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Determination of chlorinated acid herbicides and related compounds in water by capillary electrophoresis–electrospray negative ion mass spectrometry

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Abstract

Capillary electrophoresis electrospray negative ion mass spectrometry was investigated for the determination of chlorinated acid herbicides and several phenols in water. Sixteen analytes were separated as their anions in less than 40 min with a buffer consisting of 5 m*M* ammonium acetate in isopropanol–water (40:60, v/v) at pH 10. A sample stacking technique was used to provide lower detection limits and a fortified drinking water sample was made pH 10 and analyzed without further processing. Quantitative analyses with an internal standard gave recoveries in the 91–124% range and replicate measurements of a calibration standard gave relative standard deviations in the range of 3–10%.

Keywords: Water analysis; Environmental analysis; Capillary electrophoresis–mass spectrometry; Pesticides; Phenols; Organochlorine compounds

1. Introduction

Chlorinated carboxylic acid herbicides have been recognized as environmental pollutants for many years [1] . Table 1 shows the structures and common names or abbreviations for 12 of these compounds and several acidic phenols that are also common environmental contaminants. In 1984 the US Environmental Protection Agency (EPA) approved test procedures for six of these compounds (2,4-D; 2,4,5-T; 2,4,5-TP; dicamba; 4-nitrophenol; and, pentachlorophenol) in waste water discharges [2]. Dinoseb, 2,4,5-T, and 2,4,5-TP are banned from use in the USA but they may be exported and used in other countries and they may persist in the environment [3]. In the USA picloram and pentachlorophenol are restricted to use by certified pesticide applicators [4]. Maximum contamination levels in drinking water have been established in the United States for dinoseb, 2,4-D, 2,4,5-TP, picloram, and pentachlorophenol [5].

Most published measurements of the chlorinated carboxylic acid herbicides in environmental samples have been made by liquid–liquid extraction of the acids into an organic solvent, conversion of the acids

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Table 1
Structures and major ions in electrospray mass spectra of chlorinated acid herbicides

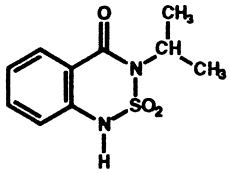
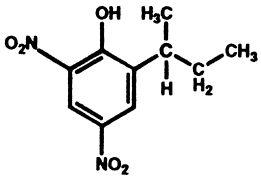
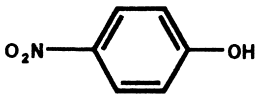
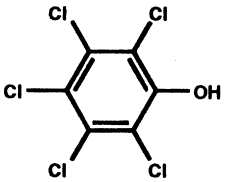
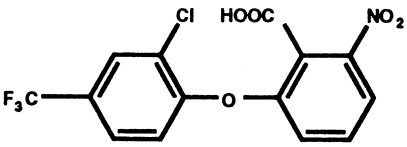
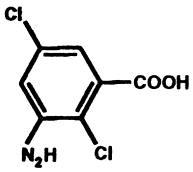
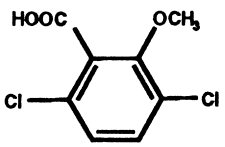
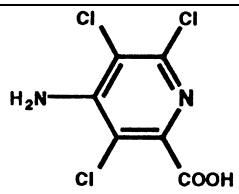
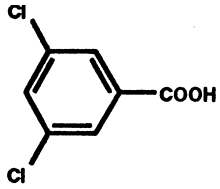
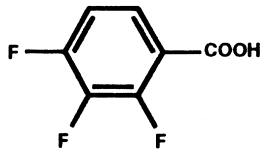
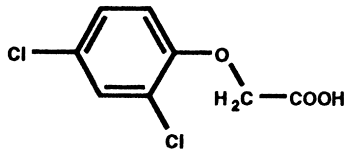
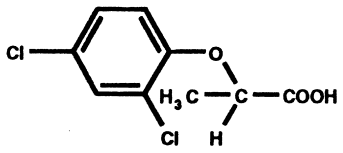
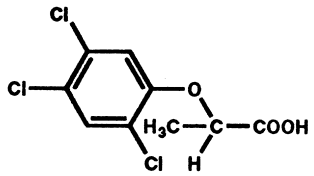
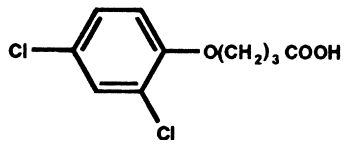
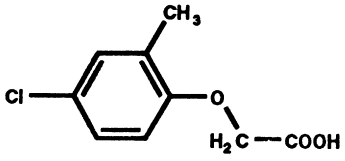
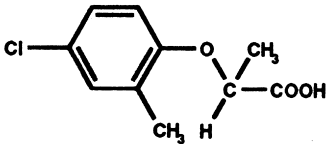
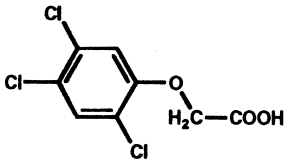
Compound	Structure	M_r [a]	Potentials on CapEx			
			−65 V		−100 V	
			Base peak	Second ion	Base peak	Second ion
Bentazon		240	239	–	239	–
Dinoseb		240	239	–	239	–
4-Nitrophenol		139	138	–	138	107 (9%)
Pentachlorophenol		264	265	–	265	–
Acifluorfen		361	360	316 (10%)	316	360 (14%)
Chloramben		205	204	160 (16%)	160	204 (38%)
Dicamba		220	219	175 (80%)	175	219 (11%)

