set of conditions based on the use of two internal standards for assessing changes in the apparent mobility. From the basic equation of electrophoresis, the apparent mobili-

TABLE IV

CORRECTED MIGRATION TIMES OF ARYL ALIPHATIC ACIDS UNDER MEKC AND CZE CONDITIONS

Buffer, borate pH 8.3; MEKC with cholic acid.

Aryl aliphatic acids	MT_c	MT_e
4-Hydroxyphenylacetic acid	8.43	3.54
3-Hydroxyphenylacetic acid	8.46	3.55
7-Hydroxycoumarin-4-acetic acid	8.82	3.74
4-Chlorophenylacetic acid	8.92	3.58
4-Nitrophenylacetic acid	8.94	3.78
2-Hydroxyphenylacetic acid	8.98	3.73
Pyrenebutyric acid	10.86	3.76

TABLE V

CORRECTED MIGRATION TIMES OF SELECTED DYES UNDER MEKC AND CZE CONDITIONS

Buffer, borate pH 8.3; MEKC with cholic acid. N.O. = Not observed.

Dyes	MT_c	MT_e
Erioglaucine	9.16	6.05
Acid Blue 113	10.17	N.O.
Erythrosin	10.26	3.61
Acid Blue 25	10.69	3.78
Orange II	10.78	3.41
Indigo Carmine	11.05	4.59
Trypan Blue	12.96	3.39
Tartrazine	15.67	3.33

TABLE VI

CORRECTED MIGRATION TIMES OF NITROGEN-CONTAINING ACOAs UNDER MEKC AND CZE CONDITIONS

Buffer, borate pH 8.3; MEKC with cholic acid.

Nitrogen-containing ACOAs	MT_c	MT_e
2-Quinolinecarboxylic acid	7.72	3.55
9-Acridinecarboxylic acid	7.87	3.35
4-Quinolinecarborylic acid	8.52	3.48
3-Quinolinecarboxylic acid	8.75	3.55
2,3-Pyridinedicarboxylic acid	8.79	6.04
2-Pyridineethanesulfonic acid	8.80	3.69
2-Pyridylacetic acid	8.87	3.69
2,4-Pyridinedicarboxylic acid	9.76, 10.61	3.84
Picolinic acid	9.78	3.65
Nicotinic acid	10.17	3.90
Isonicotinic acid	10.18	3.91
4,9-Acridinedicarboxylic acid	10.71	3.39

ty of a substance can be calculated based on its observed MT; its apparent mobility is the combination of the mobility of the electroosmotic flow (EOF) and the mobility of the substance as modified by the capacity factor under MEKC conditions. Mobility is proportional to 1/MT so that corrections can be developed from the use of a single internal standard (IS) by relating 1/MT(sample) to 1/MT(IS). The inclusion of two internal standards allows the immediate assessment of changes in conditions independent of an EOF marker via the difference $\delta = 1/MT(IS1) - 1/MT(IS2)$. This is particularly convenient under MEKC conditions where EOF may be difficult to determine exactly. All MT values in the tables are corrected to an EOF of 2.30 min (determined by pyridine) under CZE conditions and MT of 8.43 and 14.92 for IS1 and IS2, respectively, under MEKC conditions. Generally, we have found that corrections via IS1[a] alone usually bring the MT within a 1% reproducibility. Additional corrections based on scaling the δ values could further reduce variability but were not used for the corrected MT given in the tables.

Difficult separations achieved

A number of separation problems are implicitly solved by the experimental data reported in Tables I–VI. For example, a useful approach to the separation and determination of phenoxy acid herbicides, aromatic carboxylic acids and aromatic sulfonic acids (Tables I–III) is provided under MEKC conditions. The MT_c reveal that many of the compounds would be separated. MEKC also provides a general approach to the separation of azo dyes (Table V) and the analysis of the purity of dye standards. Fig. 2 presents the electropherogram of trypan blue, which reveals nine major peaks for the standard. This equates to 72% purity based on the assumption of equal molar absorptivity among the compounds. The first peak, because of its early MT, is probably a neutral compound.