Table 1. Continued

Compound	Structure	M_r [a]	Potentials on CapEx			
			−65 V		−100 V	
			Base peak	Second ion	Base peak	Second ion
Picloram		240	239	195 (10%)	195	239 (40%)
3,5-Dichlorobenzoic acid		190	189	–	189	145 (78%)
2,3,4-Trifluorobenzoic acid		176	175	131 (50%)	131	175 (10%)
2,4-D		220	219	161 (20%)	161	219 (7%)
Dichloroprop		234	233	161 (6%)	161	233 (20%)
2,4,5-TP		268	267	195 (16%)	195	267 (13%)
2,4-DB acid		248	247	161 (58%)	161	247 (8%)

(Continued overleaf)

Table 1. Continued

Compound	Structure	M_r [a]	Potentials on CapEx			
			−65 V		−100 V	
			Base peak	Second ion	Base peak	Second ion
MCPA acid		200	199	141 (3%)	141	199 (80%)
MCPP acid		214	213	141 (3%)	141	213 (58%)
2,4,5-T acid		254	253	195 (9%)	195	253 (22%)

[a] Monoisotopic molecular weight calculated from the atomic masses of the isotopes with the smallest masses.

into methyl esters, and gas chromatography with an electron-capture detector [1,6]. The acids also can be partitioned at low pH onto a cross-linked styrene–divinylbenzene resin contained in a cartridge or embedded in an inert filter disk [7]. To avoid the use of the toxic and explosive diazomethane for preparation of methyl esters, other reagents are used but these are less efficient and require longer reaction times, heating, and produce lower yields of the methyl esters of some analytes [8]. To obviate the derivatization reaction a high-performance liquid chromatography (HPLC) separation procedure with a ultraviolet diode array detector was developed [9]. In this method the herbicide acids are partitioned onto a C_{18}-silica cartridge or disk at pH 2, then separated on a C_{18}-silica HPLC analytical column with a gradient elution under acidic conditions. However all the analytes listed in Table 1 have not been separated on a single column and separate calibrations are conducted using two subgroups of analytes. In an environmental sample there is a real potential for coelution of several analytes and a high probability of interferences from anthropogenic and naturally occurring UV-absorbing substances.

Liquid chromatography–mass spectrometry (LC–MS) has been explored for these analyses and the three compounds 2,4-D, 2,4,5-T, and 2,4,5-TP were separated using reversed-phase LC with negative ion thermospray detection [10,11]. A thermospray interface was used by three laboratories in a multilaboratory comparison of LC–MS measurements of nine of the herbicide acids shown in Table 1 [12]. Although all the acids could be measured in simulated extracts at a concentration of 5 mg/l with mean recoveries in the 87–108% range, the measurements were variable with relative standard deviations (R.S.D.s) in the 12–31% range. Particle beam LC–MS systems have been used to measure the herbicide acids but some of the acids show evidence of surface adsorption and thermal decomposition which results in relatively high detection limits using typical particle beam operating conditions [13,14]. Improved performance has been obtained with a Teflon coated particle target in the ion source [15]. With this

technique 12 of the 17 compounds in Table 1, and six other compounds, were partially separated with a packed microbore LC column in 37 min with low ng sensitivity. Pneumatically assisted electrospray LC–MS has been applied by several investigators to this important class of compounds, but abundant positive ions are not produced in the electrospray ion source. Six acidic herbicides, including four in Table 1, and two oxidation products were separated at low pH on a 3 mm I.D. reversed-phase column [16]. Post-column addition of a tripropylamine solution produced abundant negative ions and excellent low ng/l sensitivity was achieved using an automated on-line solid-phase preconcentration device and selected ion monitoring. Electrospray LC–MS was applied to 20 acidic herbicides and related compounds, including 10 of the analytes in Table 1 [17]. In this work the compounds were partitioned from water onto graphitized carbon black, eluted using a series of washes and solvent mixtures, and separated as anions with ion pair chromatography on a 4.6 mm I.D. reversed-phase column. The effluent from the analytical column was split and 3% admitted to the electrospray interface and mass spectrometer and the remainder to a UV detector. Because of variabilities in LC–MS signals after several hours of continuous use it was concluded that analyte quantitation could be better performed using the UV detector. Although supercritical fluid chromatography–MS was investigated as an analytical technique for several herbicide acids, no detailed quantitative studies have been reported and some of these compounds may be too polar for the commonly used supercritical carbon dioxide mobile phase [18,19].

The purpose of this research was to explore capillary electrophoresis combined with electrospray negative ion mass spectrometry (CE–ES-MS) which has the potential of providing a simpler and faster method for the determination of a variety of acidic herbicides and phenols in water. CE is a powerful technique for the separation of both positive and negative ions in solution [20–22]. For the easily ionized carboxylic acids in water, CE has an important advantage over reversed-phase LC which is best suited for polar but neutral species. An important practical advantage of CE is that uncoated fused-silica capillary columns used with the technique are very inexpensive, typically under US$ 10 each, compared to US$ 360 or more for a standard bonded phase GC or reversed-phase HPLC column. A few chlorinated acid herbicides have been separated by CE using UV detection which is of minimal value with environmental samples [23,24]. The low flow-rates used with CE, typically nl/min, are well suited to the introduction of the effluent into a mass spectrometer through an electrospray interface. Another potential advantage of the CE–ES-MS technique is that it may be possible to analyze environmental water samples directly without an extraction, extract concentration, derivatization or any other sample manipulation except pH adjustment. Elimination of these steps would significantly shorten and simplify the analysis and avoid complications caused by the use of solvents, glassware, reagents, and other apparatus. Relatively low-cost bench-top electrospray mass spectrometer systems with sufficient sensitivity are commercially available [25].

A recent publication described the determination of another carboxylic acid, ethylenediaminetetraacetic acid (EDTA), in human plasma and urine using CE–ES-MS–MS [26]. Tandem mass spectrometry and selected reaction monitoring were employed to provide a highly specific direct determination of EDTA in the complex biological matrices. With a relatively clean water matrix, for example drinking water, the determination of the herbicide acids should be feasible with a simpler and somewhat less costly single mass analyzer approach. A major question about the CE–ES-MS strategy concerns sensitivity because CE injection volumes are extremely low and detection limits in the 1–5 μg/l range are required to measure some of the herbicide acids in water.

2. Experimental

2.1. Materials

The sixteen individual herbicides and related compounds shown in Table 1 were obtained from the EPA's pesticide repository (now defunct) with a stated purity of 98–99% and these were used in the direct infusion ES-MS experiments without further purification. The internal standard 2,3,4-trifluoroben-

zoic acid (Table 1) was obtained from Aldrich (Milwaukee, WI, USA) with a stated purity of 98%. Two mixtures of eight analytes each in acetonitrile were purchased from Supelco (Bellefonte, PA, USA) and used without further purification. The mixtures are designated as groups A and B and the analytes in each group are given in the caption for Fig. 1 and described in EPA Method 555 [9]. Methanol, isopropanol, ammonium hydroxide, and ammonium acetate were Fisher Scientific HPLC grade. Buffers were freshly prepared using Milli-Q water (Millipore; Bedford, MA, USA). Uncoated fused-silica capillary columns (FSCCs) were obtained from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Instrumentation

A Dionex (Sunnyvale, CA, USA) model CES-1 CE system equipped with a ultraviolet (UV) detector was used for the preliminary evaluation of CE for the separation of the herbicide anions. A Crystal 300 CE system (Thermo Capillary Electrophoresis, Franklin, MA, USA) was used for all the ES-MS experiments. The electrospray ion source was a Hewlett–Packard (Palo Alto, CA, USA) model 59987A equipped with some components (model 103313 serial number 1066) manufactured by Analytica of Branford (Branford, CT, USA) including a CE-Pro CE probe. The probe is used to ensure the correct positioning of the CE capillary, sheath liquid, and sheath gas with respect to the ion entrance capillary. This electrospray interface has a design similar to that described by Fenn et al., in which the electrospray needle is at ground potential and high voltage (HV) is applied to the surrounding cylindrical and endplate electrodes and to the entrance of a dielectric capillary leading to the mass spectrometer [27]. A low voltage of opposite polarity is applied to the exit of the dielectric capillary (CapEx). The ends of the dielectric capillary were coated with a thin layer of Pt to facilitate connection of the electrical leads. The CE–ES probe and the electrospray interface have been described in some detail [28]. The original optics of the ion source were replaced with an Iris hexapole ion guide (Analytica of Branford) to improve transmission of low m/z species. The sheath liquid was delivered with a Harvard Apparatus (South Natick, MA, USA) Model 55-2222 syringe pump. The mass spectrometer was a Hewlett–Packard 5989B single quadrupole instrument equipped with a high energy conversion dynode detector and an G1034C MS ChemStation data system.

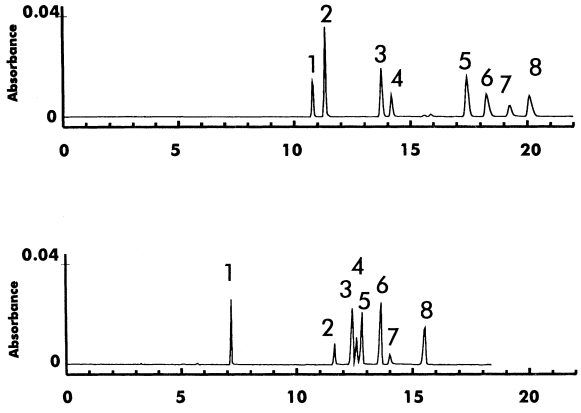


Fig. 1. The upper electropherogram shows the separation of the eight group A compounds with UV detection: 1=dicamba; 2=picloram; 3=chloramben; 4=acifluorfen; 5=bentazon; 6=2,4-D; 7=2,4,5-TP; 8=dichlorprop. The lower electropherogram shows the separation of the group B compounds: 1=4-nitrophenol; 2=2,4-DB; 3=MCPP; 4=dinoseb; 5=2,4,5-T; 6=MCPA; 7=pentachlorophenol; 8=3,5-dichlorobenzoic acid. Time scale in min.