Certain kinds of separations difficult to carry out by RPLC can be directly effected using MEKC. For example, the electropherogram of nicotinic, isonicotinic and picolinic acids is shown in Fig. 3. Nicotinic and isonicotinic acids are not resolved, but peak shapes of all acids are sharp without extreme measures to prevent peak tailing [31]. Under CZE conditions, nicotinic and isonicotinic acids are also unresolved. We have observed (shown as an inset in Fig. 3) a partial separation of nicotinic and isonicotinic acids with acetone as a 5% additive under MEKC conditions.

Spiked water and soil samples —isolation and recovery

Seven compounds, **1–7**, were chosen to evaluate a general analytical scheme for the determination of ACOAs in water and soil (Tables VII–IX). The compounds encompass phenoxy acid herbicides, aromatic carboxylic acids and sulfonic acids, and azo dyes. The separation of **1–7** is shown in Fig. 4 under MEKC conditions. The compounds are well resolved in a relatively short time in view of the wide differences in molecular structure. The electropherograms generally display between 200 000 and 250 000 theoretical plates based on peak

[a] $MT(corr) = 1/\{1/MT(IS1,S) - [1/MT(IS1) - 1/MT(S)]\}$, where MT(IS1,S) = 8.43 and MT(IS1) and MT(S) are the particular MT of the internal standard and substrate, respectively, observed in a given run.

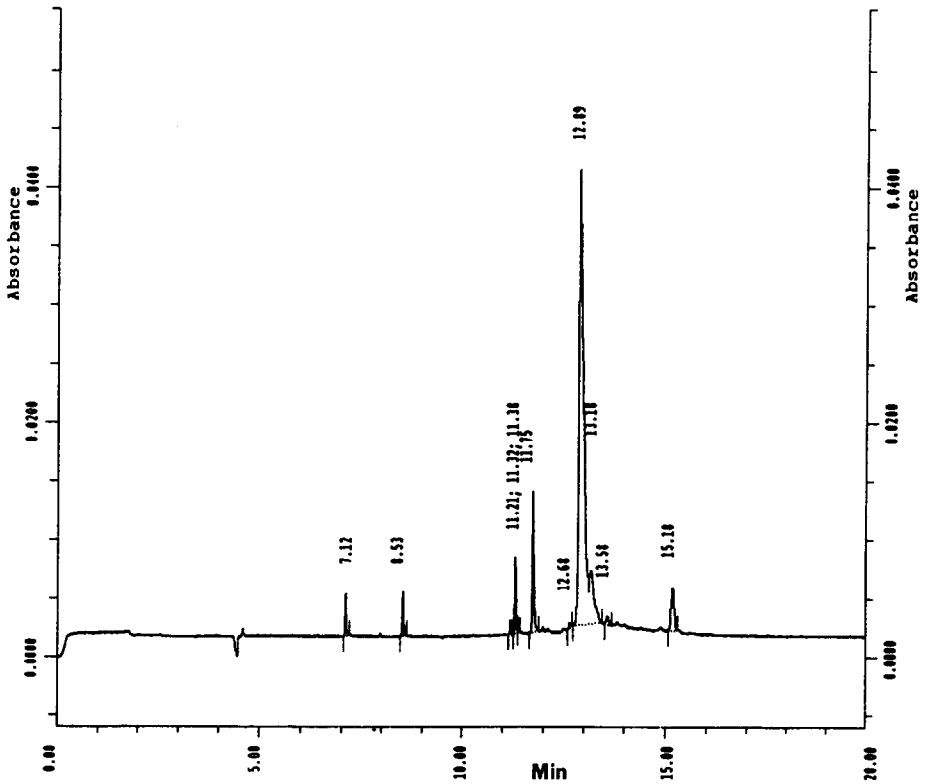

Fig. 2. Electropherogram of trypan blue standard under micellar conditions (pH 8.3, boric acid–sodium borate 50 m*M*, 100 m*M* sodium cholate). Main component MT = 12.89.

widths. The peak corresponding to 2,4,5-trichlorophenoxypropionic acid (**5**) is broadened and at times we have observed the slight separation of two peaks. We attribute this to a partial resolution of optical isomers of **5** in view of the chiral nature of cholic acid [28].