2.3. CE–UV experiments

A 60 cm×50 μm I.D. uncoated FSCC was used for the CE separation. The running buffer consisted of 20 m*M* disodium and 20 m*M* monosodium phosphate adjusted to pH 2.9 (group A) or 6.4 (group B) with phosphoric acid. The source reservoir potential was either −18 kV (group A) or 18 kV (group B), the destination reservoir was at ground potential, and the UV detector was set at 230 nm. The compounds in groups A and B were diluted in separate portions of deionized water to a concentration of 50 mg/l and 5.9 nl aliquots injected separately by the gravity mode.

2.4. Direct infusion ES-MS

The equipment and conditions for direct infusion ES-MS were nearly identical to those used for CE–ES-MS. For direct infusion, no HV was applied to the CE anode and the analyte solutions were infused

through a 75 cm×75 μm I.D. uncoated FSCC into the CE–ES probe using the pressure mode of the CE instrument. Seventeen solutions were prepared in methanol–water (50:50, v/v) with each containing a single analyte or the internal standard at 20 mg/l. The pH of each solution was adjusted to 10 with ammonium hydroxide. The syringe pump was used to deliver 4–6 μl/min of methanol to the CE probe where it is used as a sheath liquid around the outside of the FSCC. A stream of oxygen gas at about 100 ml/min flowed through the outer concentric stainless steel tube of the CE–ES probe into the ES interface where it captures stray electrons and prevents corona discharge. The electrospray HV was 3300 V on the larger outer cylindrical electrode, 3000 V on the smaller endplate electrode, and 3300 V on the capillary entrance. Spectra were measured with CapEx potentials of −65 V and −100 V. A flow of heated dry nitrogen gas (heater temperature 180°C) was maintained counter to the electrospray flow to promote the desolvation of the negative ions. The spectrometer was repetitively scanned from 100–370 m/z at a rate of 2.6 scans/s.

2.5. CE–ES-MS

An uncoated 75 cm×75 μm I.D. FSCC was used for the CE separations. The CE separation buffer was 5 m*M* ammonium acetate in isopropanol–water (40:60, v/v) adjusted to pH 10 with ammonium hydroxide. The mixture of sixteen analytes and the internal standard was diluted in either deionized water or Cincinnati drinking water and the pH adjusted to 10. The injection volume was normally about 14 nl, the anode HV +28 kV, and the injection made using the pressure mode of the CE instrument. For sample stacking, the acids were dissolved in CE separation buffer which was diluted by a factor of ten with deionized water but maintained at pH 10. The injection volume was about 440 nl and the source reservoir potential was +30 kV. The electrospray interface voltages and other conditions were the same as in the direct infusion experiments but the CapEx voltage was fixed at −65 V. The quantitative measurements used selected ion monitoring (SIM) of various analyte ions with a 30 ms dwell time and a scan rate of 1.6 cycles of the quantitation ions per second.

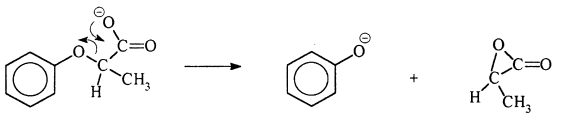
3. Results and discussion

A preliminary investigation was conducted with UV detection to determine the feasibility of separating the analytes in Table 1 with CE. The 16 compounds were available in two groups of eight as described in the HPLC method [9]. The analytes in the two groups are identified in the caption for Fig. 1 which shows the separation of the compounds in the two groups by CE. The analytes were identified by their migration times which were determined independently using solutions containing a single analyte. Fig. 1 clearly indicates the feasibility of a CE separation of most or possibly all 16 analytes plus an internal standard in a reasonable analysis time. Because of the deficiencies of this approach with real environmental samples, no further experiments were conducted using the UV detector.

3.1. Direct infusion electrospray mass spectra

The complete negative ion electrospray mass spectra of the analytes and internal standard were investigated by direct infusion of 20 mg/l solutions of the individual compounds in methanol–water (50:50, v/v) at pH 10. The purpose of these experiments was to determine optimum conditions for electrospray including conditions that minimize or eliminate adduct ions which are solvated ions or ion–molecule complexes of the analytes such as proton bound dimers. Adduct ions reduce overall analytical sensitivity by spreading abundance among several related ions. The abundances of some adduct ions are reduced or eliminated by the counter current flow of heated nitrogen in the spray chamber. Those adducts that survive and emerge from the dielectric capillary may be decomposed by collision induced dissociation (CID) in the region between the exit of the capillary and the skimmer before the ion guiding optics [29]. The pressure in this region is about 1.5 Torr from the oxygen sheath gas and nitrogen drying gas that pass through the capillary (1 Torr=133.322 Pa). Ions that emerge from the capillary are accelerated toward the skimmer by the capillary exit voltage (CapEx). This acceleration causes low energy ion–molecule collisions which produce additional desolvation, declustering, and some fragmentation of the anions.

The negative ion spectra of the analyte anions were measured at CapEx potentials of −65 V and −100 V and the major ions observed are shown in Table 1. A CapEx potential of −65 V was sufficient to cause desolvation and declustering of most ions and the base peaks in all spectra were the $[M–H]^-$ ions which are present in solution. Note that the base peak for pentachlorophenol at −65 V is 1 u more than the molecular mass because the most abundant ion of the $[M–H]^-$ cluster caused by the natural distribution of Cl isotopes has the composition $C_6O^{35}Cl_4\ ^{37}Cl$. With the exceptions of dicamba, 2,3,4-trifluorobenzoic acid (TFBA), and 2,4-DB, the second most abundant ion at CapEx-65 V had a relative abundance (RA) of 20% or less of the base peak. The nature of the second most abundant ion depended on the structure of the compound. The $[M–H]^-$ ions of bentazon, dinoseb, 4-nitrophenol, and pentachlorophenol (the first four compounds in Table 1) did not fragment at a CapEx of −65 V. Most of the herbicides with a carboxylic acid group attached directly to the aromatic ring (acifluorfen through TFBA in Table 1) gave second most abundant ions which correspond to the loss of CO_2 from the $[M–H]^-$ anion. The exception to this is 3,5-dichlorobenzoic acid whose $[M–H]^-$ ion did not fragment at −65 V. The $[M–H]^-$ ions of the phenoxyacids (the last seven analytes in Table 1) suffered losses of 58, 72, or 86 u depending on the number of carbon atoms between the phenoxy oxygen and the carboxylate group. This fragmentation of the $[M–H]^-$ ions, which is rationalized in the accompanying mechanism, is characteristic of structure and can be used to confirm the identification of analytes if necessary. The indicated formation of the three-membered ring lactic acid lactone does not imply its actual formation, but accounts for the atoms lost in the process. Alternative products could be carbon dioxide and methylcarbene. Butyrolactone is a likely product from the decomposition of the 2,4-DB anion.



[M-H] $^-$ [M-H-72] $^-$

With a CapEx of −100 V, the $[M–H]^-$ ions of bentazon and the three phenols again were the base peaks and only one low abundance fragment ion was observed, that is the loss of NOH from the $[M–H]^-$ ion of 4-nitrophenol. With the single exception of 3,5-dichlorobenzoic acid, the base peaks of the other compounds in Table 1 at a CapEx of −100 V are the same ions that were second most abundant at −65 V. Similarly, the $[M–H]^-$ ions, which were the base peaks at −65 V, are the second most abundant ions at −100 V. If additional confirmation of structure is required, a CapEx potential of −100 V can be used to enhance the abundances of the major fragment ions. For the CE–ES-MS measurements and quantitative analyses that follow, the CapEx potential was −65 V and the $[M–H]^-$ base peak ions in Table 1 were generally used for selected ion monitoring and quantitation of the analytes. An exception is pentachlorophenol where the ion containing only ^{35}Cl at m/z 263 was used as the quantitation ion although it is not the base peak. Another exception is dicamba where an 80% RA fragment ion at m/z 175 was used for monitoring and quantitation. These alternative ions were selected to avoid potential conflicts between the base peak ions of these analytes and those of 2,4,5-TP and 2,4-D respectively.

3.2. CE–ES-MS experiments

The running buffer selected for the separation of the herbicide anions consisted of 5 m*M* ammonium acetate in isopropanol–water (40:60, v/v) and adjusted to pH 10 with ammonium hydroxide. A limited number of other buffers were available for evaluation because a volatile buffer, such as an ammonium salt, is required to prevent plugging of the dielectric capillary between the spray chamber and the mass spectrometer. In addition, the conductivity of the buffer must not be too high to obtain a stable electrospray and ammonium salts perform well in this regard. With an anode HV of +28 kV, 12 of the 16 analytes and the internal standard TFBA were separated in less than 40 min. The analyte quantitation ions, migration times, and migration times relative to the internal standard TFBA are listed in Table 2. Pentachlorophenol and 2,4-DB are separated by only about 5 s, and bentazon and 2,4-D coelute under these conditions. Migration times vary somewhat because of fluctuations in the CE bulk

Table 2
Quantitation ions, migration times, and relative migration times of the carboxylic acid herbicides and related compounds

Analytes	Quantitation ions	Migration times (min)	Relative migration times
Acifluorfen	360	24.91	0.645
Pentachlorophenol	263	26.44	0.685
2,4-DB	247	26.53	0.687
Dinoseb	239	26.86	0.696
2,4,5-TP	267	27.27	0.706
2,4,5-T	253	28.31	0.733
MCPP	213	28.80	0.746
Dichloroprop	233	29.01	0.752
MCPA	199	30.07	0.779
Bentazon	239	30.61	0.793
2,4-D	219	30.60	0.793
Picloram	239	31.10	0.806
3,5-Dichlorobenzoic acid	189	31.54	0.817
Chloramben	204	34.00	0.881
Dicamba	175	34.27	0.888
4-Nitrophenol	138	36.33	0.941
Internal standard:			
2,3,4-trifluorobenzoic acid	175	38.60	1.000

flow-rate caused by slight temperature variations and changes in capillary inner surface conditions. Relative migration times are more reproducible with regard to these minor changes in conditions.