The isolation of the compounds from spiked water and soil is a unified approach that depends on SPE using extraction disks and SPE cartridges. All but the most strongly acidic, water-soluble acids are isolated at pH 2 on the C_{18} disks. This pH was chosen to remain within the operating range of stability (pH 2–7) recommended by the manufacturer[a]. The non-retained acids at pH 2 are, nevertheless, retained on disk at pH 6 using the ion-pairing agent CEMA when the aqueous acid filtrate is reextracted with a new disk. Alternatively, acid labile compounds can be extracted exclusively using the ion-pairing approach.

Extraction from soil requires the presence of water in the solvent in order to recover **4**. We found that a water–methanol (25:75, v/v) solvent was adequate to ensure reproducible recovery. Once the analytes are extracted, a reduced volume of the extracting solvent is diluted in water and subjected to the SPE isolation given previously.

Compounds **1–7** were studied at nominal levels of 1.0 and 0.1 μg/g for water and 100 and 20 μg/g for soil. The percent recoveries are fairly good, ranging from 38 to 101% for water and 26.5 to 94.4% for soil. Trypan blue (**4**) was particularly difficult to recover, a fact not surprising in view of its complex structure. It should also be pointed out that overall recovery is dependent on both extraction efficiency and recovery efficiency from the disk. Trypan blue is

[a] A referee has commented that pH 1 may be used to improve retention of analytes that are not fully retained at pH 2 (Yoo *et al.* [36]).