Because the CE anode voltage was +28 kV and the electrospray end of the CE capillary was at ground potential, the negative ions actually migrate away from the ES-MS interface toward the anode. However at pH 10 the electroosmotic flow (EOF) toward the interface is larger than electrophoretic migration and the anions are carried with the EOF toward the mass spectrometer. This effect accounts, in part, for the delay before the first eluting analyte, acifluorfen, reaches the mass spectrometer. If a faster analysis time is desired, a shorter CE column and/or a lower proportion of isopropanol should reduce the migration times, but coelution of more analytes is more likely.

Coelution of anions does not preclude accurate and precise measurements of concentrations of analytes as long as different ions are used for quantitation of unresolved analytes and care is taken to ensure that no other interferences are present in unresolved peaks. The virtually coeluting pentachlorophenol and 2,4-DB are measured using the $[M-H]^-$ ions at m/z 263 and 247, respectively. Similarly the coeluting bentazon and 2,4-D are measured using the $[M-H]^-$ ions at m/z 239 and 219, respectively. The use of the same ion for different analytes is not precluded as long as the analytes are separated in time from one another. For example, the $[M-H]^-$ ions at m/z 239 were used for monitoring and quantitation of bentazon, dinoseb, and picloram, but these analytes are separated in time. Fig. 2 shows the selected ion electropherograms of the sixteen analytes and the internal standard TFBA. The second panel from the top on the left side of Fig. 2 shows the separation of the three analytes that have common quantitation ions. Similarly, the bottom panel on the left side shows the separation of dicamba and the internal standard which have the same quantitation ions. Examination of the panels in Fig. 2 shows that most analytes are separated in time but where coelutions occur, different quantitation ions are used.

The concentrations of the analytes and the internal standard in the solution used to generate the ion current profiles shown in Fig. 2 were 5 mg/l. The injection volume was about 14 nl which corresponds to about 70 pg of each analyte. The signal-to-noise ratios in Fig. 2 are generally good suggesting that a lower concentration sample could be analyzed. Fig. 3