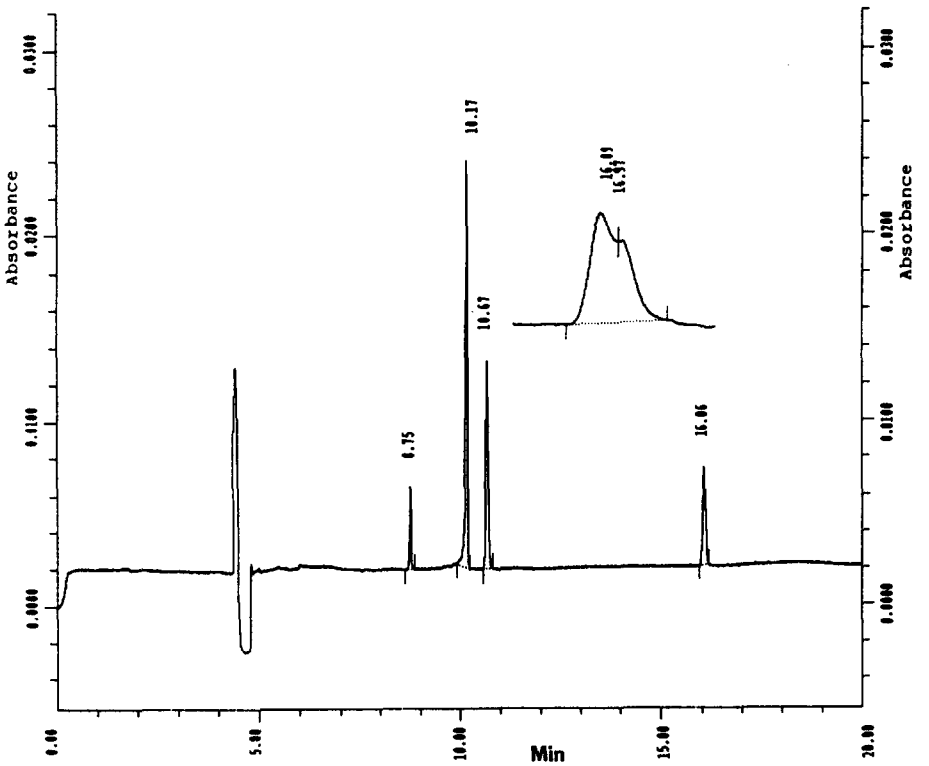

Fig. 3. Electropherograms of nicotinic, isonicotinic and picolinic acids under micellar conditions (pH 8.3, boric acid–sodium borate 50 m*M*, 100 m*M* sodium cholate). Picolinic acid MT = 10.17; isonicotinic and nicotinic MT = 10.67. Inset: nicotinic and isonicotinic acids partially resolved using 5% acetone additive in the buffer.

difficult to recover completely from the disk. The reason for the lower recovery of **5** in water is not clear to us. Again, factors related to recovery from the disk could be involved. The coextractives and minute soil particles retained on the disk apparently aided subsequent recovery of **5** from a soil matrix.

The precision of recovery indicated by the

TABLE VII

RECOVERY OF 7-STD IN SPIKED WATER

Analyte	Spike level 1.0 μg/g [a]			Spike level 0.1 μg/g [b]		
	Recovery (%)	Average	R.S.D.	Recovery (%)	Average	R.S.D.
1	88.9, 49.5, 88.1	75.5	30	42.2, 90.0, 77.3	69.8	36
2	86.0, 65.2, 44.4	65.2	32	45.3, 82.2, 74.6	67.4	29
3	101, 110, 93.0	101	8.4	44.4, 91.8, 88.7	75.0	35
4	109, 45.3, 91.5	81.9	40	48.0, 95.4, 89.5	77.6	33
5	50.8, 43.4, 20.2	38.1	42	64.9, 76.6, 42.1	61.2	29
6	91.4, 105, 98.3	98.2	6.9	36.4, 51.6, 89.1	59.0	46
7	81.9, 74.5, 72.2	76.2	6.7	41.0, 75.4, 82.7	66.4	34

[a] Exact spike level for **1–7**, respectively, in μg/g: 0.764; 0.92; 0.816; 1.592; 0.80; 1.024; 0.892.
[b] Exact spike level for **1–7**, respectively, in μg/g: 0.0955; 0.115; 0.102; 0.199; 0.100; 0.128; 0.1115.

TABLE VIII

RECOVERY OF 7-STD IN SPIKED SOIL

Analyte	Spike level 100 μg/g[a]			Spike level 20 μg/g[b]		
	Recovery (%)	Average recovery (%)	R.S.D. (%)	Recovery(%)	Average recovery (%)	R.S.D. (%)
1	93.1, 88.0, 102	94.4	7.5	89.5, 82.9, 104	92.1	12
2	90.3, 86.3, 101	92.5	8.2	80.3, 87.4, 105	90.9	14
3	89.2, 71.1, 80.7	80.3	11	90.9, 103, 76.2	89.9	15
4	25.8, 21.0, 37.5	28.1	30	11.2, 40.3, 28.1	26.5	55
5	92.7, 86.0, 103	93.9	9.1	83.2, 83.5, 105	90.6	14
6	74.7, 68.2, 41.4	61.4	29	82.9, 93.3, 93.2	89.8	6.7
7	83.3, 57.9, 41.6	60.9	34	83.3, 76.3, 86.7	83.1	7.1

[a] Exact spike level for **1–7**, respectively, in μg/g: 111; 106; 104; 226; 105.5; 117; 130.5.
[b] Exact spike level for **1–7**, respectively, in μg/g: 22.2; 21.2; 20.8; 45.2; 21.1; 23.4; 26.1.