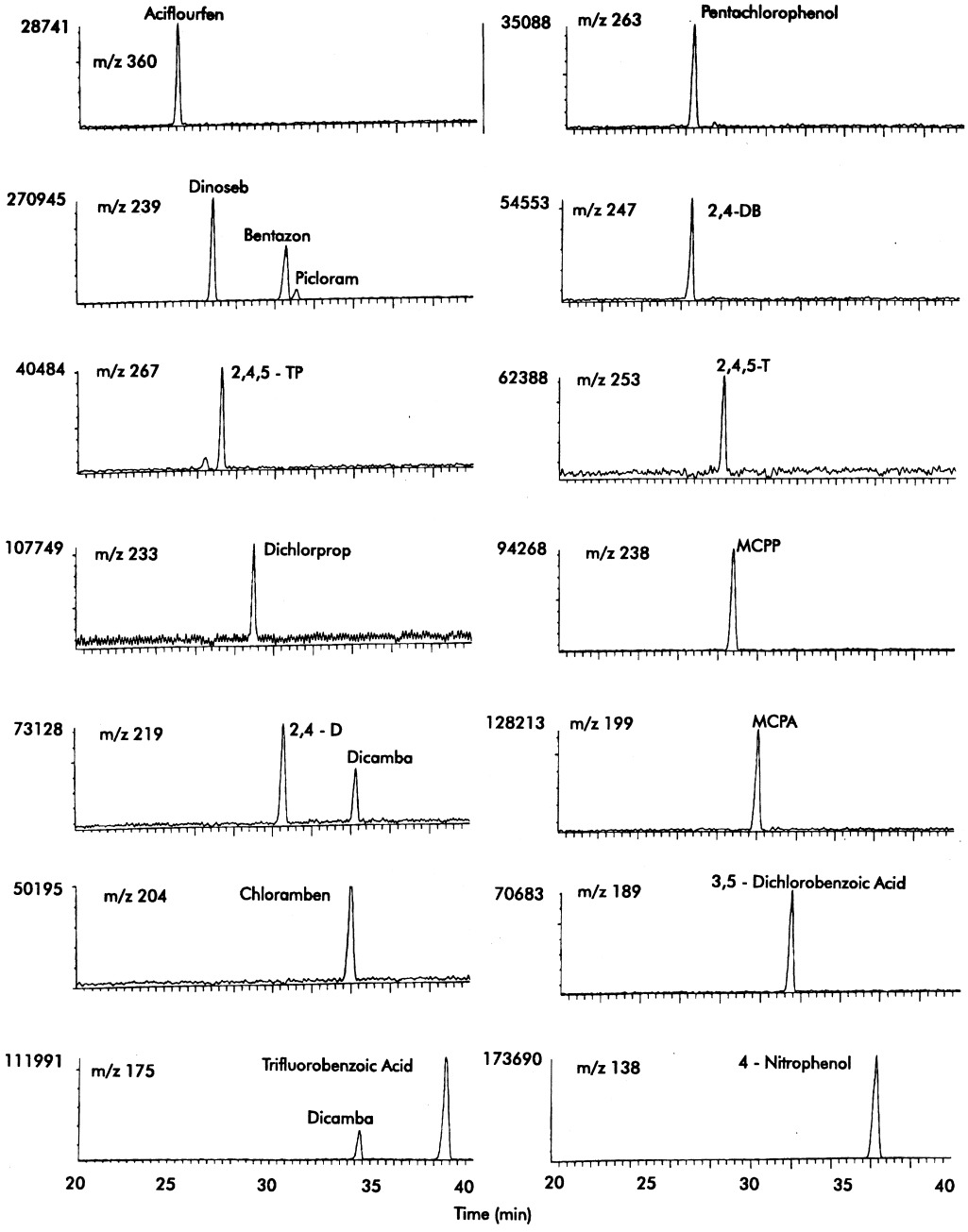


Fig. 2. Selected ion electropherograms from the CE separation of the sixteen analytes and the internal standard at a concentration of 5 mg/l.

shows the selected ion electropherograms of nine analyte anions at 50 μg/l and the internal standard TFBA at 250 μg/l. The analyte concentrations in Fig. 3 are a factor of 100 lower than shown in Fig. 2 and are well below the maximum contaminant levels for several regulated compounds [5]. The volume

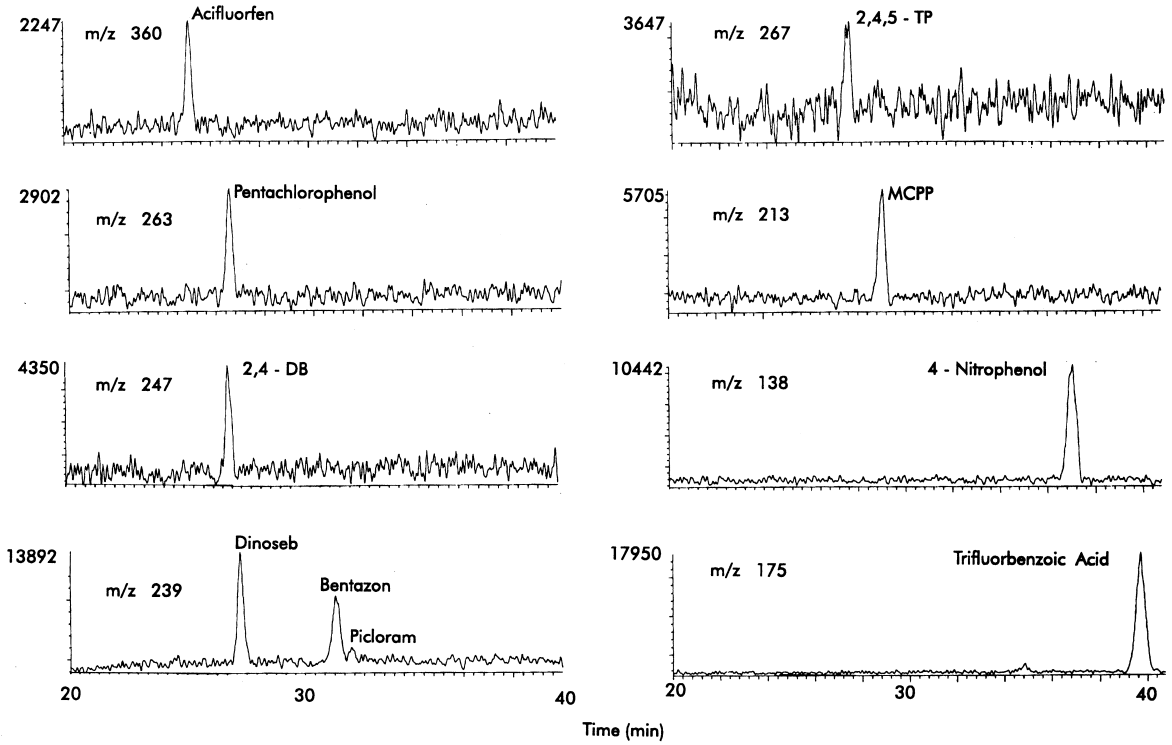


Fig. 3. Selected ion electropherograms from the CE separation of nine of the analytes at the concentration of 50 μg/l and the internal standard at 250 μg/l with sample stacking on the CE column.

injected was 440 nl with sample stacking and the peaks in Fig. 3 correspond to about 22 pg of each analyte.

Sample stacking techniques in CE have been described in detail with particular emphasis on negative ions [30,31]. Briefly, the acids were dissolved in buffer solution that was diluted by a factor of ten with deionized water. Therefore the analytes were injected in a solution containing 0.5 m*M* ammonium acetate in 4% isopropanol at pH 10. This analyte solution, 440 nl, was injected behind the normal higher concentration CE buffer and a potential of +30 KV was applied at the source reservoir. The anions in the injected sample stacked at the boundary between the lower concentration sample buffer and the higher concentration CE buffer, then were separated and measured by ES-MS in the usual way. The quantities of analytes in the stacked sample (Fig. 3) are about one-third the quantities of the standard injection (Fig. 2). The stacking procedure produced broader CE peaks, which has been reported previously with other negative ion analytes [31], but still with reasonable resolution.