R.S.D.s was higher for water than for soil, and reflects a variability that is not entirely understood. The lower recovery for water is also curious. The presence of free silanol groups on the bonded silica may be playing a role in retention and subsequent elution. These data, however, are comparable to extraction efficiencies and precision obtained with analytes as routine as organophosphorus pesticides [32]. In that study, 17 organophosphorus pesticides recovered from water ranged in recovery from 7 to 98% and the R.S.D. from 7 to 38%. Further studies published on the application of SPE to a variety of semivolatile analytes using multiresidue methodology show a great variety of recoveries and precision that are not entirely understood [33,34]. It was postulated that the presence of other analytes affects recoveries in certain compound classes. This factor may be important in understanding the better recoveries we obtained from a soil matrix. Method variability from laboratory to laboratory is a well known and not always explicable phenomenon.

TABLE IX

RECOVERY OF 7-STD IN SPIKED SOIL USING SOXHLET EXTRACTION AND REEXTRACTION AFTER SONICATION EXTRACTION

Analyte	Recovery (%)	
	100 μg/g[a]	Soxhlet reextraction of sonicated soil 100 μg/g[b]
1	96.7	13.1
2	96.5	12.4
3	112	13.1
4	48.8	10.1
5	55.3	10.3
6	113	6.9
7	96.3	6.2

[a] Exact spike level for **1–7**, respectively, in μg/g: see footnote *a* in Table VIII. [b] Exact spike level for **1–7**, respectively, in μg/g: see footnote *a* in Table VIII.

An example of an electropherogram of the OR fraction and the CL fraction is given in Fig. 5 for a spiked soil. Compound **6** is recovered in both fractions and is an example of breakthrough at pH 2. The peaks that result from analyzing the finished sample extract are less sharply focussed in a 10-s injection than those for the 7-STD. This is presumably due to the higher ionic strength of the finished extract that results in less focussing effect by field amplification [35] than that observed with standards. Drift in MT seen in comparing Figs. 4 and 5 was observed when comparing samples and standards run on different days. These variations can be minimized by doing complete changeouts of inlet and outlet buffer vials regularly. Use of internal standards

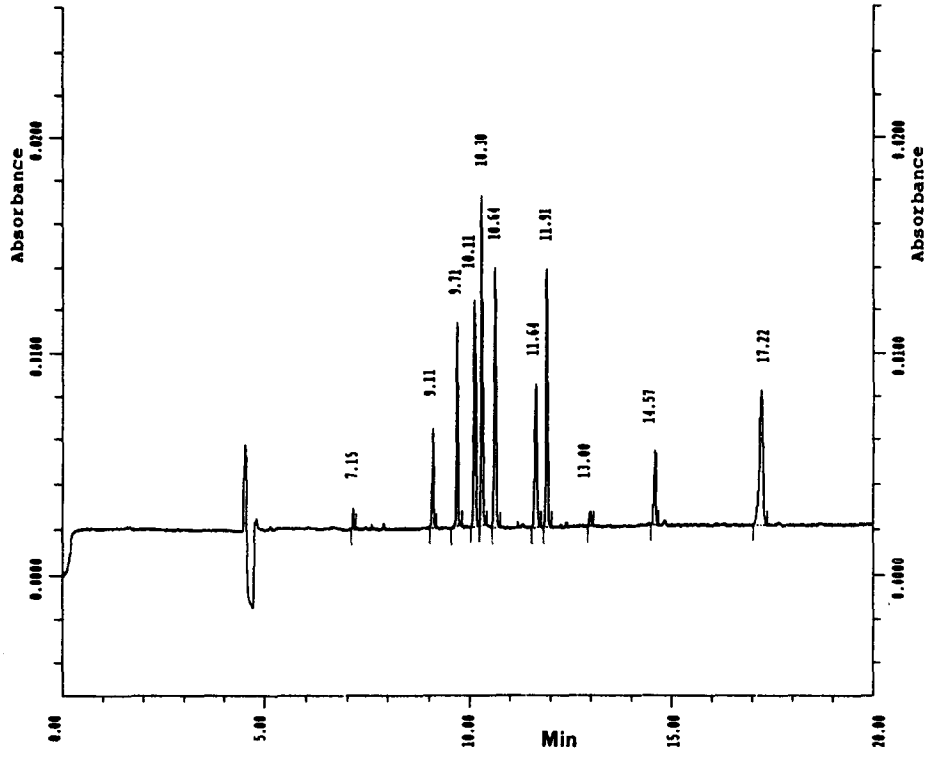

Fig. 4. Electropherogram of the 7-STD under micellar conditions. Identification of peaks: MT 9.11 = IS1; MT 9.71 = **1**; MT 10.11 = **5**; MT 10.30 = **2**; MT 10.64 = **6**; MT 11.64 = **7**; MT 11.91 = **3**; MT 14.57 = **4**; MT 17.22 = IS2.

adequately compensates for observed variations which for IS2 varied over about a 3-min window before regular changeouts of buffer were instituted.

Compounds **1–7** encompass a wide range of structures and complexity. Extraction by SPE in this work depends upon controlling pH and modifying retention via ion-pairing reagents. The critical nature of any experimental parameters including the source of the disks and effects of coextractives may need to be assessed in a more systematic way. Although specialized procedures have been developed for specific classes of compounds, such as herbicides, the general approach that we present provides the foundation for a broad monitoring tool. It integrates the procedure for both water and soil determinations based on SPE from water and determination by MEKC. Where anticipated ACOAs are unstable at pH 2, the ion-pairing procedure used for **6** and **7** should result in an efficient isolation. Although the OR and CL fractions may be combined and determined simultaneously, we feel the two-fraction determination increases the likelihood of obtaining good separations and recovery for complex samples.

Extraction comparison

Compounds were extracted from soil using a sonication method [5]. For comparison, recoveries from Soxhlet extraction of a soil are given in Table IX; both methods are comparable. In addition, a soil that was first extracted by sonication was reextracted by the Soxhlet method to estimate residues remaining. The sonication extraction performs well in terms of recovery, because we expected higher efficiency from the Soxhlet method. Trypan blue presents special difficulties as it even adheres to the final filter used before HPCE determination. It must be removed with methanol containing buffer.