3.3. Quantitative Analyses

Internal standard concentration calibrations for all sixteen analytes were evaluated using four concentrations in the range from 50 to 500 μg/l with sample stacking and four concentrations in the range from 1 to 25 mg/l without sample stacking. The concentration of the internal standard TFBA was 250 μg/l for the lower concentration range and 5 mg/l for the higher concentration range. The abundances of the quantitation ions were integrated over the widths of the selected ion electropherogram peaks for each analyte ion and the internal standard. The ratio of the integrated abundance of the analyte anion to the integrated abundance of the internal standard

anion was plotted as a function of the analyte concentration for each analyte. Linear plots were obtained with correlation coefficients better than 0.998 for all analytes. Calibration variability was determined by five replicate analyses of the calibration sample with a concentration of 5 mg/l. The R.S.D.s for these measurements are given in Table 3 and range from 3–10%.

The sixteen herbicide acids and related compounds and the internal standard TFBA were added to Cincinnati drinking water at a concentration of 5 mg/l and analyzed without sample stacking. Cincinnati drinking water has background concentrations of total dissolved solids, mostly inorganic substances, typically in the 100–300 mg/l range. Recoveries obtained in a single analysis are given in Table 3 and range from 91–124%. The estimated detection limits (EDLs) in Table 3 were determined at a signal-to-noise ratio of about 3 using sample stacking data similar to that shown in Fig. 3. The EDLs of 2,4-D, 2,4,5-TP, and picloram (60, 50, and 100 μg/l respectively) are either close to or below the USA drinking water maximum contaminant levels of 70, 50, and 500 μg/l respectively [5]. The EDLs of pentachlorophenol and dinoseb (30 and 13 μg/l, respectively) are above the maximum contaminant levels of one and seven μg/l respectively [5]. Ten of the EDLs in Table 3 are in the range of 8–32 μg/l which is an environmentally useful range for some types of samples.

Several options are available which can be used alone or in combination to lower the detection limits of the CE–ES-MS technique. Preconcentration of a drinking water sample, such as simple evaporation, lyophilization, or concentration of the analytes on a solid adsorbent, can be used to concentrate the sample by a factor of 10–1000. These techniques are well developed, widely used, and proven effective but add to the time, cost, and complexity of the analysis. Nevertheless, since they are well-developed it can be assumed that if used they would add the appropriate sensitivity factors to the CE–ES-MS technique and allow detection of all the analytes at or well below 1 μg/l. Alternatively, improvements in CE sample injection or stacking techniques which allow much larger injections and increase sensitivities by factors of 100–1000 are developed but could not be utilized in this research because of time

Table 3
Precision of replicate measurements, recoveries, and estimated detection limits for carboxylic acid herbicides and related compounds in water

Analytes	R.S.D.[a] (%)	Recovery[b] (%)	E.D.L[c] (μg/l)	E.D.L[c] (pg)
Acifluorfen	9	106	30	13
Pentachlorophenol	7	99	30	13
2,4-DB	8	104	30	13
Dinoseb	3	106	13	6
2,4,5-TP	6	119	50	22
2,4,5-T	10	116	100	44
MCPP	8	110	21	9
Dichloroprop	7	103	250	110
MCPA	9	102	32	14
Bentazon	5	124	20	9
2,4-D	8	99	60	26
Picloram	8	91	100	44
3,5-Dichlorobenzoic acid	10	122	21	9
Chloramben	10	106	24	11
Dicamba	5	100	40	18
4-Nitrophenol	7	102	8	4

[a] Relative standard deviations (R.S.Ds) from five replicate analyses of a 5 mg/l calibration standard.
[b] From a single measurement of each analyte added to Cincinnati drinking water at 5 mg/l.
[c] Estimated detection limits (E.D.Ls); the concentration or amount injected which gave a signal-to-noise ratio of about 3 for the integrated abundance of the quantitation ion.

constraints [31]. Implementation of these injection techniques should allow sub-μg/l detection limits with no sample concentration, no analyte extraction, no extract concentration, and no derivatization. Improvements in electrospray interface designs, which are already available from some manufacturers, will likely increase the fraction of ions transmitted into the mass spectrometer and increase analytical sensitivity. Electrospray interface design is still in an early stage of development and significant improvements are expected in the future. Finally, this research was conducted using a conventional single quadrupole mass spectrometer of late 1980s vintage that is no longer in production. Substantial increases in sensitivity are already available on several types of mass spectrometers and utilization of these instruments should substantially support this analytical approach [25].

4. Conclusion

The CE–ES-MS method for the determination of the herbicide acids and related substances in water is extremely simple, rapid, and uses only very low-cost supplies including a short uncoated FSCC. The sixteen acid herbicides and related compounds can be separated in time, or when coeluting measured with different ions, in about 40 min with no sample preparation besides pH adjustment. Quantitative analyses using selected ion monitoring and internal standardization provides good recoveries and precision. While some detection limits are in a useful range, standard sample concentration techniques, sample stacking, existing instrumentation improvements, and anticipated improvements in instrument design clearly indicate that environmentally useful detection limits are readily available.

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