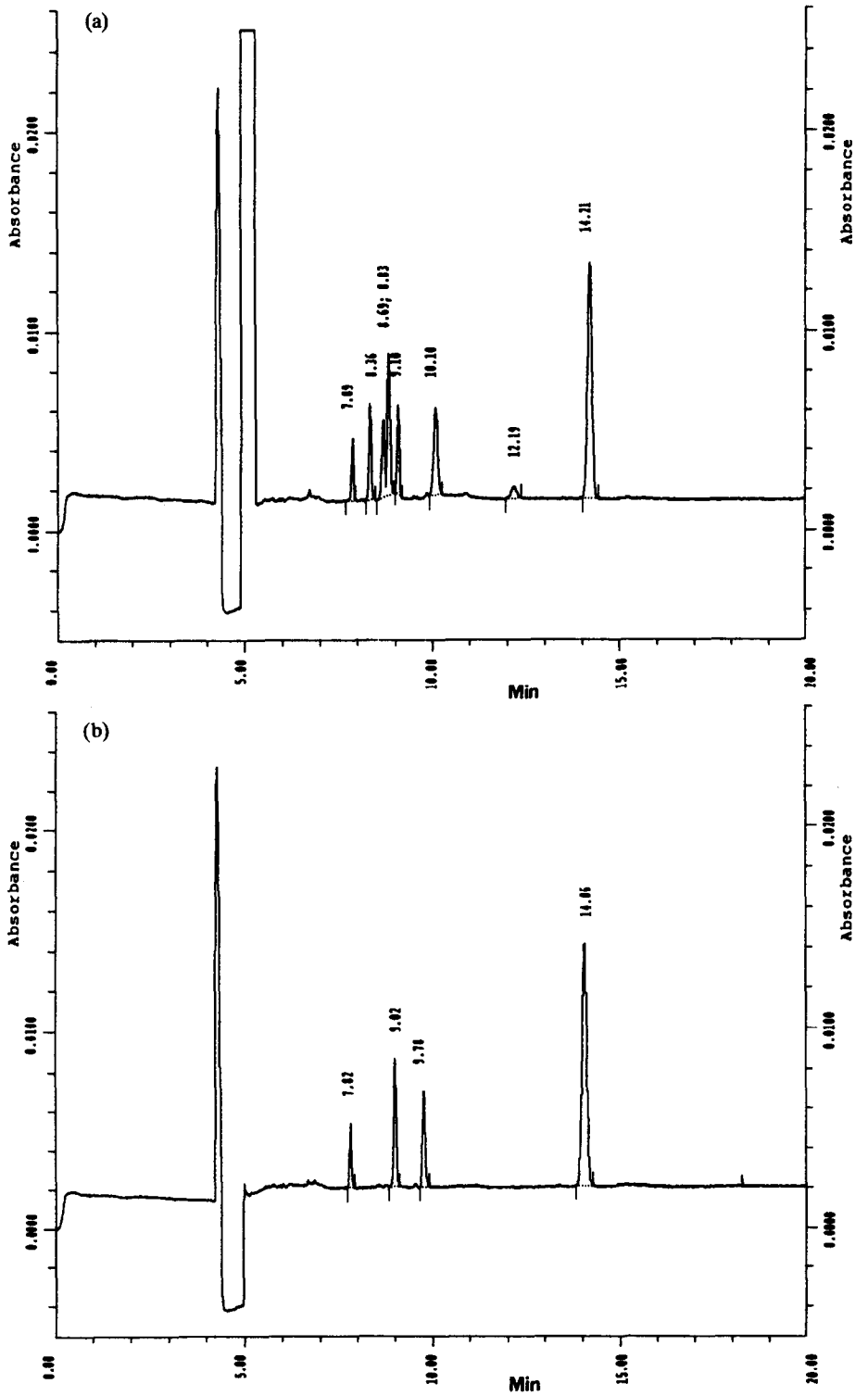

Fig. 5. Electropherogram of (a) OR fraction (**1–6** from C_{18} SPE, pH 2) and (b) CL fraction (**6** and **7** from ion pairing, pH 6) after extraction/cleanup of a 20.0 μg/g spiked soil and run under micellar conditions. Peaks (a): MT 7.89 = IS1; MT 8.36 = **1**; MT 8.69 = **5**; MT 8.83 = **2**; MT 9.10 = **6**; MT 10.10 = **3**; MT 12.19 = **4**; MT 14.21 = IS2. Peaks (b): MT 9.02 = **6**; MT 9.78 = **7**.

Interferences to MEKC determination

The UV detection under MEKC is relatively non-specific. Therefore, a number of potential interferences to the determination of ACOAs exist. Under MEKC conditions using cholate as the micellar agent, neutral compounds will usually elute before IS1. This is due to the limited affinity of hydrophobic compounds and the enhanced affinity of anions for cholic acid micelles relative to micelles of sodium dodecyl sulfate (SDS). This particular specificity of cholic acid micelles makes them particularly applicable to anionic analytes. Presumably, the great difference in aggregation numbers between micelles of SDS and cholate affect anion affinities via charge repulsion [27].

Although further cleanup is not required at the levels studied, other matrices and soil types and lower detection levels may dictate the need for additional cleanup steps. Two complementary approaches are suggested. The first employs TLC separation of dyes, herbicides, and aromatic acids from polar and non-polar neutrals. Analytes may be recovered from the TLC plates, which can also serve as a sample screening technique. A second cleanup approach can be based on an ion-pairing technique using C_{18} SPE cartridges (see Experimental section).

Cationic surfactants are removed in the SCX cleanup step. Anionic surfactants will be recovered in this scheme. If anionic surfactants interfere with the determination of ACOAs by MEKC, a separation based on RP C_{18} cartridges, ion-pairing with C_{18} cartridges, or TLC on silica should effect the necessary additional cleanup.

NOTICE

Although the research described in this report has been funded by the US Environmental Protection Agency, it has not been subjected to Agency review and, therefore, does not necessarily reflect the views of the Agency and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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